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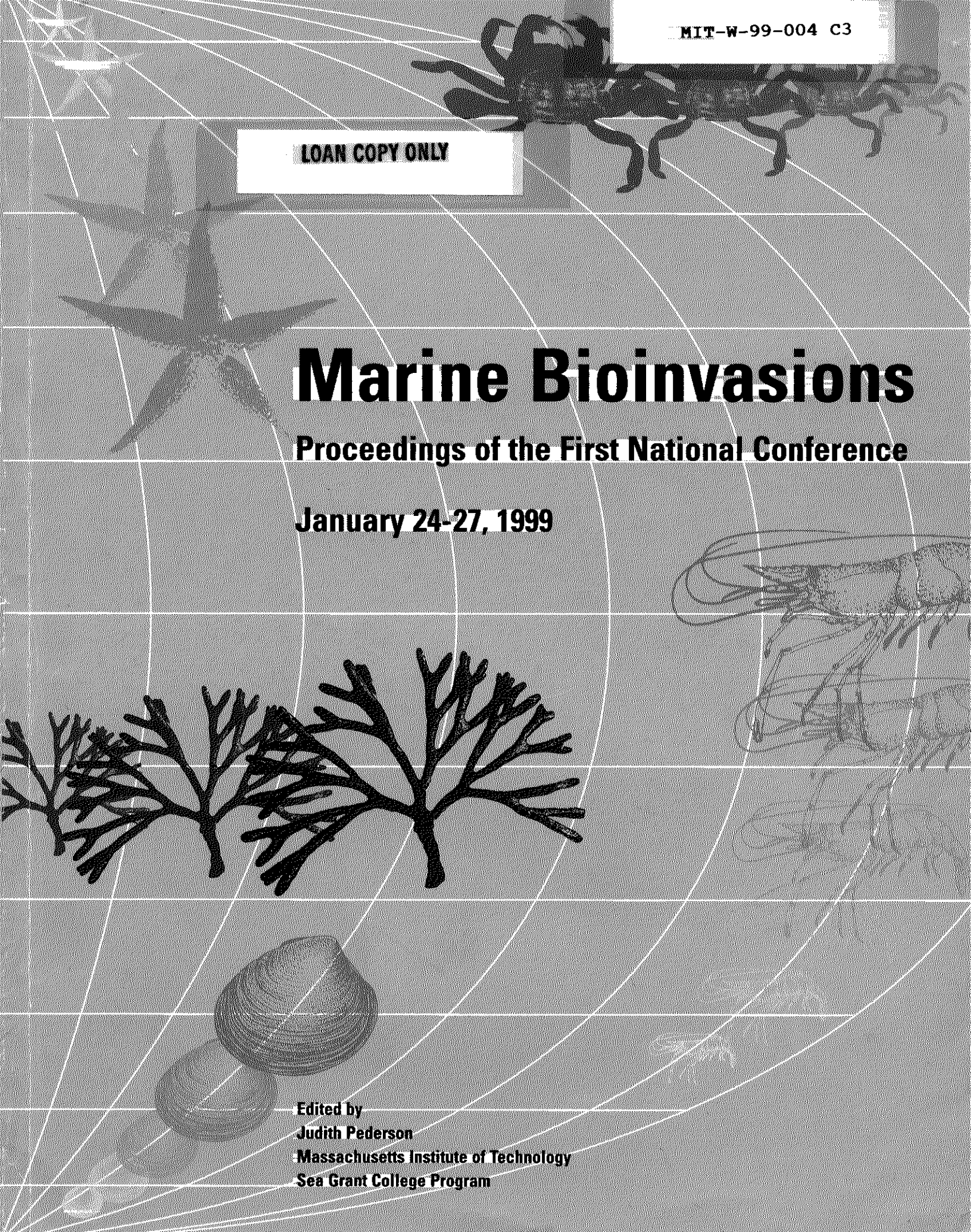

Marine Bioinvasions

Proceedings of the First National Conference

January 24-27, 1999

Edited by
Judith Pederson
Massachusetts Institute of Technology
Sea Grant College Program

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Marine Bioinvasions

PROCEEDINGS OF A CONFERENCE

JANUARY 24–27, 1999

Edited by
Judith Pederson
MIT Sea Grant College Program

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MIT SG Center for Coastal Resources
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Preface

As recently as twenty years ago, only a handful of experts was discussing marine bioinvasions or expressing concern about impacts of nonindigenous species on ocean communities or ecosystems. That situation changed around the world with the appearance in the 1980s of the Eurasian zebra mussel (*Dreissena polymorpha*) in the Great Lakes (U.S. and Canada), the American comb jellyfish (*Mnemiopsis leidyi*) in the Black Sea and Japanese dinoflagellates in southern Australia. The resultant ecological and economic impacts ushered in a new era of awareness. National legislation (the National Aquatic Nuisance Species Prevention and Control Act of 1990) was passed and called for action to prevent new invasions. Funding supported new research initiatives and managers explored options for preventing new invasions, especially through ballast water introductions. Scientists, managers and industry representatives began to meet annually to share information, identify ways to manage and control invasive species, and describe technologies designed to prevent future introductions.

With the reauthorization of nonindigenous species legislation (Nonindigenous Species Act of 1996) greater emphasis was placed on marine invasions. The conference on which this volume is based grew from a perceived need by a steering committee to convene a national meeting for those studying marine invasions to share insights into the science of invasion ecology and into managing what is a growing worldwide problem. The first National Conference on Marine Bioinvasions was held January 24–27, 1999 at the Massachusetts Institute of Technology, Cambridge, Massachusetts, USA and attracted approximately 250 national and international participants. The purpose was to bring together scientists, students, and managers to examine patterns of marine bioinvasions, ecological and evolutionary consequences, and ballast water management.

This volume, *Marine Bioinvasions: Proceedings of a Conference*, consists of many of the papers presented at the conference. It covers new and ongoing research, work in progress, current status of management options, and recommendations for new approaches to prevent and better manage biological invasions. Each submitted paper was subjected to peer review by at least two external reviewers and revised by the authors. Over half of the presenters submitted papers; abstracts of the remaining presentations have been included to provide a comprehensive view of the subject matter of the conference.

The volume is organized around three major topics: Patterns of Invasions, Ecological and Evolutionary Consequences, and Ballast Water Management. An additional section on outreach and education highlights the Sea Grant Programs' efforts to inform a broad-based audience. The papers cover a range of topics that are fundamen-

tal to understanding marine bioinvasions and their impacts. The distribution and pattern of species in space and time, molecular approaches to identifying sources, management options to prevent introductions, estimation of risk, and technological developments for managing ballast water are addressed by several authors. Together the papers represent a rich assemblage describing what is known about marine invasions and options for managing or preventing introductions.

Not all topics were addressed at the conference and their absence in this volume reflects a lack of response rather than a deliberate omission. Issues relating to aquaculture where alien species are intentionally and unintentionally released are only touched upon. However, the changes to native populations through predation, competition, and genetic alterations may be significant. The more general topic of the effects of aliens species on biodiversity is acknowledged, but not discussed in depth. The role of nonindigenous species in homogenizing communities is poorly documented but may have significant evolutionary consequences. Biological control is a topic that generates passionate debate as to its viability in marine waters, but this topic was poorly represented at the conference.

Some of the more open-ended issues were discussed as part of a panel discussion held at the end of the conference. The panelists were asked to respond to the following statement:

"*We have no evidence that we can prevent ALL bioinvasions in the long term, and with few exceptions bioinvaders are here to stay.*"

The dialogue between the audience and the panelists went beyond the individual studies and highlighted areas for further study. Ballast water management remains as a major topic of discussion with different technologies proposed but few have been field tested on ships. Nor was there consensus that biological control in marine waters is a viable option. The statement implies that all bioinvaders are unwanted, but in some regions the invasive species has become a source of income–a new resource. The proponents of "black lists" argue that risk assessments can be used to identify species most likely to become invasive, others argue that we cannot predict which species will become invaders and we should assume that all species are potential problems. There was much discussion about early detection and rapid responses to invaders that are likely to cause problems, and examples were given illustrating successes and failures of responding and not responding to early sightings. There was agreement that prevention, early detection, and eradication were more cost effective than management and control efforts once a species was established. It is anticipated that the unresolved issues will serve to focus the next conference.

This volume should be of use to marine biologists, environmental scientists, managers, students, industries that may introduce or be impacted by marine invasions, and those with an abiding interest in the sea and how humans impact it. The challenges are clear: How do we, as a society, do a better job of preventing new invasions? What is needed to more realistically document ecosystem impacts and to translate these for managers and policy makers? What are the socio-economic costs to individuals who lose their livelihoods and to society, which pays for control of marine invasions? Through sharing ideas and exchanging information, the many facets of marine bioinvasions will become more understood and lead to new insights. Collectively, this volume of papers and abstracts offers insights beyond the individual discussions and offers a holistic view that is greater than the sum of the individual parts.

JUDITH PEDERSON
Editor

First National Conference on Marine Bioinvasions

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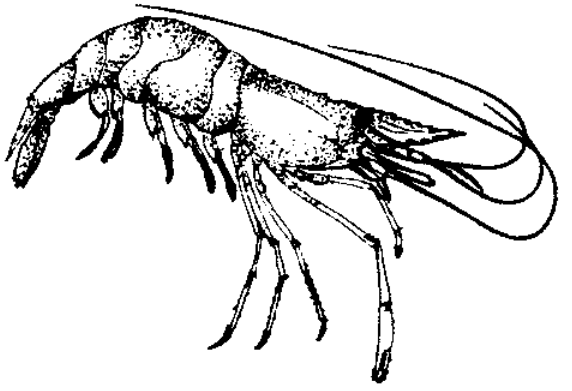
In Memoriam

We mourn the passing of Neil Richmond, Oregon Department of Fish and Wildlife, who took great interest in and concern about the impact of invasions on aquatic resources.

Index of Acronyms

AAPMA	Australian Association of Ports and Marine Authorities
ABWMAC	Australian Ballast Water Management Advisory Council
ANOVA	analysis of variance
ANS	aquatic nuisance species
APEC	Asian Pacific Economic Co-operation
APHIS	Animal and Plant Health Inspection Service (in U.S. Department of Agriculture)
AQIS	Australian Quarantine and Inspection Service
ARCO	Atlantic Richfield Company (oil company)
ASP	amnesiac shellfish poisoning
AW	abdominal width
BACI	before-after-control-impact
BAH	Biologische Anstalt Helgaland
BAT	best available technology
BFU	Baltic Floating University
BT	biological treatment
BW	ballast water
BWE	ballast water exchange
BWR	Ballast Water Reporting (Forms)
Cal-Sag Channel	Channel between Calumet River and Saganeshkee Slough, IL, U.S.
cfs	cubic feet per second
CL	carapace length
COTP	Captain of the Port (U.S. Coast Guard)
CRC	Cooperative Research Centres (Australia)
CRIMP	Centre for Research on Introduced Marine Pests (Australia)
CSIRO	Commonwealth Scientific Industrial Research Organisation (Australia)
CW	carapace width
CWA	Clean Water Act (U.S.)
DAF	dissolved air filtration
DNA	deoxyribonucleic acid
DSP	diarrhetic shellfish poisoning
DSS	decision support system
DSTO	Defense Science and Technology Organisation
DWT	dead weight metric tons (or dwt)
EEC	European Economic Community
EEZ	Economic Exclusive Zone
EGM	extragenomic markers
EMECS	Environmental Management of Enclosed Coastal Seas
EPA	U.S. Environmental Protection Agency (*see also* USEPA)
ETI	Environmental Technical Institute (Singapore)
EUCA	European Union of Concerted Action
EUMAST	European Union Marine Science and Technology Program
EXCH.	exchange
FAO	Food and Agricultural Organizations (United Nations)
Forum	1998 Forum on Ecological Surveys of Aquatic Nuisance Species
FY	Fiscal Year
GESAMP	Group of Experts on the Scientific Aspects of Marine Pollution
GLBTDP	Great Lakes Ballast Technology Demonstration Project
HAZOP	hazard and operability
HIMB	Hawaiian Institute of Marine Biology
HMSC	Hatfield Marine Science Center (OR, U.S.)
HSD	Tukey multiple range test
ICES	International Council for the Exploration of the Sea
ILTA	invasions lag-time analysis
IM Canal	Illinois Michigan Canal
IMA	ideal mechanical advantage
IMO	International Maritime Organization (United Nations)
IPM	integrated Pest Management
ITS	internal transcribed spacer
IUCN	International Union for the Conservation of Nature
LPOC	last port of call
MAF	Ministry of Agriculture and Fisheries (New Zealand)
MAFF	Ministry of Agriculture, Fisheries and Food (United Kingdom)

MANOVA	Multivariate analysis of variance
MARAD	Maritime Administration (U.S. Department of Transportation)
MARFI	Marine and Freshwater Resources Institute (Australia)
MARPOL	Convention for the Prevention of Pollution from Ships
MassGIS	Massachusetts Geographic Information System
MBL	Marine Biological Laboratory (Woods Hole, MA, U.S.)
MDH	malate dehydrogenase
MEPC	Marine Environmental Protection Committee (IMO, United Nations)
MFish	Ministry of Fisheries (New Zealand)
MIST	Marine Invasive Species Team
MITSG	Massachusetts Institute of Technology Sea Grant College Program
MLLW	mean low, low water
MLW	mean low water
MPA	Maritime and Port Authority (Singapore)
MS	motorship
MSA	mixed stock analysis
MT	motor transport (also as M.T.)
MV	motor vessel (also as M/V)
NABS	National Ballast Survey
NANPCA	Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990
NAS	National Academy of Science (U.S.)
NEMO	Nonindigenous Estuarine and Marine Organisms
NE-MWI	Northeast-Midwest Institute
NIS	nonindigenous species
NISA	National Invasive Species Act of 1996 (U.S.)
NIWA	National Institution of Water and Atmospheric Research
NMFS	National Marine Fisheries Service (U.S.)
NOAA	National Oceanic and Atmospheric Administration (U.S.)
NOBOB	no exchangeable ballast on board
NPDES	National Pollution Discharge Elimination System (issued as a permit)
NRC	National Research Council (U.S.)
NSF	National Science Foundation (U.S.)
NTIS	National Technical Information Service (U.S.)
NTU	nephelometric turbidity unit
NUS	National University of Singapore
PCR	polymerase chain reaction
P.L.	Public Law (enacted by U.S. Congress)
PGI	phosphoglucose isomerase
PGM	phosphoglucose mutase
PPB	Port Philip Bay (Australia)
ppt	parts per thousand (refers to salinity; *see also* psu, ‰)
PSP	paralytic shellfish poisoning
psu	practicality salinity unit
PVA	population viability analysis
QRA	quantitative risk assessment
R&D	research and development
RAPD	randomly amplified polymorphic DNA
RCAC	Regional Citizens Advisory Council (Prince William Sound, AL, U.S.)
RFLP	restriction length polymorphisms
RFP	request for proposal
RI	retention index
RNA	ribonucleic acid
RSHMU	Russian State Hydrometerological University
San-Ship Canal	Chicago Sanitary and Ship Canal
SCOPE	Scientific Committee on Problems of the Environment
SERC	Smithsonian Environmental Research Center (U.S.)
sp.	unknown, but assumed, single species
spp.	several species, genus not identified to individual species
ssp.	subspecies
SST	sea surface temperature
Task Force	generally refers to the ANS Task Force (U.S.)
TBT	tributlyltin
TEMA	Training Education and Mutual Assistance
TEP	transposable element polymorphism
UN	United Nations
UNEP	United Nations Environmental Programme
UNESCO	United Nations Environmental and Cultural Organization
-IOC	-International Oceanographic Commission
U.S.	United States
USA	United States of America (also U.S.)
USACE	U.S. Army Corps of Engineers
USCG	U.S. Coast Guard
USDA	U.S. Drug and Agriculture
USEPA	U.S. Environmental Protection Agency (also EPA)
USFWS	U.S. Fish and Wildlife Service (also called Service)
UV	ultraviolet
UW	University of Washington (U.S.)
VLCC	very large crude carrier
WHO	World Health Organization
wwt	wet weight
WWU	Western Washington State University (U.S.)
y.o.y.	young of the year



Plenary Lectures

'In 1962... it was beyond imagination that we would close this century with a higher level of national and international awareness of bioinvasions in the seas than ever before.'

Plenary Lectures

Launching a Counterattack Against the Pathogens of Global Commerce

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At the outset, let me congratulate the conference sponsors. You are taking the initiative in a much neglected field. Marine bioinvasions have large consequences for our food supply, our economy, our fishing industry, and human health. These invasions also threaten to degrade and homogenize coastal waters in every corner of the seven seas.

Ten years ago, just after midnight on March 24, the Exxon Valdez crashed into a reef in Prince William Sound. Eleven million gallons of crude oil poured into the pristine waters, casting a shroud over hundreds of miles of shoreline. Television crews on the scene broadcast images of seabirds, otters, and sea lions, slicked black with oil. Those images fixated the world on the dangers of oil spills and led to many new laws and regulations designed to prevent another such tragedy.

Yet the biological spills taking place in Prince William Sound from oil tankers go virtually unnoticed. Just over a year ago the U.S. Fish and Wildlife Service discovered four new species of zooplankton spreading through the Sound, released from ballast water brought by tankers from Southeast Asia via San Francisco Bay. In the long run, these zooplankton, feeding on phytoplankton utilized by the Dungeness crab, may change the Sound more extensively and permanently than any oil spill. And no one has a clue—or a dime—to contribute toward a massive clean up. Were that even possible.

With just four small bioinvasive species, Prince William Sound is relatively lucky, so far. But look farther south, where a prolific and hungry European stowaway has disembarked. The green crab has begun to infest Pacific coastal waters, devouring anything from commercially valuable oysters and clams to barnacles, algae, and snails. And it's not alone: in the northwest nearly forty percent of all aquatic species are exotic, including the *Spartina alterniflora* that has choked Willapa Bay, Washington, and decimated the shellfish industry. This particular invader came from our own Atlantic coastal estuaries.

It gets worse inside the Golden Gate. There, as Interior Secretary, I have worked with environmentalists, irrigation farmers, and cities to get more freshwater down California's main rivers into the Delta and San Francisco Bay. Our goal is to help restore endangered native fish like Chinook salmon and Delta smelt. Only now I know that it is not enough to ensure healthy flows downstream; our real threats may be coming upstream.

Specifically, some 30 species of exotic fish—Asian goby, Atlantic shad, Mississippi catfish, carp, bass, perch, sunfish, goldfish—are swarming the Bay, a veritable marine zoo. An additional 200 bioinvasive species are suffocating native fisheries and helped drive the thicktail chub to extinction. Those are only the documented cases, with new arrivals every ten weeks.

Moving eastward, the Gulf of Mexico is being mugged by the brown mussel, which displaces native mollusks, threatens mangroves, and fouls water intake systems. In the Chesapeake, a hotspot with over 150 documented bioinvasive species, oyster beds now succumb not only to polluted runoff, or overharvest, but to the new arrival of a predatory whelk. I'll let the courageous researchers detail what's happening less than a mile away from here, in North America's oldest coastal port and fishery. It's too depressing for me.

It might be easier if we could simply blame the rest of the world for our troubles. But the truth is that ballast water sloshes both ways. In the early

1980s, a small, luminescent blob called Leidy's comb jelly was pumped aboard ships along our coast, then discharged weeks later into the Black Sea. With no predators, it mushroomed into one of the most intense marine invasions ever recorded, nearly wiping out anchovies and other fisheries. Zebra mussels exchanged for jellyfish: the maritime law of reciprocity at its darkest.

No place on earth is immune from the twin threats of extinction and alien invaders. In the mid-nineteenth century, when wooden whaling ships crisscrossed the seas in bloody pursuit, Herman Melville pondered "whether Leviathan can long endure so wide a chase and so remorseless a havoc; whether he must not at last be exterminated from the waters." He took note of how we were pushing the buffalo to extinction on the prairies, but dismissed it as impossible on the high seas, rationalizing that, surely, whales could escape to polar regions and thus become "immortal in his species."

Mankind never used to navigate such frozen regions, even though the fouled wooden hulls like Ahab's surely carried a few unwelcome guests. To be sure, bioinvasion from ships is as ancient as the Vikings and the Phoenicians. Even when ballast consisted of stones, dirt, and iron, some exotic bioinvasive species hitchhiked along.

What has changed in the past half-century is the rate of spread, leading to faster, wider, more complex dispersal. We reach remote ports on a weekly, daily, hourly basis–from more diverse trade routes, loaded with much larger volumes of ballast. Discharge of that ballast is nothing more than "point source pollution" and must be treated as such.

Global aquaculture–shrimp farms, public fish hatcheries, commercial oyster beds–also bears responsibility for the spread of epibionts, parasites, predators, and pathogens. So does the aquarium industry: the outbreak of giant African snails in Florida or the *Caulerpa taxifolia* clone, an alga taking over the Mediterranean, originated not in ballast, but from aquarium tanks.

All these sources must be included in our response, both policy and research. But at a more immediate level, we must grasp the root of the problem. That root lies not in isolated incidents, but in scope: the dramatic rate of spread, the increasing vectors of pathogens that carried cholera to Alabama and seem to multiply toxic red tides around the world.

As a very crude rule of thumb, ten percent of invasive species will establish breeding populations; ten percent of those will launch a major invasion. At first, that one percent factor seems negligible. Then, consider how San Francisco Bay is approaching 300 exotics.

Consider also that ships in this century have grown from 3,000 tons to 300,000 tons, and the volume of ballast water slurry–pumped and sucked at 20,000 cubic meters an hour–has kept apace. Faster crossings let more species survive, reproduce, make connections, and take baggage. The fall of trade walls brings global exposure to once quiet seaside ports, and vice versa. In the ballast water of timber cargo ships traveling between Coos Bay, Oregon, and Japan, researchers found 367 species of living animals and plants.

That's a single route. Consider how larger ports, say Norfolk and Baltimore, receive more than 12 million metric tons of foreign ballast water per year, originating in 48 different foreign ports, and 90 percent of them carried live organisms, including barnacles, clams, mussels, copepods, diatoms, and juvenile fish. Worldwide, it is estimated that tens of thousands of ships carry several thousand species daily.

Let me put this another way: In the time it takes me to deliver this speech, two million gallons of foreign plankton will have been discharged somewhere in American waters. We'd better get busy. And fast.

How? What is our response? So far it has been pitiful. Frankly, in light of the economic and ecological devastation, we have been too timid. We restrain ourselves with voluntary guidelines, a scattered approach, and limited unenforced codes. No longer.

In 1997, President Clinton, responding to concerns of scientists like yourselves, asked the Departments of Interior and Agriculture to draft an executive order for his consideration. That order, which is now before the President, will contain two broad initiatives. First, it will require federal agencies to review their existing authorities and activities to reduce the risk of bioinvaders. Second, it will create an interagency working group to draft a plan–possibly including regulatory and legislative change–necessary for a coordinated response to bioinvaders.

What will this look like in practice? I'll sketch the rough outlines in pencil. For there are existing models, and while there is still much to learn, we do know this: the first and best line of defense against bioinvaders is to keep them out in the first place. Period. Not one marine bioinvasive species, after it has taken

hold, has ever been eliminated or effectively contained. There is simply no silver bullet. This is a sobering fact. It means our efforts must be focused primarily on prevention. And that, in turn, means effective regulation and enforcement.

In 1990, in response to the damage caused by the zebra mussel in the Great Lakes, the Congress enacted the Nonindigenous Aquatic Nuisance Prevention and Control Act. Among other provisions, the Act now requires ballast water exchange at sea rather than in the Great Lakes system. We should now move toward mandatory ballast exchange for not just the Great Lakes, but for all shipping in all American ports. In California, water districts whose systems are threatened by invaders working their way upstream out of San Francisco Bay have begun to call for ballast water regulation by federal and state agencies.

We need to mount a coordinated research program to better understand the threats posed by alien invaders including fish, crustaceans, mollusks, and pathogens and to guide programs of prevention and control. Perhaps we can find economical and safe means to decontaminate ballast water and sediments *in situ*. The Agricultural Research Service and APHIS in the Department of Agriculture, the Coast Guard, the National Oceanic and Atmospheric Administration, and the Biological Research Division of the United States Geological Service should mount a coordinated effort to understand agricultural threats, threats to natural ecosystems, and new methods of prevention and control.

Does this mean our agency budgets must catch up to, and keep pace with, the ecological devastation they target? Yes, because that devastation is economic as well. Vast as they are, the Great Lakes are easy to manage compared to the task ahead, and but offer few unqualified success stories. Yet, the results there make a strong case for why an aggressive, well-funded public response to bioinvasion is well worth the expense and effort.

We spend several million dollars a year sterilizing, catching, poisoning, and putting up barriers to suppress the sea lamprey. Well, it's still there and it may never go away. But for every dollar we invest, the Great Lakes earn $30.25 in increased fisheries revenue. Your stock portfolio should perform as well.

Global cooperation is an imperative. Our joint efforts with Canada on the Great Lakes provides an example. Two global entities—the Convention on Biological Diversity and the World Trade Organization—should play a major role in international cooperation. The Convention on Biological Diversity is the place to begin, and indeed preliminary discussions pursuant to Section 8 of the Biological Diversity Treaty are underway. Those discussions underline the need for Senate ratification of the Biodiversity Treaty. The World Trade Organization must also take an active role in the movement to develop and harmonize regulations in this area.

Let me conclude on a cautious note of hope. You've all heard that the flip side of crisis is opportunity? Well, the Exxon Valdez crash gave us such an opportunity. It led Congress to require double-hulled tankers and stiffen training, navigation, and technology within the shipping industry. It prompted state, federal, and private agencies to establish habitat restoration programs and undertake comprehensive research on the North Pacific ecosystem.

We face an even greater opportunity now. The time is at hand for scientists, policy makers, industry, and the public to join together for an intensive coordinated counterattack on the threat of bioinvasions. You have initiated that process, and we in the public sector must now respond in kind.

PLENARY LECTURE

Quo Vadimus Exotica Oceanica? Marine Bioinvasion Ecology in the Twenty-First Century[1]

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Key words: invasion ecology; historical perspective, evolutionary consequence

These are heady times in the world of marine bioinvasions, as witnessed by the gathering of over 200 persons here this morning. In January 1989, such a congregation would have been inconceivable. A new journal, *Biological Invasions*, is being launched this fall[3] that will serve as a platform for invasion science. And as we will hear tomorrow morning, a Presidential Executive Order on exotic species will be released 10 days from now.[4]

Despite this remarkable blossoming of interest, marine invasion science is a young science and challenges abound. The depth and breadth of the profound alteration to marine communities by invasions in the oceans remain, in large part, unknown and thus vastly underestimated. Invasions have occurred not only in estuaries and harbors but also in exposed rocky intertidal shores, coral reefs, mangrove communities, open continental shelves, and the deep sea. Indeed, it may be that, at the least, no shallow-water temperate or tropical marine community in the world now remains untouched by human-mediated bioinvasions, but that hypothesis remains to be tested. This morning I will suggest ways in which we need to be more rigorous, more refined, and more aggressive in our grasp of the temporal and spatial scales of the ecology of invasions in the seas.

We need to be clearer and less hesitant about the *scale* of invasions that must have occurred prior to the 19th century. We need to wash away the salty cloud of antiquity that obscures the modern history of marine communities. It is impossible to overemphasize the poor picture that we have of the nature of the ocean's biota only 100 or 200 years ago. Ships with organisms on and in their hulls and in their rock and sand ballast have moved species around the world since at least the 14th century. But we too often think of invasions as beginning, more or less, in the 19th century. If in the 300-year period between 1500 and 1800, only three species a year were spread around the world (the number, of course, may be much greater), then nearly 1000 coastal species of marine organisms that are now regarded as naturally cosmopolitan are in fact "simply" early introductions.

This estimation is not a mere historical curiosity: an understanding of the number and identity of pre-19th century invaders would profoundly impact both our understanding of modern marine community ecology and our basic assumptions about and interpretation of the natural diversity, biogeography, and rate of evolution in the seas. In terms of invasion biology itself, we can ill afford to seek patterns such as the relative susceptibility or resistance of different regions to invasions, or attempt to define guilds or

[1]This paper is the conference opening Plenary Lecture, with modifications, as presented on January 25, 1999. As such the lecture format is retained here and no references are cited in the text. However, an extensive and partially annotated bibliography of marine bioinvasions literature is presented at the end.

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[3]The first issue of *Biological Invasions* was published in October 1999.

[4]Secretary of the Interior Bruce Babbitt spoke on Tuesday morning, January 26, 1999. President William Clinton's Executive Order was released in Washington, D.C. on Wednesday, February 3, 1999.

clades of invaders that may be more or less likely to invade, if we persist in ignoring more than 75% of the modern invasion history in the ocean. It follows that at least some of the hundreds of pre-1800 invasions are likely to be the common, if not abundant, species where they were introduced long ago, and thus perhaps some of the most important organisms regulating community structure.

But which ones are they? How startled would we be if we could look back at some of our "best known" shallow marine communities–kelp beds, rocky shores, and coral reefs–and find that keystone species were absent in 1599 or 1699 or 1799? Why is it that we cannot tell if a species has been present for 100 years or 100,000 years, or are we not paying attention to what evolution is telling us? Should not the presence of certain clades or lineages in certain marine communities that appear to have evolutionary roots elsewhere–such as mussels of the northern genus *Mytilus* in the southern hemisphere–not surprise us? By using morphological, genetic, historical, paleontological, archeological, and other evidence we may be able to begin to look below this cryptic invasion iceberg:

- The ship-boring isopod, *Sphaeroma terebrans*, possibly native to the Indian Ocean, appeared in the Caribbean Sea or northwestern South American coast sometime in the 19th century. It bores into and destroys the seaward root tips of mangroves. It may have reset the lower intertidal limit, and thus the history of outward propagation, of the mangrove ecosystems of the tropical western Atlantic Ocean. It passes without notice in the literature of invasions.
- The Asian seasquirt, *Styela plicata*, was carried to the North American Atlantic coast perhaps two or more centuries ago and became one of the hallmark species in the concept of multiple stable state communities. The species falls outside of our general view of marine invasions.
- And, as hinted at above, the northern hemisphere mussels, *Mytilus galloprovincialis* and *Mytilus edulis*, were carried as fouling organisms by ships to the southern hemisphere for centuries, and there given a plethora of local names.

These are merely a few examples. We need iconoclastic invasion ecology. We need to question the "assumption of naturalness." In fact, the modern historical geography of thousands of coastal species of planktonic and benthic organisms remains unknown. Thus, such species must be removed from the category of "native until shown otherwise", and instead be placed in the rapidly growing category of cryptogenic species. Did the giant kelp, *Macrocystis pyrifera*, for example, now found in both the southern and northern hemispheres, and taken to be a classic textbook example of natural bipolarity, of necessity naturally occur in both hemispheres? Or could *Macrocystis* have been carried on the hulls of Spanish ships–since it can be a fouling organism–from the North Pacific Ocean as early as the 1500s? The early footprints of human activities across the oceans became the shipprints of the world and yet we have largely fallen virtually silent about the potential for such early invasions.

Why be concerned about earlier invasions? Why should we care about invasions of 100 or 200 or 300 years ago? There is the potential value to a greater resolution of global invasion patterns as noted earlier, but beyond that, are not such early invasions "naturalized"? Aren't they "integrated" into the community? Isn't the community "in equilibrium" by now? That we should invoke naturalization, integration, and equilibrial processes underscores another arena of ambiguity in our thinking in invasion ecology. The word "naturalized" was introduced in 19th century botanical literature to mean "reproducing in the wild"–not to mean a remarkably rapid conversion over a few decades or centuries to mirror the integration that native species achieve over tens or hundreds of thousands of years. The answer is that we cannot pronounce invasions of past decades or centuries as being well integrated: there are by and large no data to support such concepts. Simply becoming abundant and widespread is not ecological integration. Simply eating or being eaten is not ecological integration. Integration implies a vast suite of interrelated functions, rather than a functional response along one or a few axes, such as predation, space utilization, or competition. We know little about the rate of these integrative processes in invasion ecology. It may be that in terms of evolutionary processes and community integration, the European periwinkle, *Littorina littorea*, which arrived on the shores of Atlantic North America in the early 19th century, arrived only "yesterday."

Several other famous myths in invasion science are worth noting. One is that "everything that could have been introduced would have been introduced by now." This is not simply an image in the mind of

a ship's captain who is contemplating 100 years of ballast water movement, nor is it the imagination of the hopeful commercial entrepreneur. Rather, we learn that grant proposals to investigate dispersal vectors are turned down even today by a hand-wave of such statements. That everything has not been introduced by now is demonstrated every day. Were it so, all the ship fouling organisms of Europe that could survive and reproduce in American waters would be here by now.

Another myth is the following: "Invasions are part of nature. They always happen. Human-mediated invasions are only speeding up what would happen eventually." This statement is, of course, also not true. Most–perhaps all?–of the invasions now occurring would not only *not* happen sooner or later, they would *never* happen. Species are not "eventually" exchanged by natural processes between San Francisco Bay and the Black Sea, species do not "eventually" find their way in ecological time between Australia and England, and species do not "eventually" move between Argentina and Puget Sound. The fact that over geological time there is a predictable natural ebb and flow of biota along coastlines and within or between ocean basins, as barriers dissolve or are created, has little to do with the past several centuries of human-mediated alterations to the oceans.

Another myth is that phytoplankton have been and are, with a few exceptions, not part of the modern invasion story. Since just the reverse may be true, the existence of this illusion may have had profound impacts on our ability to understand the scale of invasions and invasion processes–and indeed may have caused us to be several to many decades behind in ballast management, relative to one major reason why harmful algal (toxic phytoplankton) blooms may have mushroomed in the past quarter century.

This sense of size-mediated invasion is a huge bias in our science. We recognize introductions most often among the charismatic megainvasions–clams, crabs, seastars, large seaweeds. We recognize some invasions among smaller organisms–copepods, amphipods, bryozoans, hydrozoans, and so forth. But when we get to very small organisms–the diatoms, the dinoflagellates, the pfiesterias, the brown tides (aureococuses)–we simply say, with rare exception, "no invasions here." The transparency of recognizing invasions only by size could not be clearer: not one professional phytoplankton ecologist, biogeographer, or systematist is speaking at or attending this meeting, although we will hear again and again about phytoplankton and ballast water from other workers. Ironically, one of the very first invasions to be recognized as being due to ballast water was the appearance of an Asian diatom in Europe in the early 1900s. We presume that such invasions have continued steadily, if largely unreported, around the world since.

We need, then, to increase the rigor of our overall thinking about invasions. And this rigor needs to be applied to every aspect of our science.

We need to pay more attention to the many biases in making "species lists" of marine invasions if we are to do more sophisticated comparisons. Our lists tend to be extraordinarily sensitive to the history of local taxonomic interest or current local available expertise, generating lists of very different emphases.

We must be more rigorous and focused in our thinking about whether introduced species have an "impact" or not. In terms of ecological and evolutionary science *every* invasion has an impact. The definition–the nature and extent–of impact is the question, not whether an impact did, did not or will occur. The extent to which invasions alter the diversity, abundance, distribution or phenology of previously existing species can be a measure of impact. *Who* is concerned–ecologists, the public, or politicians–about the type and scale of impact is a different question, but perhaps the question more often meant. *Why* we are concerned–for example, whether the invasion changes the ability of humans to use the oceans as a resource–is yet another question still. Because impact is a long sliding scale we would do better to abandon the concepts of the "Top 10 Invaders" or "Worst 100 Invaders." Rather, under the assumption that all invasions alter *some* aspect of the community mesh in which they find themselves embedded, we should focus on the *types and scales* of impacts that invasions have, rather than implying that only some small percent of invasions actually lead to impacts or cause "problems."

Perhaps there is no more important arena where we need to refine our thinking than in the field of prediction. The interface between the public and science insists on prediction, whether it is hours after an oil spill or hours after the discovery of a new introduced species. We are also interested in prediction in our science in and of itself, whether or not there are sociopolitical pressures, or questions from the press.

We are thus now engaged in a great search—we seek the Predictive Invasion Grail. We desire more than ever before to be able to predict who will invade, when invasions will occur, and what the impacts of the invasion will be. Thousands of invasions have occurred and yet, like the weather, it appears that we cannot predict the next invasion.

Is it all too stochastic? Can we evolve more rigorous models that better resolve the invasions sweepstakes—the roulette nature of invasions? In predicting who will invade is it ever possible to point to some species that will forever be unsuccessful invaders? Or is the match between an invading species' biology and the new prospective environment, in fact, a shapeshifter model of invasion ecology, where at times it appears to be a matter of trying to fit a round invasion into a square environment—but at other times the round invasion slips smoothly in?

Where do we look to unlock some of these questions? I suggest that we look more closely at those invasive species which, despite numerous apparent opportunities for dispersal, inoculation, and establishment, and which for centuries have failed to become introduced, suddenly become successful colonists. Rather than focusing on those species that *appear* to have permanently failed to invade, we should look more carefully at species that have failed to invade for centuries and then do so. These are the *delayed invaders*. Is it in these species that we can find answers to some of the long-term mysteries of those processes that regulate invasions?

An example is the five-centimeter-long European seasquirt, *Ascidiella aspersa*, a translucent, recumbent filter feeder in shallow fouling communities. This ascidian, common on hard bottoms throughout western and northern Europe was, we may speculate, on the bottoms of hundreds or thousands of vessels coming to America for 500 or more years. It first appeared in fouling communities about 1985 between Cape Cod (Massachusetts) and Long Island Sound, in southern New England, long after such communities would appeared to have been "filled" by previous ascidian invaders such as *Styela clava, Molgula manhattensis, Ciona intestinalis, Botrylloides* sp. and *Botryllus schlosseri*, which combined formed 100 percent cover in fouling communities prior to the arrival of *Ascidiella*.

Up until 1985, we might have chosen *Ascidiella* as an example of a permanently unsuccessful invader, and sought compelling reasons as to why it had failed to become established in North America after half-a-millennium of presumed transport. Why then did it invade in the 1980s and not the 1880s or 1780s or 1680s? Invasion lag-time analysis (ILTA) remains virtually untouched as a field of investigation, and yet may be a singularly important key to unlocking invasion processes.[5] This then is the *Paradox of Ascidiella*, a puzzle that must be solved. If we were to pay more attention to these creatures—the ascidiellas of the world—invasion science may move forward all that much faster.

We know—or we think that we know—some of the roads that we must explore when considering ILTA: were there changes in the donor region or changes in the recipient region? Did invasion windows open or were there unusual inoculation episodes? Did the dispersal vector change in some way? These are complicated phenomena, but complicated is not the same as unknowable or unpredictable. The answers to each question have striking implications relative to the ecology, biology, and evolutionary history of invaders; each question also opens the door to many more questions. We have to pursue interactive pathways and *integrative invasion ecology* much more robustly. Why do we not find, in invasion biology, more examples of subtle webs such as the one that links spirochete bacteria, acorn production, white-footed mice, black-legged ticks, white-tailed deer and climatic models all in one intricate mesh to predict the potential for Lyme disease? Are we not looking? The European marine fauna continues to dribble and leak into and invade North America over a long blue line that fades vaguely into the past 500 years and yet we are surprised at every new invasion. Is this because we rarely seek out the vast arrays of physical and chemical and oceanographic and biological data now available for coastal waters in order to detect a web of environmental change—and then combine such webs with detailed vector data and our knowledge of species' biology and ecology—that would anticipate new invasion opportunities?

For management purposes, predictive marine invasion science is now of only limited value. It may of course improve considerably. As an example, we cannot, today, look at what is inside the ballast water of a ship and imply that the contents are of little or

5. ILTA is distinct from lag times in population "explosion" (Crooks and Soule 1999).

no concern if a few recognized pests on a short list are absent from that tank or from the region from which the ballast water was drawn. Noting the absence of a few target species does not make the ship "safe" or "certified" or "clean." It may remain full of dozens or scores of species, like the ascidiellas of Europe or the potamocorbulas of China or the hemigrapsuses of Asia, of which we can predict little about whether they can become established outside their native regions, or, if they do, what impacts they may have.

Despite this, we must clearly get more serious about our regulatory framework. Whether it is ballast water, whose scale is so profound that perhaps it is not 3000 species a day being carried around the world but five times that, or whether it is ships' sea chests, or whether it is the live Mediterranean mussels, Chilean mussels, and New Zealand mussels that can be purchased globally in seafood stores, or whether it is the now web-based purveyors of marine life—such as one company whose website claims it to be the "World's Largest Marine Livestock Retailer: 1000s of species [of] fish, corals, clams, [and other] invertebrates. We ship to 65 countries."—whichever vector it may be, we remain with fundamental regulatory vacuums. We need to invest in prevention far more than we have, following the same philosophy that drives us to close the windows in a rainstorm before we start mopping—or at least while we are mopping—up the floor.

In closing, a common question is that if the vectors that we see today are indeed so fluid and so effective in transporting species, why do we not see more invasions? In part, we have already answered this: if we see invaders, they tend to be the larger species, and thus we tend to ignore the greater number of smaller taxa. But even more important is that despite the surge in interest in invasions, there are in fact fewer workers every passing year who are exploring the shore and fewer still who can identify what is found. There is a profound demise in the sheer pride of knowing about the natural world and about being able to identify its contents—as if such knowledge was mutually exclusive with being an experimental ecologist or a molecular geneticist or a cell biologist. With the exception of a relatively few sites around the world, our best eyes are not those of marine ecologists but those of the interested public who seek out experts to report novelties—and that puts most of the shores of the world outside our view. Bait fishermen called our attention to the Asian shore crab (*Hemigrapsus sanguineus*) in Long Island Sound, it took an amateur naturalist to alert the scientific world to the invasion of an abundant Caribbean barnacle (*Chthamalus proteus*) to the Pacific Islands, and another to discover the Japanese shore crab (*Pachygrapsus fakaravensis*) in Hawaii, and the public knew about zebra mussels (*Dreissena polymorpha*) in the Great Lakes at least a year before scientists found them. The answer to the question of "why are there not more invasions?" is that there are without doubt many more invasions than we have been recording. The demise in the knowledge of systematic biology and natural history is a critical hole to patch if we are to gain a more accurate picture of the scale and rate of change in coastal ecosystems.

In September 1962, I was introduced to the world of exotic marine organisms by unceremoniously stepping on what I was to learn, a few days later, was a small colony of exotic tubeworms in a lagoon off San Francisco Bay.[6] It was beyond imagination at that time that we would close this century with a higher level of national and international awareness of bioinvasions in the seas than ever before. This first conference on marine bioinvasions is very appropriately set on the edge of the 21st century. We are witnessing a vastly changing paradigm.

Annotated References on Marine Bioinvasions: A Highly Selective Bibliography

The following papers, and the papers they cite in turn, provide an entrée to the literature on marine introduced species. About 1,600 additional references are found in Carlton (1979). I use the hedgpethian method (Ricketts et al. 1968) of annotation here; thus, annotations are often telegraphic, not full sentences, leave out verbs and the occasional noun, and are often only understood as juxtapositions to the title of the paper itself.

Agard, J., R. Kishore, and B. Bayne. 1992. *Perna viridis* (Linnaeus, 1758): first record of the Indo-Pacific green mussel (Mollusca: Bivalvia) in the Caribbean. *Caribbean Marine Studies* 3:59-60.
See Hicks and Tunnel 1993.

6. The southern hemisphere serpulid polychaete worm *Ficopomatus enigmaticus*, then known as *Mercierella enigmatica*, on the small beach on Adams Point, in Lake Merritt, in Oakland, California.

Aguirremacedo, M.L. and C.R. Kennedy. 1999. Diversity of metazoan parasites of the introduced oyster species *Crassostrea gigas* in the Exe Estuary. *Journal of the Marine Biological Association of the United Kingdom* 79:57-63.

Allen, F.E. 1953. Distribution of marine invertebrates by ships. *Australian Journal of Marine and Freshwater Research* 4:307-316.
A classic paper outlining the principles of the subject.

Alpine, A.E. and J.E. Cloern. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. *Limnology and Oceanography* 37:946-955. *The Asian clam, Potamocorbula amurensis, plays an important role, along with other introduced bivalves, in controlling water column productivity in San Francisco Bay.*

Asakura, A. 1992. Recent introductions of marine benthos into Tokyo Bay (review): process of invasion into an urban ecosystem with discussion on the factors inducing their successful introduction. *Journal of the Natural History Museum and Institute (Chiba, Japan)* 2:1-14 (English abstract, pp. 13-14).
A useful discussion of the introduction into Japan of the polychaetes Hydroides elegans and Ficopomatus enigmaticus, the bryozoans Zoobotryon pellucidum and Bugula californica, the bivalves Limnoperna fortunei, Perna viridis, Mytilopsis sallei, and Mytilus galloprovincialis, the slipper limpet snail Crepidula onyx, the barnacles Balanus improvisus and B. eburneus, the crabs Carcinus aestuarii (as C. mediterraneus) and Pyromaia tuberculata, and the ascidians (sea squirts) Molgula manhattensis and Ciona intestinalis.

Bastrop, R., K. Jürss, and C. Sturmbauer. 1998. Cryptic species in a marine polychaete and their independent introduction from North American to Europe. *Molecular Biology and Evolution* 15:97-103.
Two species of the American worm, Marenzelleria, have been introduced to northern Europe; see Essink and Schottler (1997).

Bellan-Santini D., P.M. Arnaud, G. Bellan, and M. Verlaque. 1996. The influence of the introduced tropical alga *Caulerpa taxifolia* on the biodiversity of the Mediterranean marine biota. *Journal of the Marine Biological Association of the United Kingdom* 76:235-237.

Benech, S.V. 1978. Ocean transport of a community of the grapsid crab *Plagusia dentipes* (de Haan, 1833). *Crustaceana* 35:104.
A rare contribution to the role of semisubmersible self-propelled exploratory platforms in the transoceanic dispersal of marine life.

Berman, J. and J.T.Carlton. 1991. Marine invasion processes: interactions between native and introduced marsh snails. *Journal of Experimental Marine Biology and Ecology* 150:267-281.
The players are the native snails, Assiminea californica and Littorina subrotundata, and the introduced Atlantic snail, Ovatella myosotis: "the successful establishment of this Atlantic snail in the Pacific Northwest did not arise at the expense of native species."

Berman, J., L. Harris, W. Lambert, M. Buttrick, and M. Dufresne. 1992. Recent invasions of the Gulf of Maine: three contrasting ecological histories. *Conservation Biology* 6:435-441.
The insertion into marine communities in the Gulf of Maine (that body of water between Cape Cod in Massachusetts and Canada) of three recent invaders is considered: the seasquirts (ascidians), Styela clava and Botrylloides diegensis, and the bryozoan, Membranipora membranacea.

Bertness, MD. 1984. Habitat and community modification by an introduced herbivorous snail. *Ecology* 65:370-381.
An experimental demonstration of the impact of the introduced European snail, Littorina littorea, on low energy habitats of the southern New England coast: the outward growth of the Spartina marsh is compromised by Littorina eating the shoots and rhizomes of the marsh grass, while at the same time the grazing activities of the snail foreword of the marsh prevented the accumulation of soft sediments creating more exposed hard substrate (onto which the marsh cannot grow).

Bertness, MD., P.O. Yund, and A.F. Brown. 1983. Snail grazing and the abundance of algal crusts on a sheltered New England rocky beach. *Journal of Experimental Marine Ecology and Biology* 71:147-164.
More experiments on the ecological effects of the introduced European snail, Littorina littorea.

Beukema, J.J. and R. Dekker. 1995. Dynamics and growth of a recent invader into European coastal waters: the American razorclam, *Ensis directus*. *Journal of the Marine Biological Association of the United Kingdom* 75:351-362.

Boalch, G.T. 1994. The introduction of non-indigenous marine species to Europe: planktonic species. pp. 25-27 In: Boudouresque *et al.* 1994 (see below).
Professor Boalch has been one of the very few phytoplankton workers to recognize the introduction of diatoms and dinoflagellates by ballast water.

Boudouresque, C.F., F. Briand, and C. Nolan (eds.). 1994. Introduced species in European Coastal Waters. *European Commission (Luxembourg), Ecosystems Research Report* 8:1-111 (EUR 15309) (ISBN 92-826-6727-8).
A collection of 13 papers in English and French, originating from a symposium held in Monaco in early March 1993; nine papers provide histories of introductions. The color cover appears to show the spread of the Japanese brown seaweed, Sargassum muticum, across all of the European waters into the Mediterranean in compelling yellow, green, purple, and red colors, in 1966 (first report in France), 1977 (north and south of the English channel), 1988 (much of the

rest of western and northern Europe), and 1992 (Portugal, southern Spain, and into the Mediterranean)– that is, every ten years, except for the last date, when the picture was produced for the symposium. No explanation of the cover appears in the book: in fact, it is solely a computer-based projection of the spread of Sargassum, and has no bearing upon actual records! (Inger Wallentinus, pers. comm.).

Blackstone, N.W. 1986. Variation of cheliped allometry in a hermit crab: the role of introduced periwinkle shells. *Biological Bulletin* 171:379-390.
The introduced periwinkle Littorina littorea in New England and its utilization by the native hermit crab Pagurus longicarpus (see also Blackstone and Joslyn 1984).

Blackstone, N.W. and A.R. Joslyn. 1984. Utilization and preference for the introduced gastropod *Littorina littorea* (L.) by the hermit crab *Pagurus longicarpus* (Say) at Guilford, Connecticut. *Journal of Experimental Marine Ecology and Biology* 80:1-9.

Bouchain, J., E. Pradier, and M.T. L'Hardy-Halos. 1999. The introduced alga *Undaria pinnatifida* (Laminariales, Alariaceae) in the rocky shore ecosystem of the St. Malo area: Morphology and growth of the sporophyte. *Botanica Marina* 42:71-82.
This seaweed was intentionally planted on the Atlantic coast of France for mariculture purposes under the initial proclamation that it could not reproduce or spread. It did and it did.

Brenchley, G.A. 1982. Predation on encapsulated larvae by adults: effects of introduced species on the gastropod *Ilyanassa obsoleta*. *Marine Ecology Progress Series* 9:255-262.
The introduced species are the European periwinkle snail Littorina littorea and the European shore crab Carcinus maenas.

Brenchley, G.A. and J.T. Carlton. 1983. Competitive displacement of native mud snails by introduced periwinkles in the New England intertidal zone. *Biological Bulletin* 165:543-558.
Of native Ilyanassa obsoleta by introduced Littorina littorea. See also the work of Whitlatch and Obrebski (1980) and Race (1982).

Buttermore, R.E., E. Turner, and M.G. Morrice. 1994. The introduced northern Pacific seastar *Asterias amurensis* in Tasmania. *Memoirs of the Queensland Museum* 36:21-25.
There is little doubt that this seastar invasion finds its roots in Japan; the common name reflects political necessities. Millions upon millions of this omnivorous seastar have become established in the Derwent estuary of southern Tasmania, Australia. It has since spread to mainland Australia.

Calvo-Ugarteburu, G. and C.D. McQuaid. 1998. Parasitism and introduced species: epidemiology of trematodes in the intertidal mussels *Perna perna* and *Mytilus galloprovincialis*. *Journal of Experimental Marine Biology and Ecology* 220:47-65.

Calvo-Ugarteburu, G. and C.D. McQuaid. 1998. Parasitism and invasive species: effects of digenetic trematodes on mussels. *Marine Ecology Progress Series* 169:149-163.
The native mussel, Perna perna, in South Africa is commonly infected by digenetic trematodes while the introduced Mediterranean mussel, Mytilus galloprovincialis, lacks trematodes; this may lend Mytilus a competitive advantage.

Carlton, J.T. 1965. Lake Merritt fauna. *News of the Western Association of Shell Clubs (Northern California Malacozoological Club)* 6(1):N25-N26.

Carlton, J.T. 1979. History, biogeography, and ecology of the introduced marine and estuarine invertebrates of the Pacific coast of North America. Ph.D. Dissertation, University of California, Davis. 904 pp.
A species index was prepared by Todd Miller and John Chapman in 1999 and posted at the following site: http://www.hmsc.orst.edu/library/carlton.html

Carlton, J.T. 1982. The historical biogeography of *Littorina littorea* on the Atlantic coast of North America, and implications for the interpretation of the structure of New England intertidal communities. *Malacological Review* 15:146.

Carlton, J.T. 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanography and Marine Biology, An Annual Review* 23:313-371.
A monographic review of ballast water prior to most of the world's studies on ballast water.

Carlton, J.T. 1987. Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science* 41:452-465.

Carlton, J.T. 1989. Man's role in changing the face of the ocean: biological invasions and implications for conservation of near-shore environments. *Conservation Biology* 3:265-273.
The title of this paper plays off the famous monumental mid-20th century tome, "Man's Role in Changing the Face of the Earth", although a double entendre may have been intended, as few women are responsible for the current state of the oceans.

Carlton, J.T. 1992. Dispersal of living organisms into aquatic ecosystems as mediated by aquaculture and fisheries activities. pp.13-45 In: Rosenfield, A. and R. Mann (eds.). *Dispersal of Living Organisms into Aquatic Ecosystems.* Maryland Sea Grant Publication, College Park, Maryland. 471 pp.

Carlton, J.T. 1992. Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *Journal of Shellfish Research* 11:489-505.

Carlton, J.T. 1994. Biological invasions and biodiversity in the sea: the ecological and human impacts of non-

indigenous marine and estuarine organisms. Keynote Address. pp.5-11 In: *Nonindigenous Estuarine and Marine Organisms (NEMO). Proceedings of the Conference and Workshop, Seattle, Washington, April 1993.* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of the Chief Scientist. (September 1994). Government Document No. C55.2:N73, Government Printing Office No. 0208-C-04. 125 pp.

Carlton, J.T. 1996. Marine bioinvasions: the alteration of marine ecosystems by nonindigenous species. *Oceanography* 9:36-43.

Carlton, J.T. 1996. Biological invasions and cryptogenic species. *Ecology* 77:1653-1655.

Carlton, J.T. 1996. Pattern, process, and prediction in marine invasion ecology. *Biological Conservation* 78:97-106.
A table herein presents a series of six hypotheses as to why invasions occur when they do.

Carlton, J.T. (ed.). 1998. *Ballast Water: Ecological and Fisheries Implications.* International Council for the Exploration of the Sea (ICES) Cooperative Research Report No. 224. 146 pp.
From a September 1995 symposium in Aalborg, Denmark.

Carlton, J.T. 1999. The scale and ecological consequences of biological invasions in the world's oceans. pp. 195-212 In: Sandlund, O.T., P.J. Schei, and Å. Viken (eds.). *Invasive Species and Biodiversity Management.* Kluwer Academic Publishers, Dordrecht, Netherlands. 431 pp.
The giant kelp (brown seaweed) Macrocystis pyrifera is used as an example of a possible southern hemisphere invasion of centuries ago, what might have occurred on the bottoms of 18th-century ships is further explored, and estimates are made of the potential number of invasions that could have occurred between 1500 and 1800. The entire book, less than 20 mm thick, costs US$255, making it largely unavailable to most workers.

Carlton, James T. 1999. Molluscan invasions in marine and estuarine communities. *Malacologia* 41: 439-454.
Includes a summary of the names in the southern hemisphere by which the northern hemisphere fouling mussels, Mytilus edulis and Mytilus galloprovincialis, go, as well as an argument that more than a few shipworms may owe their modern distribution to the history of wooden shipping.

Carlton, J.T. and J.B. Geller. 1993. Ecological roulette: The global transport of nonindigenous marine organisms. *Science* 261:78-82.
The results of sampling the ballast water in 159 ships arriving in Coos Bay, Oregon, from Japan: 367 species of animals, plants, and protists are reported, thus having implications for the global history, biogeography, systematics, and ecology of many phyla. In Table 1 of this paper, the number of species shown for Urochordata should be 6, not 10 (however, the total of 367 remains correct). Professor Les Watling of the University of Maine (pers. comm.) has identified 4 additional species of cumaceans from these samples, and Pierce et al. (1997) report an additional 31 species of tintinnids (in addition to the 2 previously reported), making 402 species recorded to date from these samples.

Carlton, J.T. and J. Hodder. 1995. Biogeography and dispersal of coastal marine organisms: experimental studies on a replica of a 16th-century sailing vessel. *Marine Biology* 121:721-730.
What survived on experimental fouling panels attached to a replica of Sir Francis Drake's Golden Hinde as it sailed down the American Pacific coast. The vessel sailed between four bays at slow (3.5-4 knots) speeds, resided in each bay for about 30 days, and spent one to three days in the open ocean between ports. All common fouling species survived the open sea voyages; in one port, the vessel settled onto the harbor floor, and several entrained benthic organisms were transported almost 400 km to the next port.

Carlton, J.T. and K. Richardson. 1995. *International Council for the Exploration of the Sea Code of Practice on the Introductions and Transfers of Marine Organisms, 1994. Preamble and a Brief Outline of the ICES Code of Practice, 1994.* International Council for the Exploration of the Sea, Copenhagen, Denmark. iii+5 pp.
A bilingual edition of the famous ICES Code of Practice, which sets forth principles to be followed when contemplating the intentional movement of aquatic organisms. Available by writing to the International Council for the Exploration of the Sea (ICES), Palaegade 2-4, 1261 Copenhagen K, Denmark.

Carlton, J.T. and M.H. Ruckelshaus. 1997. Nonindigenous marine invertebrates and algae. pp. 187-201 In: Simberloff, D., D.C. Schmitz, and T.C. Brown (eds.) *Strangers in Paradise. Impact and Management of Non-Indigenous Species in Florida.* Island Press, Washington, D.C. and Covelo CA. 467 pp.
Wherein the argument is developed that the boring isopod Sphaeroma terebrans (= S. destructor) is native to the Indian Ocean.

Carlton, J.T., D.P. Cheney, and G.J. Vermeij (eds.). 1982. A minisymposium and workshop. Ecological effects and biogeography of an introduced marine species: the periwinkle, *Littorina littorea.* Abstracts of papers presented at the *Littorina* Minisymposium and Workshop. First North American Symposium on *Littorina*, 28-30 August 1981, Nahant. *Malacological Review* 15:143-150.

Carlton, J.T., D.M. Reid, and H. vanLeeuwen. 1995. *Shipping Study. The Role of Shipping in the Introduction of Non-indigenous Aquatic Organisms to the Coastal Waters of the United States (other than the Great Lakes) and an Analysis of Control Options.* The National Sea Grant

College Program/Connecticut Sea Grant Project R/ES-6. Department of Transportation, United States Coast Guard, Washington, D.C. and Groton, Connecticut. Report Number CG-D-11-95. Government Accession Number AD-A294809. xxviii + 213 pp. and Appendices A-I (122 pp.).
The cover bears the date April 1995, but the report contains no new information after April 1993, when it was first submitted to the United States Coast Guard.

Carlton, J.T., J.K. Thompson, L.E. Schemel, and F.H. Nichols. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis*. I. Introduction and dispersal. *Marine Ecology Progress Series* 66:81-94.

Castric-Fey, A., C. Beaupoil, J. Bouchain, E. Pradier, and M.T. L'Hardy-Halos. 1999. The introduced alga *Undaria pinnatifida* (Laminariales, Aliraceae) in the rocky shore ecosystem of the St Malo area: morphology and growth of the sporophyte. *Botanica Marina* 42:71-82.
See the comments under Bouchain et al. 1999.

Castric-Fey, A., C. Beaupoil, J. Bouchain, E. Pradier, and M.T. L'Hardy-Halos. 1999. The introduced alga *Undaria pinnatifida* (Laminariales, Aliraceae) in the rocky shore ecosystem of the St Malo area: growth rate and longevity of the sporophyte. *Botanica Marina* 42:83-96.

Chapman, J.W. 1988. Invasions of the northeast Pacific by Asian and Atlantic gammaridean amphipod crustaceans, including a new species of *Corophium*. *Journal of Crustacean Biology* 8:364-382.
A new species of amphipod, Corophium alienense, is described from San Francisco Bay; on the basis of morphology it is regarded as native to southeast Asia, where it remains unknown.

Chapman, J.W. and J.T. Carlton. 1991. A test of criteria for introduced species: the global invasion of the isopod *Synidotea laevidorsalis* (Miers, 1881). *Journal of Crustacean Biology* 11:386-400.
A Japanese isopod redescribed in the late 19th century from San Francisco Bay as Synidotea laticauda.

Chapman, J.W. and J.T. Carlton. 1994. Predicted discoveries of the introduced isopod *Synidotea laevidorsalis* (Miers, 1881). *Journal of Crustacean Biology* 14:700-714.

Chew, K.K. 1990. Global bivalve introductions. *Journal of the World Aquaculture Society* 21:9-22.

Chu, K.H., P.F. Tam, C.H. Fung, and Q.C. Chen. 1997. A biological survey of ballast water in container ships entering Hong Kong. *Hydrobiologia* 352:201-206.

Clark, P.F., P.S. Rainbow, R.S. Robbins, B. Smith, W.E. Yeomans, M. Thomas, and G. Dobson. 1998. The alien Chinese mitten crab, *Eriocheir sinensis* (Crustacea: Decapoda: Brachyura), in the Thames catchment. *Journal of the Marine Biological Association of the United Kingdom* 78:1215-1221.
The neoestablishment of Eriocheir in England, perhaps related to drought patterns.

Cohen, A.N. and J.T. Carlton. 1995. *Biological Study. Nonindigenous Aquatic Species in a United States Estuary: A Case Study of the Biological Invasions of the San Francisco Bay and Delta.* A Report for the United States Fish and Wildlife Service, Washington, D.C., and The National Sea Grant College Program, Connecticut Sea Grant, NTIS Report Number PB96-166525. 246 pp.+Appendices.
A monographic account of the introduced species of the fresh, brackish, and marine waters of the San Francisco Bay and Delta in central California. Available at the following web sites: http://elib.cs.berkeley.edu/TR/ELIB:701 and http://www.nfrcg.gov/nas/sfinvade.htm (missing Figures 1 and 8 and Tables 1 and 9).

Cohen, A.N. and J.T. Carlton. 1997. Transoceanic transport mechanisms: the introduction of the Chinese mitten crab, *Eriocheir sinensis*, to California. *Pacific Science* 51:1-11.
As with the European shore crab, Carcinus maenas, a plethora of dispersal mechanisms are available to Eriocheir.

Cohen, A.N. and J. T. Carlton. 1998. Accelerating invasion rate in a highly invaded estuary. *Science* 279: 555-558.
An average of one new invasion occurred in San Francisco Bay, California, every 14 weeks between 1961 and 1995; a total of 234 non-native species are reported. There are more there now.

Cohen, A.N., J.T. Carlton, and M.C. Fountain. 1995. Introduction, dispersal and potential impacts of the green crab, *Carcinus maenas*, in San Francisco Bay, California. *Marine Biology* 122:225-237.
Carcinus can move around the world in the 1990s by an embarrassing variety of human-mediated mechanisms, hampering the ability to decrease the probability of such invasions.

Coles, S.L., R.C. DeFelice, L.G. Eldredge, and J.T. Carlton. 1999. Historical and recent introductions of nonindigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Marine Biology* 135:147-158.

Cordell, J.R.R. and S.M. Morrison. 1996. The invasive Asian copepod *Pseudodiaptomus inopinus* in Oregon, Washington, and British Columbia estuaries. *Estuaries* 19:629-638.

Cory, R.L. 1967. Epifauna of the Patuxent River estuary, Maryland, for 1963 and 1964. *Chesapeake Science* 8:71-89.
Three of the seven "most productive" species are introduced (the hydroid Cordylophora lacustris and the bryozoan Bimeria franciscana) or cryptogenic (the seasquirt Molgula manhattensis).

Critchley, A.T. 1983. *Sargassum muticum*: a taxonomic

history including world-wide and western Pacific distributions. *Journal of the Marine Biological Association of the United Kingdom* 63:617-625.

Crooks, J.A. 1998. Habitat alteration and community level effects of an exotic mussel, *Musculista senhousia*. *Marine Ecology Progress Series* 162:137-152.
An Asian mussel introduced to California.

Crooks, J.A. and H.S. Khim. 1999. Architectural vs. biological effects of a habitat-altering, exotic mussel, *Musculista senhousia*. *Journal of Experimental Marine Biology and Ecology* 240:53-75.

Crooks, J.A. and M.E. Soule. 1999. Lag times in population explosion of invasive species: causes and implications. pp.103-125 In: Sandlund, O.T., P.J. Schei, and Å. Viken (eds.) *Invasive Species and Biodiversity Management*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 431 pp.
Includes discussion of several marine species; this is not ILTA per se (see the main body of the present paper), but rather lag times of species blooms after they have become established.

Daehler, C.C. and D.R. Strong. 1997. Reduced herbivore resistance in introduced smooth cordgrass (*Spartina alterniflora*) after a century of herbivore-free growth. *Oecologia* 110:99-108.

Daehler, C.C. and D.R. Strong. 1997. Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*Spartina foliosa*) in San Francisco Bay, California, USA. *American Journal of Botany* 84:607-611.

Den Hartog, C., F.W.B. Van den Brink, and G. Van der Velde. 1992. Why was the invasion of the river Rhine by *Corophium curvispinum* and *Corbicula* species so successful? *Journal of Natural History* 26:1121-1129.
Examines several hypotheses (biological, ecological, and temporal) to address the question. The amphipod Corophium curvispinum is a fresh- to brackish-water species to be expected in North America at any time; the clam Corbicula is a well-known bivalve invader of both Europe and North America.

De Wreede, R.E. 1983. *Sargassum muticum* (Fucales, Phaeophyta): regrowth and interaction with *Rhodomela larix* (Ceramiales, Rhodophyta). *Phycologia* 22:153-160.

De Wreede, R.E. 1988. *Lithothrix aspergillum* (Rhodophyta): regrowth and interaction with *Sargassum muticum* (Phaeophyta) and *Neorhodomela larix* (Rhodophyta). *Phycologia* 27:469-476.
This and the previous paper present quantitative studies on the Asian seaweed Sargassum in British Columbia. "One probable effect of the introduction of Sargassum has been a reduction in cover of Rhodomela."

Duda, T.F. 1994. Genetic population structure of the recently introduced Asian clam, *Potamocorbula amurensis*, in San Francisco Bay. *Marine Biology* 119:235-241.

Edwards, C. 1976. A study in erratic distribution: the occurrence of the medusa *Gonionemus* in relation to the distribution of oysters. *Advances in Marine Biology* 14:251-284.
An elegant analysis.

Elton, C.S. 1958. *The Ecology of Invasions by Animals and Plants*. Methuen, London. 181 pp.
This famous book includes a chapter, "Changes in the Sea." See Carlton (1989) for background comments by Elton.

Eno, N.C. 1996. Non-native marine species in British waters: effects and controls. *Aquatic Conservation: Marine and Freshwater Ecosystems* 4:215-228.

Eno, N.C., R.A. Clark, and W.G. Sanderson. 1997. *A Review and Directory of Non-native Marine Species in British Waters*. Joint Nature Conservation Committee, Peterborough, UK. 153 pp.
The first monographic review of marine bioinvasions in the U.K.

Essink, K. and H.L. Kleef. 1993. Distribution and life cycle of the North American spionid polychaete *Marenzelleria viridis* (Verrill, 1873) in the Ems estuary. *Netherlands Journal of Aquatic Ecology* 27:237-246.

Essink, K. and U. Schottler (eds.) 1997. Studies on *Marenzelleria* spp. (Polychaeta: Spionidae). *Aquatic Ecology* 31(2):117-258.
A collection of 10 papers on the invasion of northern Europe by two species of this North American worm, one in the North Sea and one in the Baltic (see Bastrop et al. 1998).

Farnham, W.F. 1994. Introduction of marine benthic algae into Atlantic European waters. pp.28-36 In: Boudouresque, C.F., F. Briand, and C. Nolan (eds.) 1994. Introduced species in European Coastal Waters. European Commission (Luxembourg), *Ecosystems Research Report* 8:1-111 (EUR 15309) (ISBN 92-826-6727-8).

Fitzhugh, K. and G.W. Rouse. 1999. A remarkable new genus and species of fan worm (Polychaeta: Sabellidae: Sabellinae) associated with marine gastropods. *Invertebrate Biology* 118:357-390.
See Kuris and Culver (1999). This is Terebrasabella heterouncinata, a name easier to pronounce than it looks, native to South Africa but first discovered in California.

Ford, S.E. 1996. Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: response to climate change? *Journal of Shellfish Research* 15:45-56.

Galil, B.S. and N. Huelsmann. 1997. Protist transport via ballast water–biological classification of ballast tanks by food web interactions. *European Journal of Protistology* 33:244-253.

Galil, B.S. and J. Lützen. 1998. Jeopardy: host and parasite lessepsian migrants from the Mediterranean coast

of Israel. *Journal of Natural History* 32:1549-1551.
Although the southern crab Charybdis longicollis came through the Suez Canal into the eastern Mediterranean in the 1950s, its parasite, the Gulf of Suez parasitic castrating sacculinid barnacle Heterosaccus dollfusi did not follow until the 1990s: "it is possible that (the crab) populations ... will suffer drastic perturbations" as a result. "In this case, a game of Jeopardy is played out between host and parasite."

Garbary, D.J., H. Vandermeulen, and K.Y. Kim. 1997. *Codium fragile* ssp. *tomentosoides* (Chlorophyta) invades the Gulf of St Lawrence, Atlantic Canada. *Botanica Marina* 40:537-540.
Forty years after being first detected on Long Island, this Asian kelp is reported from the Gulf of St. Lawrence.

Geller, J.B. 1996. Molecular approaches to the study of marine biological invasions. pp.119-132 In: Ferris, J.D. and S.R. Palumbi (eds.) *Molecular Zoology. Advances, Strategies, and Protocols.* Wiley-Liss, A John Wiley & Sons, Inc., Publication, New York. 580 pp.

Geller, J.B. 1999. Decline of a native mussel masked by sibling species invasion. *Conservation Biology* 13:661-664.
With the invasion of the Mediterranean mussel, Mytilus galloprovincialis, the native mussel, Mytilus trossulus, disappeared from southern California, but as the two are externally nearly identical, the passage of the latter went without notice.

Geller, J.B., J.T. Carlton, and D.A. Powers. 1994. PCR-based detection of mtDNA haplotypes of native and invading mussels on the northeastern Pacific coast: latitudinal pattern of invasion. *Marine Biology* 119:243-249.

Geller, J.B., E.D. Walton, E.D. Grosholz, and G. M. Ruiz. 1997. Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* 6:901-906.

Gerard, V.A., R.M. Cerrato, and A.A. Larson. 2000. Potential impacts of a western Pacific grapsid crab on intertidal communities of the northwestern Atlantic Ocean. *Biological Invasions* 1:353-361.
The Asian shore crab, Hemigrapsus sanguineus, invades New England shores.

Gollasch, S. and E. Leppäkoski. 1999. *Initial Risk Assessment of Alien Species in Nordic Coastal Waters.* Nord 1999: 8. Nordic Council of Ministers, Copenhagen. 244 pp.

Gosliner, T.M. 1995. Introduction and spread of *Philine auriformis* (Gastropod: Opisthobranchia) from New Zealand to San Francisco Bay and Bodega Harbor. *Marine Biology* 122:249-255.
A ballast-water-mediated introduction of this intertidal mudflat seaslug which preys on small bivalves.

Gray, P.W.G. and E.B.G. Jones. 1977. The attempted clearance of *Sargassum muticum* from Britain. *Environmental Conservation* 4:303-308.
An accounting of the famous attempts to remove this invading brown seaweed from English shores by hand picking. Sargassum remains a common British seaweed.

Greenberg, N., R.L. Garthwaite, and D.C. Potts. 1996. Allozyme and morphological evidence for a newly introduced species of *Aurelia* in San Francisco Bay, California. *Marine Biology* 125:401-440.
The authors propose that a San Francisco Bay population of this jellyfish is introduced from Japan by ballast water.

Griffiths, C.L., P.A.R. Hockey, C. Van Erkom Shurink, and P.J. Le Roux. 1992. Marine invasive aliens on South African shores: implications for community structure and trophic functioning. *South African Journal of Marine Science* 12:713-722.
Including the Mediterranean mussel, Mytilus galloprovincialis, and the European shore crab, Carcinus maenas. Mytilus has displaced the native mussel, Aulacomya ater, in many areas.

Grizel, H. and M. Heral. 1991. Introduction into France of the Japanese oyster (*Crassostrea gigas*). *Journal du Conseil* 47:399-403.

Grosholz, E.D. and G.M. Ruiz. 1995. Spread and potential impact of the recently introduced European green crab, *Carcinus maenas*, in central California. *Marine Biology* 122:239-248.

Grosholz, E.D. and G.M. Ruiz. 1995. Does spatial heterogeneity and genetic variation in populations of the xanthid crab *Rhithropanopeus harrisii* (Gould) influence the prevalence of an introduced parasitic castrator? *Journal of Experimental Marine Biology and Ecology* 187:129-145.
Spatial heterogeneity yes, genetic variation, no; the introduced castrator here in Chesapeake Bay is the Gulf of Mexico sacculinid barnacle Loxothylacus panopaei.

Grosholz, E.D. and G.M. Ruiz. 1996. Predicting the impact of introduced marine species: lessons from the multiple invasions of the European green crab *Carcinus maenas*. *Biological Conservation* 78:59-66.

Hallegraeff, G.M. 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32:79-99.

Hallegraeff, G.M. 1998. Transport of toxic dinoflagellates via ship's ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Marine Ecology Progress Series* 168:297-309.

Hallegraeff, G.M. and C.J. Bolch. 1991. Transport of toxic dinoflagellate cysts via ships' ballast water. *Marine Pollution Bulletin* 22:27-30.

Hallegraeff, G.M. and C.J. Bolch. 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. *Journal of Plankton Research* 14:1067-1084.

Hallegraeff, G.M., C.J. Bolch, J. Bryan, and B. Koerbin. 1990. Microalgal spores in ships' ballast water: a danger to aquaculture. pp. 475-480 In: Graneli, E. (ed.) *Toxic Marine Phytoplankton*. Elsevier, New York.
Professor Hallegraeff and colleagues have thoroughly documented the arrival of Japanese dinoflagellates in Australian waters.

Harding, J.M. and R. Mann. 1999. Observations on the biology of the veined rapa whelk, *Rapana venosa* (Valenciennes, 1846) in the Chesapeake Bay. *Journal of Shellfish Research* 18:9-17.
This large (15 cm tall) heavy molluscivorous Asian whelk arrives on North American shores, possibly from the Black Sea or the Mediterranean, whose populations arose from intentional releases many years ago.

Hayes, K.R. 1998. Ecological risk assessment for ballast water introductions: a suggested approach. *ICES Journal of Marine Science* 55:201-212.

Hayward, B.W. 1997. Introduced marine organisms in New Zealand and their impact in the Waitemata Harbour, Auckland. *Tane* 36:197-223.
Figure 3 of this summary paper shows ten of the most common introduced species in Waitemata Harbour. All but two bivalves (the file shell Limaria orientalis and the oyster Crassostrea gigas) are also found in San Francisco Bay.

Healy, J.M. and K.L. Lamprell. 1996. The Atlantic-Mediterranean bivalve, *Corbula gibba* (Olivi) (Corbulidae: Myoidea) in Port Philip Bay, Victoria. *Memoirs of the Queensland Museum* 39:315-318.
Another possible ballast water introduction into southern Australia; can such clams also survive long-distance transport in ships' sea chests?

Hicks, D.W. and J.W. Tunnell, Jr. 1993. Invasion of the south Texas coast by the edible brown mussel *Perna perna* (Linnaeus, 1758). *Veliger* 36:92-94.
See Agard et al. (1992).

Hines, A.H., F. Alvarez, and S.A. Reed. 1997. Introduced and native populations of a marine parasitic castrator: variation in prevalence of the rhizocephalan *Loxothylacus panopaei* in xanthid crabs. *Bulletin of Marine Science* 61:197-214.

Hirakawa, K. 1986. A new record of the planktonic copepod *Centropages abdominalis* (Copepoda, Calanoida) from Patagonian waters, southern Chile. *Crustaceana* 51:296-299.
Introduced copepods should be watched for everywhere: this Japanese species now occurs in Chilean fjords.

Jamieson, G.S., E.D. Grosholz, D.A. Armstrong, and R.W. Elner. 1998. Potential ecological implications from the introduction of the European green crab, *Carcinus maenas* (Linnaeus), to British Columbia, Canada, and Washington, USA. *Journal of Natural History* 32:1587-1598.
Carcinus maenas was discovered in British Columbia in June 1999.

Jousson O., J. Pawlowski, L. Zaninetti, A. Meinesz, and C. Boudouresque. 1998. Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea. *Marine Ecology Progress Series* 172:275-280.
See Meinesz's book.

Kelly, J.M. 1993. Ballast water and sediments as mechanisms for unwanted species introductions into Washington state. *Journal of Shellfish Research* 12:405-410.

Kimmerer, W.J., E. Gartside, and J.J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. *Marine Ecology Progress Series* 113:81-93.
The Asian clam, Potamocorbula amurensis (see Carlton et al. 1990 and Nichols et al.1990) consumes the copepod, Eurytemora affinis.

Kinzie, R.A. III. 1984. Aloha also means goodbye: a cryptogenic stomatopod in Hawaii. *Pacific Science* 38:298-311.
The mantis shrimp (stomatopod) Gonodactylus aloha, although described as a new species from Hawaii, and although treated in this paper as cryptogenic, was introduced from the Indo-Pacific after World War II to the Hawaiian Islands. The native mantis shrimp Pseudosquilla ciliata was displaced wherever Gonodactylus was abundant.

Kittelson, P.M. and M.J. Boyd. 1997. Mechanisms of expansion for an introduced species of cordgrass, *Spartina densiflora*, in Humboldt Bay, California. *Estuaries* 20:770-778.
A Chilean species introduced many years ago to North America.

Krapp, F. and R. Sconfietti. 1983. *Ammothea hilgendorfi* (Böhm, 1879), an adventitious pycnogonid new for the Mediterranean Sea. *Marine Ecology, Pubblicazioni della Stazione Zoologica di Napoli* 4:123-132.
Sea spiders are underreported as ship-mediated invasions in most community invasion studies.

Kube, J., M.L. Zettler, F. Gosselck, S. Ossig, and M. Powilleit. 1996. Distribution of *Marenzelleria viridis* (Polychaeta: Spionidae) in the southwestern Baltic Sea in 1993/94–ten years after introduction. *Sarsia* 81:131-142.

Kuris, A.M. and C.S. Culver. 1999. An introduced sabellid polychaete pest infesting cultured abalones and its potential spread to other California gastropods. *Invertebrate Biology* 118:391-403.
See Fitzhugh and Rouse, 1999. An elegant and masterful study of a South African worm making its way on the other side and other half of the world.

Lambert, C. and G. Lambert. 1998. Non-indigenous ascidians in southern California harbors and marinas. *Marine Biology* 130:675-688.

A wave of exotic seasquirts has inundated southern California at the close of the 20th century.

Kuzman, S., S. Olsen, and O. Gerasimova. 1996. Barents Sea King Crab (*Paralithodes camtschaticus*): transplantation experiments were successful. pp. 649-663 In: *High Latitude Crabs: Biology, Management, and Economics*. Alaska Sea Grant College Program, Anchorage, Alaska. Number AK-SG-96-02.
Intentional plantings in the 1960s of this edible crab from the Sea of Japan to the Arctic Ocean (Barents Sea) led to the successful establishment of this crab, a rare invader of the deep sea: "large males have been caught in fair numbers down to 330 meters."

Laruelle, F., J. Guillou, and Y.M. Paulet. 1994. Reproductive pattern of the clams, *Ruditapes decussatus* and *R. philippinarum*, on intertidal flats in Brittany. *Journal of the Marine Biological Association of the United Kingdom* 74:351-366.
"In British Columbia," says the text, this clam was "introduced from Japan in the 1930s, and then spread rapidly... In the same way, this species was introduced twenty years ago to (France) for aquaculture purposes." This is not exactly so: this clam was introduced by accident to British Columbia whereas this clam was introduced intentionally into France in the 1970s, with apparently no prior studies as to what its ecological impact might be. The authors find that the exotic clam had a more extended reproductive season and a greater number of spawning events than the native congener, R. decussatus, reminiscent of the advantages that a number of introduced species have over natives (and which eventually causes the native to become somewhat less abundant).

Lee, C.E. 1999. Rapid and repeated invasions of fresh water by the copepod *Eurytemora affinis*. *Evolution* 53:1423-1434.

Lee, C.E. 1999. Causes and consequences of recent freshwater invasions by saltwater animals. *Trends in Ecology and Evolution* 14:284-288.

Lehrer, A.M. and R.W. Whitlatch. 1997. Ecological studies on the recently introduced Japanese shore crab (*Hemigrapsus sanguineus*) in Eastern Long Island Sound. pp. 49-60 In: Balcom, N. (ed.) *Proceedings of the Second Northeast Conference on Nonindigenous Aquatic Nuisance Species*. Publication Number CTSG-95-02, Connecticut Sea Grant College Program, University of Connecticut, Groton CT.
The first detailed published ecological information on this crab. For a copy of the whole proceedings, which is not in most libraries, write to: Connecticut Sea Grant Extension Program, University of Connecticut, 1084 Shennecossett Road, Groton , Connecticut 06340 USA.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *American Naturalist* 112:23-39.
The role of the introduced European periwinkle, Littorina littorea, in regulating the intertidal flora of New England.

Malyshev, V.I. and A.G. Arkhipov. 1992. The ctenophore *Mnemiopsis leidyi* in the western Black Sea. *Hydrobiological Journal* 28:33-39.
A comb jelly introduced from the Americas.

Mann, R. (ed.). 1979. *Exotic Species in Mariculture*. The MIT Press, Cambridge, Massachusetts.
A symposium (and book) published 20 years before the 1999 popularity of this subject.

McCarthy, S.A. and F.M. Khambaty. 1994. International dissemination of epidemic *Vibrio cholerae* by cargo ship ballast and other nonpotable waters. *Applied Environmental Microbiology* 60:2597-2601.

McDermott, J.J. 1998. The western Pacific brachyuran (*Hemigrapsus sanguineus*: Grapsidae), in its new habitat along the Atlantic coast of the United States: geographic distribution and ecology. *ICES Journal of Marine Science* 55:289-298.
First discovered in New Jersey in 1988, by the late 1990s the crab occurred from north of Cape Cod to North Carolina. It may have been introduced by ballast water, although other ship-related mechanisms (such as sea chests or external hull fouling, with small crabs in empty barnacles in the fouling matrix, for example) are possible.

McDermott, J.J. 1998. The western Pacific brachyuran *Hemigrapsus sanguineus* (Grapsidae) in its new habitat along the Atlantic coast of the United States: reproduction. *Journal of Crustacean Biology* 18:308-316.

McDermott, J.J. 1999. The Western Pacific brachyuran *Hemigrapsus sanguineus* (Grapsidae) in its new habitat along the Atlantic coast of the United States: feeding, cheliped morphology and growth. pp. 425-444 In: Shram, F.R. and J. C. Von Vaupel Klein (eds.) *Crustaceans and the Biodiversity Crisis. Proceedings of the 4th International Crustacean Congress*. Koninklijke Brill NV, Leiden.

Meinesz, A. 1999. *Killer Algae*. University of Chicago Press. 376 pp.
The English translation (by Daniel Simberloff) of the remarkable, emotional, political story of how the aquarium seaweed Caulerpa taxifolia was released into the Mediterranean at the foot of the Monaco Aquarium and the sordid events of denial and obfuscation that followed. The title, "Killer Algae", is a partial translation of that of the original French book, "Le roman noir de l'algue tueuse" (The Black Novel of the Killer Alga), and refers to the erroneous name–applied by the French press–by which the French public knows of this invasion; it means nothing outside of France, and the alga doesn't kill anything.
Although the advertising website produced by the publisher in advance of the book proclaims several times the alga doesn't kill, the publisher persisted with the title, evidently for sales purposes, doing little good to improve an

understanding of invasions by the public or political world.

Meng, L. and J.J. Orsi. 1991. Selective predation by larval striped bass on native and introduced copepods. *Transactions of the American Fisheries Society* 120:187-192.

Mills, C.E. and F. Sommer 1995. Invertebrate introductions in marine habitats: two species of hydromedusae (Cnidaria) native to the Black Sea, *Maeotias inexspectata* and *Blackfordia virginica*, invade San Francisco Bay. *Marine Biology* 122:279-288.

Minchin, D. 1996. Management of the introduction and transfer of marine molluscs. *Aquatic Conservation: Marine and Freshwater Ecosystems* 6:229-244.

Monniot, C., F. Monniot, and P. Laboute. 1985. Ascidies du port de Papeete (Polynesie francaise): relations avec le milieu naturel et apports intercontinentaux par la navigation. *Bulletin du Museum National d'Histoire Naturelle (4)* 7:481-495.
The Monniots have produced numerous papers demonstrating the role of ships in moving seasquirts as fouling organisms around the world. Many of these species have now become the aspect dominant organisms in many shallow-water communities.

Moyle, P.B. and T. Light. 1996. Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation* 78:149-161.
Includes brackish water invasions, particularly those in California's Sacramento-San Joaquin estuary.

National Research Council. 1996. *Stemming the Tide. Controlling Introductions of Nonindigenous Species by Ships' Ballast Water.* National Academy Press, Washington, D.C., 141 pp.
The committee nature of this book inevitably bubbles up occasionally.

Nehring, S. 1998. Establishment of thermophilic phytoplankton species in the North Sea: biological indicators of climatic changes? *ICES Journal of Marine Science* 55:818-823.

Newman, W.A. 1967. On physiology and behaviour of estuarine barnacles. pp. 1038-1066 In: *Proceedings of the Symposium on Crustacea held at Ernakulam from January 12 to 15, 1965.* Symposium Series 2, Part III. Marine Biological Association of India.
The distributional ecology and physiology of the introduced barnacles, Balanus improvisus and Balanus amphitrite, in San Francisco Bay, California.

Nichols, F.H. and J.K. Thompson. 1985. Persistence of an introduced mudflat community in South San Francisco Bay, California. *Marine Ecology Progress Series* 24:83-97.
Of more than 20 introductions, the Atlantic clam, Gemma gemma, the Atlantic amphipod, Ampelisca abdita, and the Atlantic worm, Streblospio benedicti, are the most abundant.

Nichols, F.H., J.K. Thompson, and L.E. Schemel. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis*. II. Displacement of a former community. *Marine Ecology Progress Series* 66:95-101.

Noël, P., E. Tardy, and C. D'Ukem D'Acoz. 1997. Will the crab *Hemigrapsus penicillatus* invade the coasts of Europe?. *Comptes rendus de l'academie des sciences de la vie* 320:741-745.
Yes.

Ofoighil, D., P.M. Gaffney, A.E. Wilbur, and T.J. Hilbish. 1998. Mitochondrial cytochrome oxidase I gene sequences support an Asian origin for the Portuguese oyster *Crassostrea angulata. Marine Biology* 131:497-503.
The Japanese oyster Crassostrea gigas was introduced by the Portuguese from Japan to southern Europe probably in the 16th century; it was described as a species native to Europe in the 18th century, where even now it continues to most often be referred to by the synonymous name Crassostrea angulata, under the guise of being a native species! Ofoighil put a final nail into the junior synonymy of angulata, a matter nicely discussed by Edwards in 1976.

Olenin, S. and E. Leppakoski. 1999. Non-native animals in the Baltic Sea: alteration of benthic habitats in coastal inlets and lagoons. *Hydrobiologia* 393:233-243.
Introduced species "have significantly altered ecosystems of the southeastern Baltic coastal lagoons": one of these is Vistula Lagoon, whose modern-day dominance by invasions bears comparison to similar environments elsewhere around the world, such as the l'etang de Thau (Thau Lagoon) in Sete, on the south coast of France; the Ala Wai Canal in Waikiki, Honolulu, on the south coast of Oahu, Hawaii; and Lake Merritt in Oakland, California, on the east shore of San Francisco Bay.

Orsi, J.J. and T.C. Walter. 1991. *Pseudodiaptomus forbesi* and *P. marinus* (Copepoda: Calanoida), the latest copepod immigrants to California's Sacramento-San Joaquin estuary. *Proceedings of the Fourth International Conference on Copepoda. Bulletin of the Plankton Society of Japan Special Volume* (1991):553-562.

Paula, E.J. and V.R. Eston. 1987. Are there other *Sargassum* species potentially as invasive as *S. muticum*? *Botanica Marina* 30:405-410.
Yes there are.

Petersen, K.S., K.L. Rasmussen, J. Heinemeler, and N. Rud. 1992. Clams before Columbus? *Nature* 359:679.
Extends the arrival of the North American clam, Mya arenaria, in Europe back to the 1200s, and thus within the realm of Viking transport.

Petraitis, P.S. 1983. Grazing patterns of the periwinkle and their effect on sessile intertidal organisms. *Ecology* 64:522-533.

The impacts of the European periwinkle Littorina littorea in New England.

Petraitis, P.S. 1987. Factors organizing rocky intertidal communities of New England: herbivory and predation in sheltered bays. *Journal of Experimental Marine Biology and Ecology* 109:117-136.

Petraitis, P.S. 1989. Effects of the periwinkle *Littorina littorea* (L.) and of intraspecific competition on growth and survivorship of the limpet *Notoacmea testudinalis* (Muller). *Journal of Experimental Marine Biology and Ecology* 125:99-115.

The removal of the introduced European snail Littorina littorea in lower intertidal areas in sheltered bays enhanced the growth, weight gain, and survival of the native limpet Tectura testudinalis.

Pierce, R.W., J.T. Carlton, D.A. Carlton, and J.B. Geller. 1997. Ballast water as a vector for tintinnid transport. *Marine Ecology Progress Series* 149:295-297.

Identifies 33 species of tintinnids in ballast water arriving in Coos Bay from Japan; as Carlton and Geller (1993) had already reported 2 tintinnid species, this adds 31 to the total number of ballast species reported in Carlton and Geller.

Por, F.D. 1978. *Lessepsian Migration: The Influx of Red Sea Biota into the Mediterranean Sea by Way of the Suez Canal.* Berlin: Springer-Verlag.

This is the classic summary up to the mid 1970s; see Spanier and Galil (1991).

Posey, M.H. 1988. Community changes associated with the spread of an introduced seagrass, *Zostera japonica*. *Ecology* 69:974-983.

The Japanese eelgrass in Oregon: experimental manipulations demonstrate that the plant has changed the physical habitat (mean sediment grain size declined with Z. japonica patches as compared to unvegetated areas) and the richness and density of resident invertebrate infauna (higher in than outside of the patch).

Planes, S. and G. Lecaillon. 1998. Consequences of the founder effect in the genetic structure of introduced island coral reef fish populations. *Biological Journal of the Linnean Society* 63:537-552.

Allozyme studies on what it takes in terms of minimum population size inoculation to make for a successful planting of non-native fish into the Hawaiian Islands. In the case in hand, only 3 of 11 fish from French Polynesia purposefully released between 1955 and 1961 in Hawaii became established; of the three successful species, even though "only a few individuals bequeathed their characteristics to subsequent generations, no significant change in genetic diversity was observed."

Prince, J. and W. LeBlanc. 1992. Comparative feeding preference of *Strongylocentrotus droebachiensis* (Echinoidea) for the invasive green seaweed, *Codium fragile* ssp. *tomentosoides* (Chlorophyceae) and four other seaweeds. *Marine Biology* 113:159-163.

Race, M.S. 1982. Competitive displacement and predation between introduced and native mud snails. *Oecologia* 54:337-347.

The players are the introduced Atlantic mudsnail, Ilyanassa obsoleta, and the native California mudsnail, Cerithidea californica. The former eats the eggs of the latter and otherwise eliminates Cerithidea from the lower shore. See also the work of Whitlatch and Obrebski (1980) and Brenchley and Carlton (1983).

Recher, H.F. 1966. Some aspects of the ecology of migrant shorebirds. *Ecology* 47:393-407.

Although not mentioned by the author, virtually all of the prey of these native birds are species introduced to San Francisco Bay .

Reise, K. 1999. Exotic invaders of the North Sea shore. Preface. Proceedings of a workshop held on the island of Sylt, 19-22 February 1998. *Helgolander Meeresuntersuchungen* 52 (3/4):217-218.

A symposium of 16 papers:

- *Introduced marine species of the North Sea coasts*
- *Exotic flagellates of coastal North Sea waters*
- *Red algal exotics on North Sea coasts*
- *Introduced brown algae in the North East Atlantic, with particular respect to Undaria pinnatifida (Harvey) Suringar*
- *From introduced species to invader: what determines variation in the success of Codium fragile ssp. tomentosoides (Chlorophyta) in the North Atlantic Ocean?*
- *On the population development of the introduced razor clam Ensis americanus near the island of Sylt (North Sea)*
- *Introductions and developments of oysters in the North Sea area: a review*
- *Mya arenaria - an ancient invader of the North Sea coast (see Strasser, 1998)*
- *Rapid colonization of new habitats in the Wadden Sea by the ovoviviparous Littorina saxatilis (Olivi)*
- *The neozoan Elminius modestus Darwin (Crustacea, Cirripedia): Possible explanations for its successful invasion in European water*
- *The recent arrival of the oceanic isopod Idotea metallica Bosc off Helgoland (German Eight, North Sea): an indication of a warming trend in the North Sea?*
- *The Asian decapod Hemigrapsus penicillatus (de Haan, 1835) (Grapsidae, Decapoda) introduced in European waters: status quo and future perspective*
- *Dispersal and development of Marenzelleria spp. (Polychaeta, Spionidae) populations in NW Europe and the Netherlands*
- *Ecophysiological capability of Marenzelleria populations inhabiting North Sea estuaries: an overview*
- *Styela clava Herdman (Urochordata, Ascidiacea), a successful immigrant to North West Europe: ecology, propagation and chronology of spread*
- *Exotic invaders of the meso-oligohaline zone of*

estuaries in the Netherlands: why are there so many?

Reusch, T.B.H. and S.L. Williams. 1998. Variable responses of native eelgrass *Zostera marina* to a non-indigenous bivalve *Musculista senhousia*. *Oecologia* 113:428-441.
The introduced Japanese mussel, Musculista, when experimentally added to Zostera beds, causes eelgrass rhizomes to grow 40% less than controls.

Ribera, M.A. and C.F. Boudouresque. 1995. Introduced marine plants, with special reference to macroalgae: mechanisms and impact. pp. 188-268 In: Round, F.E. and D.J. Chapman (eds.) *Progress in Phycological Research 11*.
The first thorough global review of introduced marine algae.

Ricciardi, A. and H.J. MacIsaac. 2000. Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. *Trends in Ecology and Evolution* 15:62-65.
Species from the Black and Caspian Seas are often euryhaline and have formed a conspicuous global element in the invasions picture.

Ricketts, E.F., J. Calvin, and J.W. Hedgpeth. 1968. *Between Pacific Tides*. Fourth Edition. Stanford University Press, Stanford, CA. 614 pp.

Rosenfield, A. and R. Mann (eds.). 1992. *Dispersal of Living Organisms into Aquatic Ecosystems*. University of Maryland and Maryland Sea Grant College Program, College Park, Maryland. 436 pp.

Rueness, J. 1989. *Sargassum muticum* and other introduced Japanese macroalgae: biological pollution of European coasts. *Marine Pollution Bulletin* 20:173-176.

Russell, D.J. and G.H. Balazs. 1994. Colonization by the alien marine alga *Hypnea musciformis* (Wulfen) J. Ag.(Rhodophyta: Gigartinales) in the Hawaiian Islands and its utilization by the green turtle, *Chelonia mydas* L. *Aquatic Botany* 47:53-60.

Ruiz, G.M., J.T. Carlton, E.D. Grosholz, and A.H. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* 37:621-632.

Ruiz, G.M., P. Fofonoff, and A.H. Hines. 1999. Non-indigenous species as stressors in estuarine and marine communities: assessing invasion impacts and interactions. *Limnology and Oceanography* 44:950-972.
Uses Chesapeake Bay as a model system, with 196 introduced and cryptogenic taxa used for analysis: while 39 (20 %) of these species were believed to have had "some significant impact", the authors could find quantitative data on impacts for only 12 of the 39, representing only 6% of the 196 species surveyed!

Sheath, R.G. 1987. Invasions into the Laurentian Great Lakes by marine algae. *Archive fur Hydrobiologie Beiheft Ergebnisse der Limnologie* 25:165-186.
An all-too-rarely cited paper; with rare exception usually cited as Arch. Hydrobiol. Beih. Ergebn. Limnol., *a mysterious set of abbreviations to most English-speaking workers.*

Shushkina, E.A., G.G. Nikolaeva, and T.A. Lukasheva. 1990. Changes in the structure of the Black Sea planktonic community at mass reproduction of sea gooseberries *Mnemiopsis leidyi* (Agassiz). *Oceanology* 51:54-60.
An early paper on the invasion of this comb jellyfish (ctenophore) from the Americas to the Black Sea.

Sindermann, C.J. 1993. Disease risks associated with importation of nonindigenous marine animals. *Marine Fisheries Review* 54:1-10.

Skerman, T.M. 1959. Marine fouling at the Port of Auckland. *New Zealand Journal of Science* 2:57-94.
A mid-century look at a fauna dominated by introduced species.

Smith, L.D., M.J. Wonham, L.D. McCann, G.M. Ruiz, A.H. Hines, and J.T. Carlton. 1999. Invasion pressure to a ballast-flooded estuary and an assessment of inoculant survival. *Biological Invasions* 1:67-87.

Southward, A.J., R.S. Burton, S.L. Coles, P.R. Dando, R. De Felice, J. Hoover, P.E. Parnell, T. Yamaguchi, and W.A. Newman. 1998. Invasion of Hawaiian waters by an Atlantic barnacle. *Marine Ecology Progress Series* 165:119- 126.
The Caribbean barnacle, Chthamalus proteus Dando and Southward, 1980, invades the Hawaiian archipelago, but exactly when it did so in the previous 20 years is surprisingly unclear.

Spanier, E. and B.S. Galil. 1991. Lessepsian migration: a continuous biogeographical process. *Endeavour* 15:102-106.
...through the Suez Canal; see Por 1978.

Stachowicz, J.J., R.B. Whitlatch, and R.W. Osman. 1999. Species diversity and invasion resistance in a marine ecosystem. *Science* 286:1577-1579.
"In experimental communities of sessile marine invertebrates, increased species richness significantly decreased invasion success, apparently because species-rich communities more completely and efficiently used available space, the limiting resource in this system." Species contributing to this richness and said to be native in this Long Island Sound, Connecticut, fouling community were the blue mussel Mytilus edulis, the ascidian seasquirts Molgula manhattensis, Ciona intestinalis and Botryllus schlosseri, and the bryozoan Cryptosula pallasiana: with the exception of the mussel, all of these species, however, are either cryptogenic or introduced from Europe.

Stigzelius, J., A. Laine, J. Rissanen, A.B. Andersin, and E. Ilus. 1997. The introduction of *Marenzelleria viridis* (Polychaeta, Spionidae) into the Gulf of Finland and the Gulf of Bothnia (northern Baltic Sea). *Annales Zoologi Fennici* 34:205-212.

Strasser, M. 1999. *Mya arenaria*–an ancient invader of the North Sea coast. *Helgolander Meeresuntersuchungen* 52:309-324.
A clam that was once widespread through the cold waters of the Northern Hemisphere until the Pleistocene glaciers wiped it out from the Eastern Pacific and the Eastern Atlantic, only to be introduced (not reintroduced!) to the Eastern Pacific and the Eastern Atlantic by humans so that its modern distribution parallels its ancient distribution. American readers will note that the author's reference to the "Pacific west coast" means Asia.

Sutherland, J.P. 1974. Multiple stable points in natural communities. *American Naturalist* 108:859-873.
A major controlling species focused upon in this foundation paper is the stalked seasquirt (ascidian), Styela plicata, which forms one of the stable points in the fouling community at Beaufort, North Carolina, and directly and indirectly impacts many other species in the community. Unbeknownst to the author, this is an Asian species introduced in the 18th or 19th centuries to Atlantic North America.

Sutherland, J.P. 1981. The fouling community at Beaufort, North Carolina: a study in stability. *American Naturalist* 118:499-519.
See Sutherland 1974. Introduced or cryptogenic species (not so noted by the author) included among the fouling community's "foundation species" include the seasquirts (ascidians) Styela plicata, Molgula manhattensis, and Botryllus schlosseri, the barnacle Balanus amphitrite and Balanus reticulatus (identified as Balanus tintinnabulum), and the bryozoan Anguinella palmata.

Swennen, C. and R. Dekker. 1995. *Corambe batava* Kerbert, 1886 (Gastropoda: Opisthobranchia), an immigrant in the Netherlands, with a revision of the family Corambidae. *Journal of Molluscan Studies* 61:97-107.
An interesting case wherein the "endemic" brackish water Zuiderzee seaslug (once listed in the endangered species Red Book) is found to be the introduced North American species Doridella obscura.

Trowbridge, C.D. 1996. Introduced versus native subspecies of *Codium fragile*: how distinctive is the invasive subspecies *tomentosoides*. *Marine Biology* 126:193-204.

Trowbridge, C.D. 1998. Ecology of the green macroalga *Codium fragile* (Suringar) Hariot 1889: invasive and non-invasive subspecies. *Oceanography and Marine Biology: An Annual Review* 36:1-64.

Utting, S.D. and B.E. Spencer. 1992. Introductions of marine bivalve molluscs into the United Kingdom for commercial culture - case histories. *ICES Marine Science Symposia* 194:84-91.

Vadas, R.L. 1992. Littorinid grazing and algal patch dynamics. pp. 197-209 In: Grahame, J., P.J. Mill, and D.G. Reid (eds.). *Proceedings of the Third International Symposium on Littorinid Biology*. Malacological Society of London, London, 324 pp.
A thorough demonstration of the control that the European periwinkle, Littorina littorea, exerts on upper and mid intertidal algal patches.

Vallarino, E.A. and R. Elias. 1997. The dynamics of an introduced *Balanus glandula* population in the southwestern Atlantic rocky shores. The consequences on the intertidal community. *Marine Ecology, Pubblicazioni della Stazione Zoologica di Napoli* 18:319-335.
An experimental study in this winter-recruiting species in Argentina. The authors argue that a combination of reproductive phenology, the absence of predators, the neutral or positive interaction with algae, and "spatial and temporal partitioning of the substrate allow this barnacle to successfully outcompete intertidal mussels and other barnacles species of both sheltered and exposed" sites. Remarkable too is the very presence of this barnacle in eastern South America: Balanus glandula finds it original home in the Eastern Pacific (from Alaska to Mexico), and is one of the very few native western North American species to ever leave home.

Verlaque, M. 1994. Inventaire des plantes introduites en Méditerranée: origines et répercussions sur l'environnement et les activités humaines. *Oceanologica Acta* 17:1-23.

Verlaque, M. 1996. L'etang de Thau (France), un site majeur d'introduction d'especes en Mediterrannee–relations avec l'ostreiculture. pp. 423-430 In: Ribera, M.A., E. Ballasteros, C.F. Boudouresque, A. Gomez, and V. Gravez (eds.) *Second International Workshop on Caulerpa taxifolia*. Publicacions Universitat. Barcelona, Spain.
A few French ecologists are steadily documenting the many Asian species that came in with massive inoculations of Japanese oysters commencing in the 1970s.

Villalard-Bohnsack, M. and M.M. Harlin. 1997. The appearance of *Grateloupia doryphora* (Halymeniaceae, Rhodophyta) on the northeast coast of North America. *Phycologia* 36:324-328.
A large red seaweed established in Narragansett Bay, Rhode Island, south of Cape Cod; first record July 1996.

Wasson, K. 1997. Systematic revision of colonial kamptozoans (entoprocts) of the Pacific coast of North America. *Zoological Journal of the Linnean Society* 121:1-63.
Includes a detailed review of the fouling kamptozoan, Barentsia benedeni, widely spread by ships and perhaps oysters.

Werner, I. and J.T. Hollibaugh. 1993. *Potamocorbula amurensis*: comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. *Limnology and Oceanography* 38:949-964.
In San Francisco Bay; see Carlton et al. 1990 and Nichols et al. 1990.

Whitlatch, R.B. and S. Obrebski. 1980. Feeding selectivity and coexistence in two deposit-feeding gastropods. *Marine Biology* 58:219-225.
The interactions between the native California mudsnail Cerithidea californica and the introduced Japanese mudsnail Batillaria attramentaria: small Batillaria are absent in the presence of Cerithidea, a species more specialized for feeding on small particulate material. See also the work of Race (1982) and Brenchley and Carlton (1983).

Williams, R.J., F.B. Griffiths, E.J. Van der Wal, and J. Kelly. 1988. Cargo vessel ballast water as a vector for the transport of non-indigenous marine species. *Estuarine and Coastal Shelf Science* 26:409-420.
One of the first studies on the biology of ballast water.

Wolff, T. 1977. The horseshoe crab (*Limulus polyphemus*) in north European waters. *Videnskabelige meddelelser fra Dansk naturhistorisk forening i Kjobehavn* 140:39-52.
A multicentury record of the appearance of this North American horseshoe crab in European ports.

Woods Hole Oceanographic Institution. 1952. Marine fouling and its prevention. United States Naval Institute, Annapolis, Maryland. 388 pp.
A sine qua non for those interested in the role of ships in dispersing marine life around the world.

Yamada, S.B. and R.A. Mansour. 1987. Growth inhibition of native *Littorina saxatilis* (Olivi) by introduced *L. littorea* (L.). *Journal of Experimental Marine Biology and Ecology* 105:187-196.
On Cape Cod in New England, USA; exactly how the introduced snail inhibits the growth of the native snail is not clear.

Zettler, M.L. 1996. Successful establishment of the spionid polychaete *Marenzelleria viridis* (Verrill, 1873) in the Darss-Zingst estuary (southern Baltic) and its influence on the indigenous macrozoobenthos. Archives of Fishery and Marine Research 43:273-284.

Zhang, F.Z. and M. Dickman. 1999. Mid-ocean exchange of container vessel ballast water. 1: Seasonal factors affecting the transport of harmful diatoms and dinoflagellates. *Marine Ecology Progress Series* 176:243-251.

Zhang, F.Z. and M. Dickman. 1999. Mid-ocean exchange of container vessel ballast water. 2: Effects of vessel type in the transport of diatoms and dinoflagellates from Manzanillo, Mexico, to Hong Kong, China. *Marine Ecology Progress Series* 176:253-262.

Zibrowius, H. 1974. *Oculina patagonica*, scleractiniaire hermatypique introduit en Mediterranee. *Helgoländer wissenschaftliche Meeresuntersuchungen* 26:153-173.
A remarkable and unusual story: this coral remains known only from the fossil record in South America, with living populations known only from the Mediterranean Sea, where it is introduced. Professor Zibrowius reports (pers. comm.) that he should not have used the term hermatypic: the coral in question has zooxanthellae but is not a reef-builder; see Zibrowius and Ramos (1983).

Zibrowius, H. 1992. Ongoing modification of the Mediterranean fauna and flora by the establishment of exotic species. *Mesogee [Bulletin du Museum d'histoire naturelle de Marseille]* 51:83-107.
The most thorough summary to date of Mediterranean invasions. Not 1991 as often cited.

Zibrowius, H. and A.A. Ramos. 1983. *Oculina patagonica*, Scleractinia ire exotique en Mediterranee - nouvelles observations dans le Sud-Est de l'Espagne. *Rapports Commission internationale de la Mer Mediterranee* 28:297-301.
See Zibrowius (1974).

Zibrowius, H. and C.H. Thorp. 1990. A review of the alien serpulid and spirorbid polychaetes in the British Isles. *Cahiers de Biologie Marine* 30:271-285.

Zolotarev, V. 1996. The Black Sea ecosystem changes related to the introduction of new mollusc species. *Marine Ecology, Pubblicazioni della Stazione Zoologica di Napoli* 17:227-236.
The Japanese snail, Rapana venosa (here called Rapana thomasiana), the North American clam, Mya arenaria, and the Indo-Pacific clam, Scapharca inaequivalvis, were detected in the Black Sea in 1947, 1966, and 1968 respectively: "The resulting changes in the structure of the bottom biocoenoses after these introductions are in many cases comparable with or exceed the consequences of other episodic environmental events and other kinds of anthropogenic activity."

INVITED LECTURE

Key Threats from Marine Bioinvasions: A Review of Current and Future Issues

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ABSTRACT: **Australia has been actively researching and developing management strategies for invasive marine species since the mid-1980s, following the discovery that several species of toxic dinoflagellates were likely of foreign origin. While the problem of introduced marine pests is far from solved, an evaluation of the results of efforts to date suggest four key points. First, exotic species have been, and continue to be, introduced by a range of vectors; priorities for management action need to be based on a critical evaluation of the real risks posed by each vector, and encompass an understanding that even major effort directed at a few vectors will not prevent new incursions of major pest species. Second, eradication of new incursions is achievable, but is uncommon and limited to those situations where the pest was either detected quickly or otherwise still had a limited distribution. For most species, practical options for rapid eradication still need to be developed. Third, long-term options for pest management have to take into account social and cultural issues that make some options unfeasible. And fourth, groups likely to pose major threats in the future include pathogens, marine macroalgae, and genetically enhanced production lines developed for use in mariculture. The development of options to deal with these issues will rely heavily on an integration of techniques for management strategy evaluation, fundamental marine ecology, and the emerging science of marine bioinvasions.**

Key words: Australia, ballast water, eradication, hull fouling, introduced marine pest, pathogen, pest management

INTRODUCTION

For the last decade, Australia has had a national program explicitly to deal with ballast water introductions and their management. Australian government agencies (and particularly the Australian Quarantine and Inspection Service–AQIS) have long recognized the threat posed by exotic marine organisms introduced by shipping, and have led the agenda at the International Maritime Organization to do something about the problem (Paterson 1994). Domestically, Australia has had a continuous program of research and management into ballast water and other potential vectors since 1989 and undertook world-first studies on ballast water exchange and heat treatment as partial solutions to the ballast water problem (Manning *et al.* 1996). The recently (1999) released Australian government Oceans Policy emphasizes the country's continued commitment to managing ballast water as a vector, including support for a nationally integrated management regime, the development of practical management tools, and implementation of a national process for identifying and responding rapidly to new pest incursions and outbreaks. This process is an extension of Australia's existing programs to deal with exotic terrestrial pests such as rabbits, cats, and a plethora of weeds.

Some aspects of the Australian situation are unusual to it, such as the strong social commitment to protecting its unique biota, but the vectors for marine invaders (Carlton 1996) and many of the species themselves are shared problems world-wide (*e.g.*, Cohen and Carlton 1997; Clark *et al.* 1998; Trowbridge 1998). In this paper, I review some of the conclusions that we have gleaned from dealing with these vectors and pests over the last decade, presented as an assessment of the critical threats we currently and are likely to face in the near future. The issues covered and ideas presented are idiosyncratic, but also reflect to an extent emerging priorities in Australia.

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Table 1. Introduced marine species in Australian waters, divided by state and likely mode of introduction, as compiled through January 1998. The table includes some species of uncertain taxonomic status and some cryptogenic species; species are listed independently if they occur in more than one state; and most species are allocated to more than one transport mechanism as they could have been transported in each. Key: WA – Western Australia, SA – South Australia, Vic – Victoria, Tas – Tasmania, NSW – New South Wales, Qld – Queensland, NT – Northern Territory.

State	Number of Species	Hull fouling and boring	Mariculture	Dry Ballast	Ballast Water	Intentional
WA	53	36	23	12	18	3
SA	48	30	24	7	10	2
Vic	104	61	52	13	23	4
Tas	42	23	21	8	19	4
NSW	56	36	23	8	12	2
Qld	21	17	10	0	2	0
NT	2	0	0	0	0	2

Invasion mechanisms

Cohen and Carlton (1997) listed ten broad categories of mechanisms theoretically available for transoceanic transport, many of which have numerous subcategories (*e.g.*, Cohen and Carlton 1995; Eno *et al.* 1997). The significance of each is debatable, doubtless varies among sites, and has changed over time. For many species, transport could have occurred by any one of several vectors. International shipping simultaneously offers transport opportunities via hull fouling, sea chests, and ballast, and species prone to transport as hull foulers are often also amenable to transport in mariculture shipments. Determining with certainty the vector for a particular unintentional introduction is impossible, and in all cases has to be decided on the basis of probability (although in some instances, the probability approaches 1, *e.g.*, the introduction of *Mnemiopsis leidyi* into the Black Sea in ballast water). Data on the number of larvae in ballast tanks or the number of species attached to hulls or in a mariculture shipment only tell us that a particular transport mechanism is operating, but say little about consequent rates of successful invasion and impacts.

One measure of the relative importance of the different transport vectors is the proportion of invasive species attributed to each by different studies. Cohen and Carlton (1995) estimated that four major vectors were historically of roughly equal importance in San Francisco Bay: ship fouling (26% of introduced species), ballast water (24%), accidental introductions due to mariculture (22%), and deliberate introductions (20%). Their study included a large number of freshwater species, however, which inflated the last category. Eno *et al.* (1997) suggested the largest single identifiable transport mechanism for introduced marine species in Britain (31% of the species) was accidental introduction associated with mariculture. Fouling accounted for about 26% and ballast water for another 18%, with an additional 12% of species equally likely to have been introduced by either of these shipping-related vectors. Deliberate introductions accounted for a further 8% of the introduced species. Cranfield *et al.* (1998) stated that "most (69%) of the adventive species...arrived in New Zealand as part of hull fouling communities," attributing only 3% to ballast water and 21% to either fouling or ballast water. It is not clear from the report whether vectors other than hull fouling, such as mariculture shipments, were considered in detail. Our evaluation of the introduced species in Australian waters (Table 1) suggests that the dominant modes of introduction to Australia historically are hull fouling and accidental releases associated with mariculture, followed by ballast water, dry ballast, and intentional releases. Ballast water accounts for 15-20% of the invasive marine species we have thus far found in Australia.

From a management perspective, a more useful analysis is the relative importance of transport vectors for pest species, here defined as those species likely to cause significant social, health, economic, or environmental damage. The Australian Joint Ministerial Taskforce on Managing Marine Pest Incursions recently (1999) reviewed the known invasive species in Australian waters and overseas against a set of criteria (Table 2), to produce a list of 12 species against which incursion response plans would be developed. This list excluded freshwater species, and also excluded pest species already widely distributed in Australian waters. The latter include the New Zealand screw shell (*Maoriculpus roseus*), the European shore crab (*Carcinus maenas*), the Mediterranean fan worm (*Sabella spallanzanii*), the Pacific oyster (*Crassostrea gigas*) and three species of toxic dinoflagellates (genera *Gymnodinium* and *Alexandrium*).

Of the established pest taxa, only the toxic dinoflagellates almost certainly arrived in ballast tanks (Hallegraeff and Bolch 1991). Of the remaining species, the Pacific oyster was deliberately intro-

Table 2a. Interim selection criteria developed by the National Taskforce on Managing Marine Pest Incursions

Criteria

Necessary and sufficient information to justify including a species on the trigger list (all four need to be satisfied)

1. Demonstrable invasive history.
2. One or more relevant transport vectors are still operating.
3. Demonstrable impact in native or invaded ranges on:
   - economy
   - environment
   - human health
   - amenity
4. Inferred as likely to have major impacts in Australia based on the overseas data and characteristics of Australian environments and marine communities.

Necessary and sufficient information to justify removing species from the trigger list (any one needs to be satisfied)

1. Scientific, empirical data show that impacts overseas are less than previously thought.
2. Scientific, empirical data show that impacts in Australia are likely to be less than previously thought.
3. Already is or becomes widely distributed in Australia.

Table 2b. Interim trigger list developed by the National Taskforce on Managing Marine Pest Incursions

Interim List

Species	Common Name	Native Distribution	Introduced Distribution
Caulerpa taxifolia Aquarium strain	Marine Algae	Native strains circumtropical	Invasive 'hybrid" in Mediterranean Sea
Eriochir sinensis	Chinese Mitten Crab	North West Pacific	Europe; West Coast North America
Mnemiopsis leidyi	Comb Jelly	Western Atlantic	Black Sea; Mediterranean
Mytilopsis sallei	Black Striped Mussel	Caribbean	Hong Kong; India; Singapore; [Darwin, NT]
Pfiesteria piscicida	Dinoflagellate	North West Atlantic	?? (proposed as introduced to N America)
Potamocorbula amurensis	Asian clam	North West Pacific	North East Pacific (SF Bay)
Rapana thomasina	Gastropod	North West Pacific	Black Sea, East Coast North America
Sargassum muticum	Asian Seaweed	North West Pacific	North West Pacific; England
In Australia, but not widespread			
Asterias amurensis	Northern Pacific Seastar	North West Pacific	Tasmania, Victoria
Codium fragile ssp. *tomentosoides*	Broccoli weed, Dead man's fingers	North East Pacific	Tasmania, Victoria
Musculista senhousia	Asian Date or Bag mussel	North West Pacific, South Asian Seas	Tasmania, Victoria, Western Australia
Undaria pinnatifida	*Undaria* Seaweed, wakame	North West Pacific	Tasmania, Victoria

duced, the European crab likely arrived in dry ballast, the screw shell was accidentally introduced in oyster shipments from New Zealand, and the rest were most likely fouling organisms in the broad sense of the term (including, for example, transport in sea chests). Of the "dangerous, but not yet here" species, two (*M. leidyi and Pfiesteria piscicida*) are clearly ballast water species, two (*Rapana thomasina* and *Potamocorbula amurensis*) are most likely to be introduced in ballast water, one (*Sargassum muticum*) is a fouling species, *Eriochier sinensis* would likely be introduced intentionally or in ballast water (Cohen and Carlton 1997), and the hybrid form of *Caulerpa taxifolia* will most likely be introduced in the aquarium trade, though it is also easily transported fouled in fishing gear, anchors, and the like (Meinesz *et al.* 1998).

This analysis suggests two points. First, no single vector or small subset of vectors accounts for all pest species; targeting any single vector will, therefore, not stop the introduction of species with significant pest potential. But second, by far the single most active transport mechanism historically for pest species is fouling, which accounts for five of the nine established pests. Among threatening species, ballast water is more significant, accounting clearly for two species, the most likely vector for two more, and a potential vector for another.

The distinction between fouling, in a broad sense, as the dominant historical vector and ballast water as a major recent threat is consistent with our analysis of invasion patterns in Port Phillip Bay (Victoria, Australia) (Hewitt *et al.* 1999). Even so, fouling appears to currently be a threat equal or greater to ballast water, in Port Phillip Bay and elsewhere in Australian waters. Two additional observations appear to support this point.

First, Australian scientists have now surveyed 15 ports for exotic species. All ports surveyed had exotic

marine species. However, ports receiving very high levels of ballast water are not generally any more invaded than those receiving little ballast water (Hewitt, in prep.). The exotic species found typically have been in Australian waters since prior to the use of ballast water, and appear to have been introduced into the high ballast water ports by domestic transport, rather than international shipping.

Second, the major invasion events in Australia over the last decade can be attributed to fouling, mariculture operations, and natural dispersal. None appear to be unambiguously a consequence of ballast water transport. These events include the introductions to Australia of *Asterias amurensis*, *Undaria pinnatifida*, *Codium fragile* ssp. *tomentosoides*, and *Mytilopsis sallei*; the domestic translocation of *A. amurensis* and *U. pinnatifida* from Tasmania to Victoria; the spread of *Sabella spallanzanii* and *Maoriculpus roseus*; and the invasion of *C. maenas* from the mainland to Tasmania.

Of these, the only invasions debatably mediated by ballast water are those involving *A. amurensis*. Evidence for this is the presence of *A. amurensis* larvae in ballast water of ships (Martin and Sutton, in prep.). However, we have also collected adults in sea chests of these same vessels, have had several apparently reliable reports of deliberate attempts to spread the species, and small juveniles are cryptofauna in fouling communities and hence routinely found in aquaculture equipment and on mussel ropes, which are moved by the aquaculture industry among sites. With regard to the initial introduction into Australia, the probable point of introduction (the Derwent estuary in Tasmania) receives little ballast water from the original source location in Japan (*e.g.*, in 1991, the only year for which hard data are available, there was only one visit to Hobart from Japan that resulted in a ballast water discharge, and that vessel was from well outside the area that genetic analysis indicates as the probable source location; qualitative information for other years indicate a similar picture). Large volumes of Japanese sourced ballast water are discharged at sites near the Derwent, but improbable scenarios are required to explain why the animals are common in the Derwent, but not at these sites (Ward and Andrew 1995). In contrast, each year the Derwent harbors a sizable fleet of Japanese fishing vessels from areas that genetic analysis (Ward and Andrew 1995) suggests as the probable source location for the invaders. These vessels, which historically were often heavily fouled (Hobart Port Authority, pers. comm.), dock in the Derwent for several weeks at a time. We conclude that although most of the media and many scientific reports have reported *A. amurensis* as a ballast water introduction in Australia, the evidence suggests otherwise.

There are several likely reasons why the assumption was made that ballast water was the relevant vector for *A. amurensis*, and why Australia has emphasized managing this vector, despite evidence of the historical and current role of hull fouling, sea chests, and associated vectors as sources of invaders, and pest species in particular.

First, ballast water unambiguously results in the introduction of exotic species, some of which achieve pest status. Several of the more prominent invasions can be linked to ballast water: in Australia, Gustaff Hallegraeff's work on the transport of toxic dinoflagellates in ballast water (see Hallegraeff and Bolch 1991) was a key discovery that stimulated much of the Australian effort. The zebra mussel (*Dreissena polymorpha*) and its likely introduction in freshwater ballast had a similar effect in North America, as did *M. leidyi* in Europe. The predominance of ballast water as a likely vector for the threatening species not yet in Australia at least in part justifies the current emphasis.

Second, ballast water is conspicuous and the scale of the vector sounds threatening. The perceived threat and the conspicuous nature of ballast water as a vector have made it the transport mechanism to which new invasions are often quickly linked in public and political arenas. With regard to the latter, media emphasis and recent high-profile technical publications have alerted both managers and environmentalists to the problem, and prompted an emphatic reaction.

Third, the prospect of a technical/operational solution to the problem for an industry used to dealing with such issues (and that acknowledges a problem that needs to be solved) contrasts with the more complex solutions that are likely to be required to address fouling, intentional introductions, and accidental and casual releases from mariculture operations. National and international processes are being developed and implemented to deal with these other vectors, but they often lack the focus or prominence attached to ballast water.

Uncertainty about the relative importance of different vectors as a source of invasive species is not a

viable excuse to do nothing. Societal and political pressure to respond to these invasions forces managers to make decisions in the face of uncertainty about underlying biology or effectiveness of policy settings. In this environment, I suggest we need to deliver three messages.

1. Provide realistic expectations to management agencies attempting to solve the problem. The diversity of vectors means that even a perfect system of sterilizing ballast tanks will not prevent new, damaging, and high-profile invasions. In the Australian context, even if such a system was available, it is debatable whether it would have had any effect on the invasions and recent range expansions by *U. pinnatifida*, *S. spallanzanii*, or possibly even *A. amurensis*.
2. Manage the manageable. If the technology and political, social, and industrial will exists to deal with ballast water, but not yet other vectors, then deal with ballast water. But at the same time, we should continue to emphasize the multifaceted nature of the threat, and seek to ensure that a focus on ballast water does not preclude the availability of resources to deal with other vectors.
3. Develop and help implement management structures and strategies that are compatible with, if not also actually effective against, multiple invasion paths. In so doing, we can help ensure that effort invested now will be equally useful in the future, should the evidence cause a shift in the emphasis of response actions.

Reflecting these messages, the Australian Ballast Water Management Advisory Council is likely to shortly be re-configured as the Australian Introduced Marine Pests Advisory Council. The AQIS has developed action plans for the next several years that address a range of vectors, rather than continuing to focus solely on ballast water.

Managing Pest Populations

Responding to established pest populations has three logically distinct components: (1) early detection of and, if possible, eradication of new incursions, (2) containing infections by minimizing the rate of spread of established pest species, and (3) long-term pest management.

Logically, the most effective time to eradicate a new pest is before it is well established and has spread from the point of initial infection. Three recent examples demonstrate the viability of the approach. In 1998, early detection and rapid response by South Australian Fisheries led to the elimination of a patch of about 20 New Zealand greenlip mussels, *Perna canaliculus*, detected by chance during a research survey. This action appears to have eradicated the invader from South Australian waters (J. Gilliland, pers. comm.). Joint action by scientists and industry appears to have recently eradicated an undescribed South African sabellid that infested *Tegula funebralis* and *Haliotis rufescens* in California (Culver and Kuris, in prep.). In 1999, a large-scale, coordinated program led by the Northern Territory government and involving most Australian states, several Commonwealth agencies, and a number of industry and community groups eradicated an incursion of a dreissenid, *Mytilopsis sallei*, from three Darwin marinas (Bax 1999). The incursion response involved closing the infested marinas, a prolonged program of poisoning using chlorine and copper sulphate, and the tracking and checking of every vessel that had left the marinas since the estimated date at which the dreissenid invaded. The eradication program cost A $2.8 million, and has led to a whole-of-government review of incursion response mechanisms.

Such attempts often fail, however. A recent effort to trap out *A. amurensis* from Port Philip Bay, Victoria, proved to be too little, too late, as did earlier attempts to physically eradicate infections of *S. muticum* in England, *C. taxifolia* in Spain, and *U. pinnatifida* in Tasmania. The practicality of an eradication attempt critically depends on the nature of the invader, the scale of the infestation (and hence the rapidity with which it was detected), and the willingness of relevant authorities and the community to invest the often considerable effort required. Our experience has been that expectations regarding the effort involved are typically unrealistic, so that insufficient resources are made available for the eradication attempt to have any real hope of success. In response, we are currently preparing a management-oriented guide to rapid response options (Bax, in prep.), that will review what has and has not been successful in the past, recommend response actions for different groups of organisms, specify the likely costs (human and financial), and outline the theoretical and conceptual underpinnings for the response action.

Detection of new pest incursions also frequently leads to demands for it to be contained until effective countermeasures can be developed. In Australia,

public education programs and some management actions have been instituted in an attempt to reduce the rates of spread of *U. pinnatifida*, *C. fragile* spp. *tomentosoides* and *A. amurensis*. A similar program against *U. pinnatifida* is underway in New Zealand. The critical issues clearly relate to potential transport vectors, the extent to which they can be managed, and, again, the willingness of government to act. Our experience has been that marine quarantine zones are difficult politically to establish, are often not maintained once the original flurry of activity has passed, and rarely incorporate a community awareness program sufficiently well designed and coordinated as to generate the level of voluntary compliance typically required. The notable exception was the quarantine erected to contain *M. sallei* in Darwin. The very rapid and strong response by government agencies, which included declaring a state of emergency, impounding vessels, at-sea hull inspections, and a well-coordinated public relations campaign, was effective, but also expensive. Legal action for compensation arising from the quarantine is still pending.

Once a pest species is established, the options for its long-term management are still few. In Australia, two crucial sets of issues emerge almost immediately when control options are discussed. The first is an attitude of defeatism. Most managers have stated implicitly or explicitly that once a pest is established, we have to learn to live with it. The reasoning behind this attitude flows from the second issue: the social milieu in which control needs to be undertaken differs fundamentally from those for land or freshwater-based control programs (Lafferty and Kuris 1996). There are three critical differences. First, the ocean is perceived by much of the public as pristine; this perception is illogical and easily refuted in principle, but difficult to overturn in practice. Because of it, suggestions of releasing a local biocide or an exotic biological control organism sometimes evoke strong, negative reactions, based on a perception that it would degrade the pristine ocean. The second difference is the perceived fenceless ocean, which has two important consequences: because marine organisms are perceived to have unlimited dispersal potential, (1) managers assume that local actions are not likely to have local impacts on the target organism, and (2) a segment of the community assumes that any management action, but particularly biological control, will impact adjacent areas, and more to the point, their adjacent areas (a manifestation of the "not in my backyard" syndrome). The third critical difference is that the ocean is utilized by hunter-gatherers (fishermen) who (1) are suspicious of any perceived threat to their independence or fishing success and (2) harvest dispersed resources, which makes it difficult to assign a dollar value to pest impacts or recover cost of control actions. There are obvious exceptions to the last point, such as mariculture operations and pests that affect industrial operations, but these are a minority. Lafferty and Kuris (1996) also raise the point that the level of control required for a marine pest may often be less than required for terrestrial agricultural pests. This is probably true in principal, but may not be true in practice; conservation groups typically push a strong agenda for complete eradication, even if this is currently impractical with available technology for widely distributed pests.

Table 3. Evalution of potential control options for *Asterias amurensis*, using the criteria proposed by Norton (1988). Based on Goggin (1998). The criterion of economic desirability is assumed to have been answered in the positive before any of these options are applied.

Method	Effective	Environmentally Acceptable	Practical	Politically Advantageous
Physical Control				
Trapping	Small scale only	Yes	Yes	Yes
Hand collection	Small scale only	Yes	Yes	Yes
Dredging	Small/Medium scale	No	Yes	No
Mopping	Small/Medium scale	?	Yes	?
Fencing	Small scale only	Yes	Yes	Yes
Chemical Control				
Broadcast	Medium scale only	No	Yes	No
Injection	Small scale only	Yes	?	Yes
Barriers	Farm scale only	Yes	?	No
Enviromental Remediation				
Rehabilitation	?	Yes	?	Yes
Redulate nutrients	?	Yes	?	?
Biocontrol				
Native predator	?	?	?	Yes
Native parasite	?	?	?	Yes
Exotic predator	?	No	?	No
Exotic parasite	?	Yes	Yes	?
Genetic Control				
Programmed fatality	Yes	Yes	?	?
Inducible fatality	Yes	Yes	?	?
Vectored sterilization	Yes	?	?	?

Norton (1988) provides a useful process to evaluate the conflicting objectives of pest eradication and the pristine ocean syndrome. He suggested that for any pest management program to be successful it must fulfill all of five criteria: it must be (1) technically possible, (2) practically feasible, (3) environmentally acceptable, (4) economically desirable, and (5) politically advantageous. The last is perhaps the most important and the most often overlooked. The crucial standard is not that a management approach be politically acceptable, but rather that the politicians and/or bureaucrats who ultimately will approve application of a control mechanism must benefit from this decision. A good recent Australian example is the proposed use of ichthyocides to kill carp in rivers. Although it appears to be technically feasible to develop a carp-specific biocide, approving the release of such a "poison" into waters in which children swim and farm stock and human communities draw drinking water would be a "brave" decision by a minister, and hence one that may never be taken.

We have applied Norton's (1988) approach to evaluate possible control options for *A. amurensis* in the Australian cultural context (Table 3) (Goggin 1998). From this and similar exercises we have undertaken for other species, pest management options can be ranked on the basis of political and social likelihood of being supported. In descending order of acceptability, these are

1. Do nothing; the problem might go away.
2. Rehabilitate the environment, in the belief that pests are only problems in degraded areas.
3. Physically remove pests from important sites (fish farms, marine reserves) and ignore the rest.
4. Utilize the pests commercially.
5. Deploy species-specific biocides, reproductive inhibitors, etc.
6. Encourage native predators.
7. Deploy general biocides, selectively applied.
8. Encourage native diseases and parasites.
9. Apply novel genetic approaches that affect only the pest.
10. Apply classical biocontrol, using exotic parasites.
11. Apply classical biocontrol, using exotic nonviral diseases.
12. Apply novel genetic approaches that involve modification of native species (*i.e.*, to use them as vectors).

On the basis of our discussions, two additional approaches are unlikely to be supported in Australia under any circumstances: biocontrol using an exotic predator and biocontrol using a viral disease (or even worse, a genetically modified virus). I suspect these options would not be supported anywhere.

A key element in this ranking is reversibility. Up to option 8, if things go wrong, no permanent change to the system has been made due to the response action itself. From option 9 onwards, participants in our workshops were very loathe to commit, which is reasonable given uncertainties on the specifics of each application. However, there was very strong resistance to the permanent introduction of "another" exotic species—a disease or parasite—to address a problem caused by the original introduction. This contrasts remarkably with Australia's relatively frequent importation and release of insect biological control agents against terrestrial weeds, and reflects the social considerations discussed above.

This ranking does not reflect the likelihood of success. Options 1 and 2 are largely wishful thinking, though option 2 has benefits in its own right and constitutes a "no-regrets" attempt at pest remediation. Physical removal is only likely to be successful against species early in an invasion, and will be limited to those species that can be easily identified and removed. Application of physical removal on a large scale, *e.g.*, commercial harvesting, can generate strong advocates, but was not supported by fisheries and marine environmental agencies on the basis of institutionalizing a pest and encouraging its translocation to areas not already infested. Biocidal approaches were close to the nervousness threshold, but were generally considered acceptable if suitable safety tests were done, collateral damage was slight, and an effective delivery mechanism could be found; the last requirement was considered a major technological difficulty. Among biocontrol options, the only broadly supported approach was enhancing native species to combat the invader, though it was also agreed this would probably not be effective in the long term. Genetic approaches that only modified the target species was also considered likely to be widely supported. Classical biocontrol were broadly seen as an option of last resort, which would require extensive public consultation before it was approved.

Next Pests: What are the Key Threats

The social, economic, and political factors that define a marine pest species are rarely based on a quantitative assessment of real impacts. More often,

pest status is conferred on the basis of perceived impacts in other areas and aspect dominance. The central issue, unexamined for most species, is whether a pest does something substantially different from the endemic species it displaces or co-exists with, and, ultimately, whether it distorts nutrient and energy flows and shifts community composition to the point where the effects are conspicuous and/or local species face extinction. Although any exotic species must have an impact, this statement alone is clearly inadequate to justify the cost of reducing its impacts. Invasive species offer huge opportunities to investigate in a quantitative and robust way the dynamics of marine communities, but the extent to which the impacts of a particular species justify remediation can be difficult to determine.

In that light, what are the real threats? I suggest three groups of organisms that not only have a high likelihood of invading, but also are likely to cause substantial ecological and economic impacts.

1. Marine pathogens, parasites, and fungi–Hallegraeff (1993) noted the apparent recent increase in the frequency of toxic algal blooms, which he attributed to the introduction of exotic species in ship's ballast. Since then, outbreaks of marine pathogens, often unexplainable, have occurred with increasing frequency. Examples range from the pilchard kills off southern Australia and New Zealand (Jones *et al.* 1997), which might be the result of an as-yet-unidentified viral agent, well-publicized *Pfisteria* outbreaks on the U.S. east coast, toxigenic *Vibrio cholera* in the U.S. Gulf states (McCarthy and Khambaty 1994), lobster kills attributable to *Vibrio fluvialis* off Maine, and seal kills in the Mediterranean, suggested to be the result of blooms of introduced toxic dinoflagellates (Hernandez *et al.* 1998).

Marine pathogens are particularly dangerous in two respects. First, the vectors that can transport them are diverse, defenses against them are difficult to develop, and legislative barriers to minimize risks may be difficult to enforce. Australian efforts to prevent importation of fresh Canadian salmon products, for example, as a means of protecting the current disease-free status of the stocks has been rejected by the World Trade Organization as an unjustified trade barrier. This decision is being appealed. Second, pathogens have the potential to fundamentally alter the dynamics of marine systems, perhaps more so than any other group. The decimation of the Caribbean urchin, *Diadema antellarum*, in the 1980s, due apparently to a marine pathogen of unknown origin (Lessios *et al.* 1984), had a profound effect on algal-coral dynamics throughout the region and fundamentally altered the composition of Caribbean reef communities (Hughes 1994). There are similar reports in other regions. Duncan *et al.* (1982) reported on a mass die-off of a large keystone predator seastar in the Sea of Cortez, attributed to unusually warm temperatures and the action of an as-yet-unidentified pathogen, and suggested major changes in benthic communities as a result. A similar die-off of the seastar, *Asterias rubens*, off the coast of the northeastern United States occurred in the 1990s, again for unknown reasons ("ray rot disease"), but attributed at least in part to stress due to water temperatures. Anthropogenically enhanced dispersal of marine pathogens to naive populations may prove to be one of the major challenges globally to marine industries and ecosystems, and is one that we are particularly poorly prepared to handle.

2. Invasive marine macroalgae–Introduced macroalgae are already common and causing substantial concern: *U. pinnatifida* in Australia, New Zealand, and Europe; *C. fragile* ssp. *tomentosoides* in America, Australia and New Zealand; *S. muticum* in Europe; and a number of species of *Caulerpa* at sites worldwide. As well, there are increasingly more frequent reports of pest macroalgal blooms at both temperate and tropical sites (Raffaelli *et al.* 1998), often involving broadly distributed genera and attributed, possibly incorrectly, to outbreaks by native species (as per arguments in Carlton 1996). Introduced macroalgae have a number of features that facilitate their invasion, most notably an ability to easily transport by a variety of vectors and, in many instances, limited dispersal abilities of motile reproductive stages (facilitating population establishment), as well as vegetative and clonal reproduction. Invasive plants may often do little more than increase local diversity or replace native congenerics (Trowbridge 1998), but in at least some cases they clearly occupy habitats and reach such high densities that they become space dominants and fundamentally change community dynamics. Again, preventative options against such invasions are poorly developed, nor do we have any effective means to combat such species once they have invaded. Physical removal has proven unsuccessful in a number of instances, and herbicidal and biological options are still far from being developed.

3. Genetically enhanced production species—The invasion of the Mediterranean by an artificial hybrid of *C. taxifolia*, selectively bred for increased growth and environmental tolerances (Jousson *et al.* 1998), is likely to be only the first of what may in the long term prove to be one of the major problems facing marine systems. Work is underway worldwide to produce species for marine mariculture that grow faster and are more environmentally tolerant than existing species. At least some of these species, such as Pacific oysters (*C. gigas*), are already considered pests in Australia when feral, a situation likely to only worsen when "super-oysters" are introduced. Unlike terrestrial systems, where production lines are often competitively inferior because they are selected for rigidly controlled farm conditions, mariculture often relies on what are essentially natural and unregulated environments, and, hence, in the short term at least, will seek organisms capable of increased production under natural conditions. When these enhanced plants and animals are introduced, it may well be impossible to stop their spread and consequent impacts on native communities. Although the problem has been recognized and some work to contain such production organisms is underway (*e.g.*, the Australian "sterile ferals" project), it is very unclear that caution, regulations, and technological solutions will be adequate to counter advocates driven by increased profit margins and increasing demand worldwide for seafood products. The vectors associated with the introduction of these super-competitors at first are likely to be quite different from those with which we are currently concerned, but as shown in the Mediterranean, once such a taxon is established, the familiar vectors, such as fouling on anchor chains, rapidly come into play in spreading the pest (Meinesz *et al.* 1998).

The prospects for managing these threats are mixed. Marine pathogens are likely to be manageable by reducing the likelihood of transport and by modifications of mariculture and human health operations post-invasion to minimize impacts. As ballast water appears to be a very suitable vector for pathogens, it is crucial that treatment processes for it are effective against them. Treatments that deal only with metazoans and their larvae not only may be targeting the lesser threat, but may even exacerbate the threat due to pathogens (Desmarchalier 1997). Dealing with marine invasive plants, although technically challenging, is likely to be able to borrow from the Integrated Pest Management (IPM) approaches developed for terrestrial weeds, including topical application of specialized herbicides, physical control, and classical biological control. The information we require to implement IPM for any marine plant is lacking, but the conceptual approaches appear to be in place. This is not likely to be true for genetically enhanced invaders. For these, as is the current situation with *C. taxifolia* in the Mediterranean, problem species will need to be approached on a case-by-case basis.

Conclusions

Australia's decade of concerted and coordinated attempts to manage the problem of introduced marine pests has resulted in some successes, some failures, and a far better understanding of the scope of the problem and the scope for management action. A principle outcome of such knowledge is a much greater public and political appreciation of the problem. But this appreciation has led to demands that scientists and managers solve the problem, which has proven difficult at best.

Australia has structured its approach to introduced marine species around a zonal defense system. The first zone—up-take and transport—is targeted by the Australian Ballast Water Management Advisory Council and the Australian Quarantine and Inspection Service, as well as several states. The Northern Territory, for example, evaluates the risk posed by arriving recreational yachts and fishing vessels, and, when in doubt, requires a hull survey and sterilization of any plumbing open to seawater prior to allowing international vessels into berths.

Zone 1 is permeable. Even assuming we could sterilize ballast tanks and clean hulls, sea chests, and internal plumbing, pests would still arrive. To the extent that we have done none of that, or demonstrated that what management actions we have initiated, such as exchanging ballast at sea, are even effective at reducing the rate of invasions, we have barely slowed the invasion rate, if at all. But the preconception that once a species arrives, you have lost the game is not only unacceptable, but wrong. Several successful eradication attempts have been launched in the last few years, though all combined an element of good luck, good planning, and a suitable, still contained incursion. Australia is formalizing a process to maximize its luck, by establishing a nationally coordinated system to manage its second defense zone—the receiver ports. Action is seen to be primarily a state

responsibility and, since the successful eradication of the black-striped mussel in particular, focuses on rapid detection of new pest species, development of tactical control options, and the establishment of an effective system of communication among state and commonwealth agencies that would need to be involved. Public awareness campaigns have been put in place in all Australian states, and several are developing programs for routine surveillance of high-risk environments. As well, work has begun at developing more effective and better targeted biocides than the broad spectrum chemicals employed in Darwin.

The third zone of defense is long-term pest control. We have begun testing commercial harvesting as a means of reducing pest numbers, are assessing the potential of environmental remediation to reduce the numbers of *A. amurensis* and *U. pinnatifida*, and have projects underway looking into both biological control and the development of novel biomolecular techniques for pest control. Which, if any, of these approaches will prove useful is still to be determined.

At times, the biological, bureaucratic, and political complexity of the problem is daunting. But, slowly, management structures are being put in place that encourage (and in some instances) require protocols to lower risks of new introductions; programs have begun to be better integrated nationally, particularly through the actions of the recently established Australian National Taskforce on Managing Marine Pest Incursions; and managers are beginning to appreciate the scale of the resources required to solve the problem. The cost of eradicating the dreissenid, *Mytilopsis sallei*, in Darwin, at just under A $3 million, drove home not only the cost of poor barrier controls, but also the threat that even one particularly bad pest species posed to Australia's biodiversity and marine industries.

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Patterns of Invasions

'There is a fundamental need to develop and implement national-scale baseline measures to evaluate the patterns and rates of invasion.'

PATTERNS OF INVASIONS OVERVIEW

Toward Understanding Patterns of Marine Invasions in Space and Time

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INTRODUCTION

In the past decade, our knowledge about the extent and potential impacts of marine invasions has increased rapidly. For North America alone, hundreds of marine and estuarine species are known to have established non-native populations, resulting from human-mediated transfer beyond their historical ranges (*e.g.*, Carlton 1979, Cohen and Carlton 1995, Fuller *et al.* 1999, Carlton 2000, Carlton and Wonham 2000, Ruiz *et al.* 2000a, Fofonoff *et al.* 2000). Scores to hundreds of marine invasions are reported for many other global regions as well (*e.g.*, Por 1978, Jansson 1994, Eno 1996, Cranfield *et al.* 1998, Hewitt *et al.* 1999, Reise *et al.* 1999, Olenin and Leppäkoski 2000). Furthermore, recent work has begun to measure the effects of marine invasions on population, community, and ecosystem processes, underscoring both their ecological and economic significance (*e.g.*, Bertness 1984, Grosholz *et al.* 2000, Byers 2000; for review see Ruiz *et al.* 1997).

Despite recent advances in describing the scope and consequences of marine invasions, many conspicuous gaps exist in our knowledge in both aspects. For example, the ecological impacts of most marine invasions remain unknown (*e.g.*, Ruiz *et al.* 1999). Perhaps more surprising, the spatial and temporal patterns of invasion remain poorly resolved, due to many confounding factors in the quantity and quality of existing data. These gaps in knowledge present many challenges for both (a) understanding invasion processes and (b) developing effective management strategies to reduce the risks associated with invasions in marine systems.

Here, I discuss briefly the state of knowledge for marine invasion patterns of North America, highlighting general patterns and limitations. In addition, I review next steps to address these limitations. This abstract is intended to provide only a short summary of much more comprehensive reviews (as cited below). I have also excluded further discussion of invasion impacts, which are reviewed by others in this volume.

APPARENT PATTERNS OF INVASION

A recent review by Ruiz *et al.* (2000b) summarized information for 298 nonindigenous species (NIS) of invertebrates and algae that are established in marine/estuarine coastal ecosystems of North America, describing the patterns that emerge from these data:

- Significant spatial variation exists in the number of NIS known among different coasts and different bays (Table 1);
- Most known NIS are crustaceans and molluscs, which are relatively large and conspicuous;
- Most known NIS invasions have been attributed to shipping;
- The rate of reported NIS invasions has increased exponentially over time.

These emergent patterns must be considered with great caution and may be misleading (Ruiz *et al.* 2000b). The existing data are derived primarily as "by-catch" (i.e., synthesis of reports) from the literature and do not result from standardized, contemporary measures. Although they provide a useful

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Table 1. Number of non-native species of algae and invertebrates reported as established in coastal marine/estuarine waters of North America. Reported numbers are shown for (A) each of the three coasts and (B) selected bays in North America. [See Ruiz *et al.* 2000b for more detailed information, including sources, for species and localities.]

Locality/Region	Number of Species
(A) Coast	
West Coast	228
East Coast	108
Gulf Coast	39
(B) Selected Bays	
Prince William Sound, AK	10
Puget Sound, WA	57
Coos Bay, OR	55
San Francisco Bay, CA	163
Chesapeake Bay, MD/VA	49

minimum estimate for the number of invasions, underscoring their importance in marine communities, the quality and quantity of these data are extremely uneven. In essence, search effort and the resolution of taxonomy/biogeography has been highly variable in space and time, and by taxonomic group. Significant biases may therefore exist in the data, confounding interpretation. Thus, the available data indicate many "apparent patterns" that largely await verification through unbiased measures.

Next Steps: Toward Interpreting Patterns of Invasion

Despite the present limitations in existing data, there is presently no plan to implement the standardized and quantitative measures needed to interpret patterns of marine invasion. As recognized at the 1998 Forum on Ecological Surveys of Aquatic Nuisance Species (hereafter the Forum; Ruiz *et al.* 2000c), this approach is necessary not only to understand invasion patterns and processes but also to inform and evaluate management strategies. More specifically, quantitative analyses of invasion patterns remove the biases discussed above and allow direct measures of the rate of invasion, relative contribution of various invasion mechanisms (or vectors), and effectiveness of management strategies. Without this approach, we simply lack the key indicators to adequately evaluate what management is needed and whether it is working (see Ruiz *et al.* 2000b,c for discussion).

The Forum group concluded that advancing both scientific and management goals in marine invasion ecology requires direct, contemporary measures of invasion that include:

1. *Standardized and quantitative measures.* This serves to remove bias, allow statistical comparisons (and confidence), and make the measures repeatable in space and time.
2. *Replication across multiple coasts, sites, and habitats.* This provides a robust measure that tests both for generality and variation among locations. For example, it is possible (if not probable) that invasion patterns and processes differ by latitude, biogeographic province, or habitat type.
3. *Repeated and regular measures over time.* It is only repeated, standardized measures that can adequately assess rate of invasions with statistical confidence.
4. *Establishment of taxonomic verification and voucher system(s).* Since reliable identification and reference collections are at the core of invasion analysis, expert consultation and maintenance of voucher materials are essential.
5. *Establishment of database system(s).* Although invasion measures are the primary focus, data management and standard database formats are of fundamental importance to promote analyses, access, and comparisons among core sites and research groups.

Overall, there is a fundamental need to develop and implement national-scale baseline measures to evaluate the patterns and rates of invasion. The Forum recommended key criteria for establishing this baseline across a network of core sites. Clearly, additional measures (*e.g.*, vector operation, experimental assessment of invasion processes) are also needed to advance invasion science. However, a premium should be placed upon measuring the number of invasions in space and time, because this remains the dependent variable of central focus to both scientists and managers.

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Invasions Status and Policy on the U. S. West Coast

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ABSTRACT: Over the past 20 years, a rapidly accumulating body of knowledge has demonstrated that invasions by non-native organisms threaten the aquatic flora and fauna in the world's coastal regions and the human activities and economies that depend on them. Intensive research in the San Francisco Bay/Delta Estuary has revealed striking alterations in biodiversity and ecosystem functioning, and provided evidence that the rate of invasion is increasing. This increase appears linked to the expansion and globalization of commerce, and the wider and faster movement of goods and people around the world. The consequences are not limited to the oceans. On land as in the sea, exotic predators and competitors alter ecosystems, while parasites or diseases of crops, livestock, native animals or plants, or human beings, may arrive with devastating effect on populations that lack genetic or acquired immunities. Although methods exist to reduce the frequency of invasions by exotic organisms, we have thus far done little to apply them. Failures at the international and national level to effectively control a major transport vector of exotic species, the transoceanic relocation of aquatic organisms via ships' ballast water, have led to increasing state and local efforts to regulate ballast water discharges.

Key words: invasion, exotic species, nonindigenous species, ballast water, San Francisco Bay

INTRODUCTION

The marine waters of the globe are sometimes regarded as constituting a single, interconnected ecosystem—the world ocean. However, biogeographers have long recognized that the organisms inhabiting temperate zone coastal waters are distributed in distinct bioregions, separated by continents, by zones of tropical water, and by reaches of deep ocean inimical to the survival of coastal life. Each of these regions, developing in relative isolation from the others, has evolved its own unique assemblage of native endemic organisms.

These assemblages, and potentially the species that comprise them, are increasingly threatened by exotic organisms transported in association with human commerce and travel. Over the past decade and a half, several spectacular invasions—European mussels (*Dreissena polymorpha*) clogging Midwestern water pipes, a tropical seaweed (*Caulerpa taxifolia*) "astroturfing" the Mediterranean, Atlantic comb jellies (*Mnemiopsis leidyi*) vacuuming the zooplankton from the Black Sea—have persuaded limnologists and oceanographers that something is seriously amiss. Known vectors transporting invasive species into aquatic habitats are expanding in scale, new vectors are being created, and bays, estuaries and freshwater ecosystems are progressively becoming invaded by exotic organisms.

AN INVADED ESTUARY

Recent studies in the San Francisco Bay/Delta Estuary provide a glimpse of just how bad things can get. We have now documented over 230 exotic plants, protists, and animals that have become established within the Estuary, with over 160 of these found in the salt- and brackish-water portion of the estuary (Cohen and Carlton 1995, 1998). We have identified another 100-200 species as cryptogenic—meaning that we do not know whether they are native or exotic (Carlton 1996). Perhaps even more impressive than the sheer number of exotic species is the frequency with which they visually and numerically dominate habitats in the Estuary. Exotic species account for 40 to 100% of the common organisms in several biotic communities at various sites, whether calculated as a percentage of the number of species present, a percentage of the number of individuals, or a percentage of biomass.

For example, the organisms clinging to the sides of the docks and burrowing in the sediment on the bottom of the Bay are nearly all exotic, primarily

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from North Atlantic or Asian coasts. Common crabs in the Bay are from Europe, the eastern United States, and China; common mussels derive from the Atlantic, the Mediterranean, and the southern hemisphere; the most common clams come from the Atlantic and from Asia; and the most common snails are from the Atlantic (Cohen and Carlton 1995). Most of the fish in the Delta are native to the eastern United States (Moyle 1976). The crustacean zooplankton are increasingly dominated by Asian species (Orsi 1995), while several exotic jellyfish have arrived from the Black Sea (Mills and Sommer 1995). These introductions have dramatically reduced some native populations, altered habitat structure and energy flows, and caused direct economic damage amounting to billions of dollars (Cohen and Carlton 1995; Cohen 1996).

One recent invasion demonstrates the potential for exotic species to damage ecosystems in myriad ways. In October 1986, three small, nondescript clams of a type never before seen on the Pacific coast of North America were collected in San Francisco Bay by a community college biology class. By the summer of 1987, nine months later, this clam had become the most abundant clam in the northern part of the Bay, averaging over 2000 clams per m^2. The clam (an Asian species, *Potamocorbula amurensis*) also turns out to be a highly efficient filter feeder, ingesting bacteria and small zooplankton as well as phytoplankton. At the densities in which it occurs in the Bay, virtually the entire water column may pass through the filtering apparatus of these clams between once and twice a day. Since its arrival the clam has eliminated annual phytoplankton blooms that had previously characterized the ecosystem, disrupted food webs, reduced the populations of existing zooplankton species, and possibly increased the vulnerability of the ecosystem to invasions by exotic zooplankton, many of which have since occurred (Carlton *et al.* 1990; Nichols *et al.* 1990; Alpine and Cloern 1992; Werner and Hollibaugh 1993; Kimmerer *et al.* 1994; Orsi 1995). Changes at higher trophic levels, though less clear, may also have resulted. The clam may also be acting as an accumulator of contaminants, possibly concentrating selenium in the diets of bottom-feeding fish and birds at levels that might be high enough to cause reproductive defects (Thompson 1997).

Exotic species are present not only in San Francisco Bay, but are common as well in other harbors and bays in California and along the Pacific Coast. For example, recent compilations indicate about 50 exotic species known to be established in Puget Sound (Cohen *et al.* 1998), about 25 exotic species in Morro Bay in central California, and about 80 exotic species in the bays and harbors of southern California. Once established in one bay, exotic organisms may quickly spread to another through either natural or anthropogenic transport. For example, the European green crab *Carcinus maenas*, first observed in San Francisco Bay in 1989-90, had spread to Bodega Bay by 1993, to Elkhorn Slough in Monterey Bay by 1994, to Humboldt Bay by 1995, to Coos Bay in southern Oregon by 1997, and to Willapa Bay and Grays Harbor in Washington and Morro Bay in California by 1998, and to southern Vancouver Island in British Columbia by 1999 (Cohen *et al.* 1995; Grosholtz and Ruiz 1995; Miller 1996; N. Richmond, B. Dumbauld, E. Grosholtz, G. Jamieson, pers. comm.). Exotic species initially established in bays may also move out of them to invade the open coast. A predatory New Zealand sea slug, *Philine auriformis*, that was collected in San Francisco Bay in 1992, appears subsequently to have spread north to Bodega Bay and south to near San Diego, becoming the most commonly collected sea slug along the southern California coast (Gosliner 1995; D. Cadien, pers. comm. 1996—although the presence of one or more additional exotic sea slugs in the same genus may confound this picture [M. Chow, T. Gosliner, M. Behrens, pers. comm.]).

On Land As in the Sea

Biologic invasions in California are by no means limited to aquatic environments. Some 1,023 species[2] of exotic plants, at least 18 species of exotic

[2] This includes both terrestrial and aquatic plants. The number is from the most recent manual of California flora (Hickman 1993), and includes both "naturalized" and "waif" species. An estimate based on the prior manual (Munz 1968) had been criticized for including plants that are not fully established and capable of persisting without human interference, such as narrow garden escapes, taxa that are only sparingly established, and agricultural weeds that are maintained though summer irrigation, and a more conservative estimate was calculated that excluded these (Howell 1972 and Raven 1988, cited in Rejmanek *et al.* 1991). Reducing the estimate of 1023 species by the same proportion yields a more conservative estimate of 686 introduced and established plants in California.

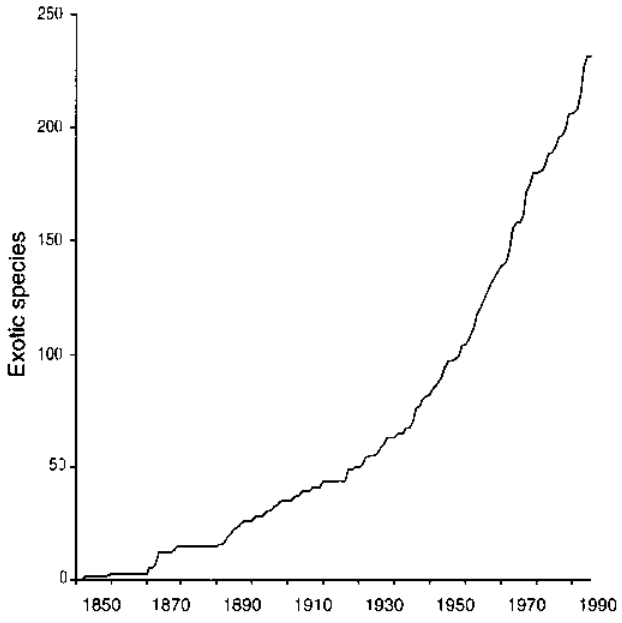

Figure 1. Increasing rate of invasions. Cumulative number of exotic species established in the San Francisco Bay/Delta Estuary. Adapted from Cohen and Carlton 1998.

land snails and slugs, 24 species of exotic birds, 22 species of exotic mammals, and an unknown but certainly large number of exotic insects and other terrestrial arthropods have been reported as established in California (Hanna 1966; Hickman 1993; Laudenslayer *et al.* 1991 as updated by R. Jurek, pers. comm.). The data for plants have been interpreted as indicating a decline in the rate of invasion since the 1950s (that is, a decline in the rate at which new plants become established in California; many previously established plants continued to expand their range and abundance) (Rejmanek *et al.* 1991).

The data from aquatic habitats, however, reveal a substantial acceleration in invasions (Figure 1). Roughly half of all invasions documented for the San Francisco Estuary occurred in the last 35 years. Overall, the rate of invasion increased from an average of one every 55 weeks between 1851 and 1960, to one every 14 weeks from 1961 to 1995 (Cohen and Carlton 1998). In the decade from 1986 to 1995, for example, 43 exotic species were newly collected in the Estuary, with at least 33 of these apparently becoming established (Cohen 1997). Similarly, a study of the Great Lakes also found the rate of invasion to be increasing, though not as steeply, from one every 64 weeks between 1840 and 1959, to one every 39 weeks from 1960 to 1990 (Mills *et al.* 1993).

Pathways and Policies

Within aquatic and especially marine ecosystems, important pathways for the introduction of exotic species include transport with transoceanic shipping (in ballast tanks and other components of ships' seawater systems, and as fouling on hulls, anchor chains, etc.) and transport via aquaculture activities (including both the intentional or accidental release of fish, shellfish or other cultured species in areas where they may become established, and the accidental transport and introduction of accompanying organisms, including predators, parasites, or diseases of fish and shellfish). Both of these areas of activity are expected to increase substantially over the coming decades.

The transport of exotic organisms in ships' ballast water has received a good deal of attention in recent years. Various data suggest that the relative importance of this pathway has been increasing in recent decades, so that currently it is probably responsible for the transport and introduction of more aquatic species than any other mechanism. For example, it appears that none of the exotic organisms established in San Francisco Bay that were reported on the Pacific coast of North American prior to 1920 were initially introduced via ballast water; however, ballast water was the initial introduction pathway for between 6 and 62% of the species that were first reported in the 1950s, and for 53–88% of the species first reported in the 1990s (Figure 2).

One method that could reduce the introduction and establishment of exotic organisms via ballast water discharges is the exchange of ballast water at sea. If done properly, the ballast water later discharged in or near bays or harbors should contain primarily oceanic organisms, which are thought to be unlikely to survive or become established in coastal waters. For reasons related to vessel architecture, sea conditions, and ship safety, some ships cannot exchange ballast water at sea under some conditions, and even when exchange is conducted, the completeness of exchange will vary. Thus, at-sea ballast water exchange is at best a partial solution, though still worthwhile where better alternatives are not available.

For several years, U. S. federal regulations have required the at-sea exchange of ballast water for

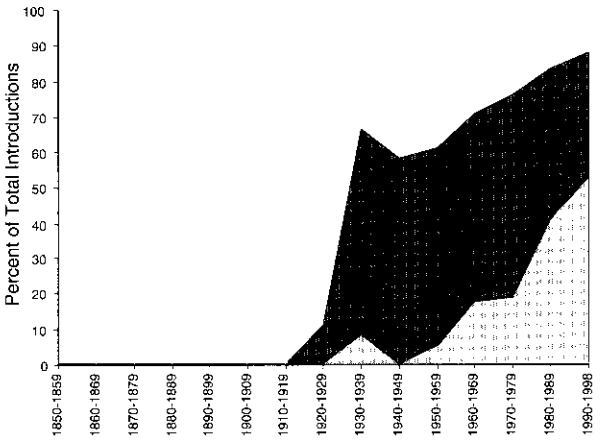

Figure 2. Ballast water introductions as a percentage of total introductions. Based on the date of the first records on the Pacific coast of North America for exotic organisms in the San Francisco Bay data set. Lower line indicates probable ballast water introductions: organisms for which no other mechanism but ballast water seems likely as the pathway for initial introduction. Upper line indicates possible ballast water introductions: includes organisms for which other mechanisms as well as ballast water appear likely. Based on data updated from Cohen and Carlton 1995).

ships entering the Great Lakes and upper Hudson River from overseas ports; in 1996 similar requirements were placed on oil tankers transporting Alaskan oil overseas and returning in ballast. However, despite mounting evidence of the extent of marine invasions and of ballast water introductions in other parts of the country, the 1996 National Invasive Species Act (NISA) failed to extend these requirements to the rest of the country. The NISA did mandate a process that could provide for the eventual promulgation of regulations by the Secretary of Transportation, but this process has been greatly delayed (Table 1).

Responding to the display of federal inaction under NISA, several efforts have been initiated outside of Washington, DC to regulate the discharge of exotic species into the nation's coastal waters, either through state laws or through other federal laws (Figure 3). Most of these intiatives would use water pollution laws to regulate the release of exotic organisms in ballast water–as a waste discharge of a biological pollutant–and there appears to be a developing consensus around this approach as an effective mechanism for managing the problem. This

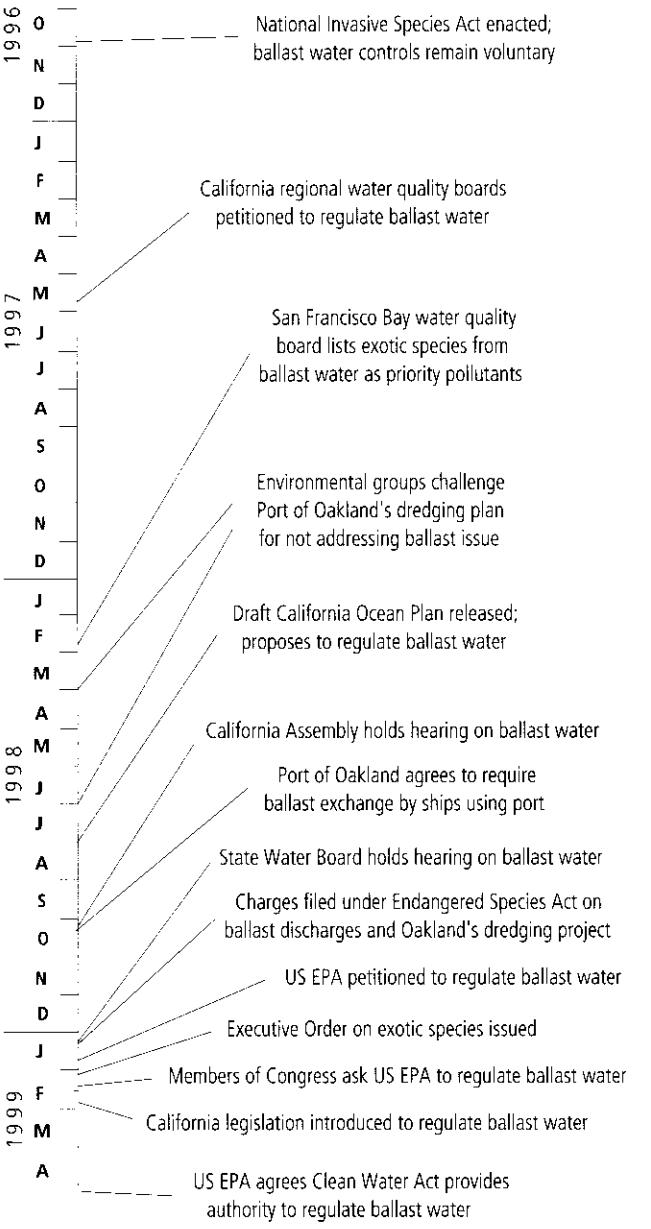

Figure 3. Policy initiatives regarding ballast water management in the United States since the enactment of the National Invasive Species Act.

approach is likely to lead beyond requiring ballast water exchange, and require the treatment of ballast water discharges to remove or kill the exotic organisms in them, much as other wastewater discharges are treated to prevent the introduction of pollutants into the nation's waters.

Table 1. Delay in implementation of actions directed by the National Invasive Species Act (NISA) regarding the consideration and promulgation of mandatory regulations for ballast water exchange.

Action Directed	Action Taken
April 16, 1996: NISA enacted into law Oct. 16, 1997: Secretary of Transportation to issue voluntary guidelines.	Draft guidelines were published in the Federal Register on April 10, 1998, and final guidelines were published as an Interim Rule on May 17, 1999 (over 18 months behind schedule).
April 16, 1998: Aquatic Nuisance Species Task Force to submit criteria for assessing compliance with and effectiveness of guidelines.	In April 1999 the Task Force appointed a committee to develop recommendations for criteria, which were to be submitted in initial form to the Task Force and made available for public review by Nov. 11, 1999, with final recommendations submitted to the Task Force in May 2000. The Task Force would then develop and submit criteria to the Secretary of Transportation. However, as of May 31, 2000, the committee had not yet submitted draft criteria or made them available for public review. (The schedule set by the Task Force when appointing the committee was thus over 2 years behind NISA's schedule; and the work has now fallen at least six months further behind.)
April 16, 2000: Secretary of Transportation to submit report to Congress on guideline compliance and effectiveness.	Because of delays to date, the report will likely be delayed until at least November 2001 (a minimum of over 18 months behind schedule).
Oct. 16, 2000: Secretary of Transportation to assess guideline compliance and effectiveness and, if criteria are not met, promulgate mandatory regulations.	Because of delays to date, the Secretary's consideration of mandatory regulations will likely be delayed until at least May 2002 (a minimum of over 18 months behind schedule).

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Patterns of Range Expansion, Niche Shift and Predator Acquisition in *Codium fragile* ssp. *tomentosoides* and *Membranipora membranacea* in the Gulf of Maine

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ABSTRACT: Introduced species often undergo stages of expansion in range, niche breadth, and the acquisition of predators as established organisms recognize a new food source. The invasive green alga, *Codium fragile* ssp. *tomentosoides*, and the encrusting bryozoan, *Membranipora membranacea*, illustrate these patterns in the Gulf of Maine. *C. fragile* ssp. *tomentosoides* became common in several protected areas in the early 1980s and it has continued to expand within protected habitats. More recently, *C. fragile* ssp. *tomentosoides* has begun to occupy exposed habitats and it is now the dominant canopy species to 8 m in depth in both former urchin barrens and established kelp beds at both protected and exposed locations. The saccoglossan *Placida dendritica* has become an important herbivore on *C. fragile* ssp. *tomentosoides*, and it may facilitate dispersal of this alga by increasing fragmentation. *M. membranacea* first appeared at the Isles of Shoals, New Hampshire/Maine, in 1987 and has expanded its range throughout the Gulf of Maine, first as an epizooite on kelps and then on a wide diversity of algae, including arborescent, cylindrical algae such as *Desmarestia aculeata*. Over the last five years, the dorid nudibranch *Onchidoris muricata* has begun to recognize *M. membranacea* as a food source in the settling larval stage. *O. muricata* is an annual winter predator of dormant *M. membranacea* populations and it has the potential to significantly alter the impact of this competitively dominant encrusting species, particularly in the cooler northeastern portions of the Gulf of Maine. Patterns of habitat expansion and interaction for these two introduced taxa within the Gulf of Maine are discussed.

Key words: *Codium*, *Membranipora*, range expansion, niche shift, predator

INTRODUCTION

Interest in the impacts of human activities in the marine environment has been increasing along with an awareness of parallel human influences in terrestrial and freshwater systems (Carlton 1996, 1998; Vitousek *et al.* 1996, 1997; Strayer *et al.* 1999; Watling and Norse 1998). The Gulf of Maine has seen the introduction of numerous species (Mathieson and Hehre 1986; Berman *et al.* 1992; Carlton 1998; Villalard-Bohnsack 1998) and additional species, such as the Asian shore crab, *Hemigrapsus sanguineus*, are expected to soon join this list (McDermott 1998; Tyrrell 1999). As noted by Villalard-Bohnsack (1998), most successful nonindigenous organisms follow a typical pattern of invasion consisting of three phases: settlement, expansion and persistence (*cf.* Mollison 1986). The phases are highly variable and intricately dependent upon the species' individual traits, as well as those of the host environment (*cf.* Ribera and Boudouresque 1995). Settlement, in addition to attachment, includes the ability of the initial inoculum to reproduce in its new environment, usually over several generations. The expansion phase involves spreading through aggressive reproduction and growth. The persistent phase that follows this vigorous colonization often consists of a plateau or a decline, leading to stabilization and balance within the new ecosystem. Abiotic factors and biotic interactions with other members of the new communities can alter the spread and roles played by these new species within a newly colonized system (Schluter 1996).

Two of the more conspicuous recent arrivals in the southwestern Gulf of Maine are the invasive green alga, *Codium fragile* ssp. *tomentosoides* (Fralick and Mathieson 1973; Carlton and Scanlon 1985), and the bryozoan, *Membranipora membranacea* (Berman *et al.* 1992). *C. fragile* ssp. *tomentosoides* first appeared within the Gulf at Boothbay Harbor,

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Maine, in 1964 (Coffin and Stickney 1966; Boerner 1972) and subsequently at the Isles of Shoals (NH/ME) by 1982 (Prince 1987, 1988). It is now dispersed throughout the southwestern portion of the Gulf of Maine, as well as being common along the coast of Nova Scotia (Bleakney 1996) and into the Gulf of St. Lawrence (Garbary *et al.* 1997). *M. membranacea* was first observed at the Isles of Shoals in 1987 (Berman *et al.* 1992) and has since spread throughout the Gulf of Maine and along the coast of Nova Scotia (Scheibling, pers. comm.). The two species have proven to be highly successful invaders and they share similarities in that both species have expanded their habitat selection over time and acquired opisthobranch predators that may influence their continued success and respective roles within local communities.

The objectives of this report are threefold: (1) to describe dispersal patterns of *C. fragile* ssp. *tomentosoides* and *M. membranacea* within the Gulf of Maine, (2) to document habitat expansion for the species, and (3) to compare the respective roles of the two predators on each introduced species. We will use a combination of previous observations, studies, and published reports to provide a historical background, plus a series of recent collections, observations, and quantitative sampling to describe the current status of each species.

Materials and Methods

The present study gives a synthesis of previous data documenting the dispersal and habitat distribution in both *C. fragile* ssp. *tomentosoides* and *M. membranacea*, plus a recent quantitative sampling describing patterns and changes. Most of the information is obtained from two Gulf of Maine sites: a station off Star Island, NH, at the Isles of Shoals, and the New Hampshire/southern Maine coastal zone (Figure 1). Mathieson has been studying algal populations along the coast of New Hampshire since 1965 (Fralick *et al.* 1974; Mathieson 1979; Mathieson and Hehre 1986; Mathieson and Penniman 1986a,b; Mathieson *et al.* 1991; Mathieson *et al.* 1998). The Star Island site has been studied by Harris and his students since 1974 (Hulbert 1980; Witman 1985; Martin *et al.* 1988; Berman *et al.* 1992; Harris *et al.* 1994; Harris and Chester 1996; Harris *et al.* 1998; Harris and Tyrrell, in prep.). A series of additional stations at the Isles of Shoals have been utilized since 1992 as part of an expanded study of sea urchin recruitment (Harris *et al.* 1994; Harris and Chester 1996) and changing sea star populations (Harris *et al.* 1998). All stations used were upper horizontal rocky substrates that were historically composed of algal dominated communities including crustose coralline algae (Hulbert 1980; Witman 1985; Sebens 1985; Martin *et al.* 1988; Ojeda and Dearborn 1989).

Information on previous patterns of community structure were obtained from published and unpublished material. The most complete documentation was available for a site (Figure 1) on the south side of Star Island (Hulbert 1980; Witman 1985; Martin *et al.* 1988). More descriptive observations were utilized for the other stations, including photographic slides taken at various times. In order to document current patterns of canopy, algal distribution, and relative abundance at the six sites shown in Figure 1, a set of 36 photographs was taken with a 15-mm lens on a Nikonos V underwater camera with flash. Slides were taken over a depth gradient from -3 to -12 m in order to show changes in relative abundance and community structure with depth. Counts of canopy species were made from these pictures by projecting them on a screen.

The distribution of *C. fragile* ssp. *tomentosoides* and other seaweeds was documented by making systematic collections within the littoral and sublittoral zones of diverse open coastal and estuarine sites within New Hampshire and southern Maine. That is, voucher samples of all taxa per site, including *C. fragile* ssp. *tomentosoides*, were made with these then being identified and established as herbarium voucher specimens (*cf.* Mathieson and Hehre 1986). Specific details on collection sites, voucher preparation, and documentation of temporal and spatial distribution between 1986 and 1998 is available for coastal and estuarine sites from southern Maine through New Hampshire and Massachusetts. The 1998 results presented here include the results of sampling more than 300 sites.

Temporal patterns of *M. membranacea* presence on different algal species was enumerated based upon surveys conducted throughout the year at Cape Neddick, York Beach, Maine. The animals' abundance (% occurrence) on various algae was determined by censusing all canopy seaweeds within a random patch of bottom approximately a meter square and then repeating the census after moving 1-3 m at the same depth. At least 20 sets of samples were collected each time. Each canopy species was identified to species and the presence of *M. membranacea* was

noted and the coverage estimated as 0, 1-10%, 11-50%, and 51-100%. On 4 December 1998, all canopy species with 10% or greater *M. membranacea* were also surveyed for the presence of the nudibranch *Onchidoris muricata*, including the number of nudibranchs found on each plant. A similar count of presence of *O. muricata* on *M. membranacea*-encrusted canopy species was conducted at the eastern Star Island site on 16 November 1998, but the survey was less detailed than the one conducted on 4 December at Cape Neddick.

Results and Observations

Three major stages in community composition have occurred at the Isles of Shoals and throughout much of the Gulf of Maine since the early 1970s. Prior to 1980, the dominant shallow water community to -10 m was a canopy of *Laminaria* spp., an understory of red algae, particularly *Chrondrus crispus*, *Corallina officinalis*, and several crustose coralline algae (Sebens 1985; Mathieson *et al.* 1991). About 1980, populations of the green sea urchin *Strongylocentrotus droebachiensis* increased in many areas, resulting in a conversion of vast areas into barren areas in which crustose coralline algae were the only conspicuous algae (Witman 1985; Ojeda and Dearborn 1989). Large scale harvesting of urchins began in 1987 and many portions of the Gulf of Maine then began to revert to algal-dominated communities (Mathieson and Hehre 1986; Harris *et al.* 1998). Some areas, such as the kelp bed site off White Island and Gosport Harbor (Figure 1), did not undergo this shift to urchin-dominated communities, but they were still subjected to the impacts of new species introductions. *C. fragile* ssp. *tomentosoides*, *M. membranacea*, and the tunicate *Diplosoma sp.* were all initially seen in Gosport Harbor before spreading to other locations in the New Hampshire/southern Maine coastal zone (Prince 1987; Berman *et al.* 1992; Harris *et al.* 1998).

Codium fragile ssp. tomentosoides

The original population of *C. fragile* ssp. *tomentosoides* documented from the Gulf of Maine was found at a protected area in Boothbay Harbor (Coffin and Stickney 1966; Carlton and Scanlon 1985). Drift specimens of *C. fragile* ssp. *tomentosoides* were collected at the Isles of Shoals in 1982 and attached plants were observed in Gosport Harbor in the summer of 1983 (Prince 1987). Table 1 provides a temporal synopsis of *C. fragile* ssp. *tomentosoides*' dispersal within the Gulf of Maine between 1964 and 1998. As noted previously, Mathieson has made detailed seasonal and spatial collections at over 300 locations along the open coastal and estuarine zones of southern Maine and New Hampshire, finding *C. fragile* ssp. *tomentosoides* at only 11 open coastal sites between Cape Neddick, Maine and the New Hampshire coast. No attached plants have been found within the Great Bay or Hampton-Seabrook

TABLE 1. A synopsis of *C. fragile* ssp. *tomentosoides* dispersal within the Gulf of Maine based upon collections by Mathieson at more than 300 locations within Southern Maine and New Hampshire during 1965–1998.

1964	Initial attached specimens found at Boothbay Harbor, Maine near U. S. Bureau of Commercial Fisheries Biological Laboratory (Fralick and Mathieson 1973).
1982	Drift populations initially found on Appledore Island, Maine, Isles of Shoals (A. Borror personal communication; Prince 1988).
1984	Second attached populations found at the Isles of Shoals from Babb's Cove and other sheltered sites on Appledore Island.
1985	Detached populations recorded from Rye Ledge (NH, nearshore open coast) and Weeks Point (NH, Great Bay).
1986	Dense subtidal beds (1-13 m below MLW) found at several locations in Gosport Harbor.
1989	Attached populations recorded from the nearshore open coast of southern Maine at Cape Neddick, York.
1992	Abundant at several locations in Gosport Harbor and first attached populations recorded from the nearshore open coast of New Hampshire at Fort Stark (Jaffrey Point).
1993	Well established on the exposed side of Appledore Island.
1994	First attached populations found at Duck, Malaga, White, Lunging and Seavey Islands at the Isles of Shoals. Attached populations also found on the nearshore open coast at Rye Ledge and drift populations found at Long Sands, York and Odiornes Point, Rye, NH.
1995	Abundant populations found on the exposed side of Smuttynose Island and initial attached populations found at Odiornes Point, Rye, NH.
1997	Attached populations at Star Island, NH, Isles of Shoals and also in the nearshore open coast at Seapoint, Kittery, Maine, and two nearby sites within the outermost part of Brave Boat Harbor, Kittery/York, ME.
1998	Attached populations found at a third outer Brave Boat Harbor site, as well as nearby Sisters Point and Fort Foster on Gerrish Island, Kittery, Maine. Initial collections also found at Great Island Common, Newcastle, NH, and Concord Point, Rye, NH.

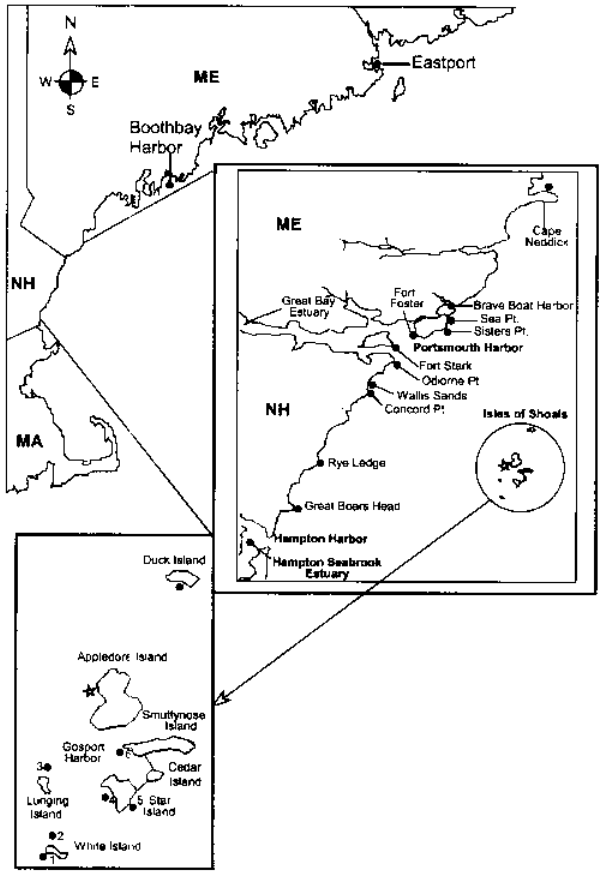

Figure 1. A map of the coast of New Hampshire and Maine (43°–45° N by 67°– 71° W). The full map shows the location of Boothbay Harbor where *C. fragile* ssp. *tomentosoides* was first seen. The first insert shows the locations of *C. fragile* ssp. *tomentosoides* collections at the Isles of Shoals and along the southern Maine/New Hampshire coast. The star (*) indicates the site of the first observation of *C. fragile* ssp. *tomentosoides* at the Isles of Shoals. The second insert shows sites at the Isles of Shoals where *C. fragile* ssp. *tomentosoides* has been observed and the numbers indicate the stations sampled for Figure 2.

estuarine systems (Figure 1). *C. fragile* ssp. *tomentosoides* was primarily limited to Gosport Harbor at the Isles of Shoals through 1990, but between 1991 and 1993 it underwent a major expansion, spreading throughout all of the islands (Figure 1). Extensive sampling along the coast of Maine has documented *C. fragile* ssp. *tomentosoides* populations at a number of sites between New Hampshire and Chamberlain, Maine, just to the north of Boothbay Harbor. No attached *C. fragile* ssp. *tomentosoides* plants have been observed north of Chamberlain, though a drifting specimen was collected in Cobscook Bay, near Eastport, in 1994.

C. fragile ssp. *tomentosoides* plants found within nearshore open coastal sites in New Hampshire and Maine tend to be smaller in stature and in more limited numbers versus insular sites like the Isles of Shoals. For example, several small aggregations of *C. fragile* ssp. *tomentosoides* have occurred at Cape Neddick since at least 1995, but most of the plants are short (<10 cm) and only slightly branched. Such a pattern contrasts with the dense populations found at protected sites such as Boothbay Harbor, Casco Bay, and Gosport Harbor, where large (>40 cm), heavily branched plants produce dense stands. Populations of *C. fragile* ssp. *tomentosoides* at the Isles of Shoals are consistently larger in size and denser, regardless of exposure. Such patterns contrast strongly to those found in nearshore open coastal sites.

Figure 2 shows the relative abundance of canopy species at two depths (0 to -7 m and -8 to -12 m) at six sites at the Isles of Shoals. *C. fragile* ssp. *tomentosoides* shows a consistent dominance at all shallow water depths. The relative abundance of *C. fragile* ssp. *tomentosoides*, which is based upon counts from over 30 photographs/site, is an underestimate, since small plants are hard to distinguish among adults. The sporophytes of *Laminaria saccharina* and *Agarum clathratum* are more conspicuous and thus are more accurately represented in these assessments. By contrast, sporophytes of *Desmarestia aculeata* often grow closely together, hence their abundance may also be underestimated. Overall, *C. fragile* ssp. *tomentosoides* is the dominant canopy species above 7 m at all sites, including the kelp bed site at White Island, where there has been no disturbance of community structure by urchins. The relative abundance of canopy species below 8 m varies by site. Gosport Harbor data were not included in the 8-12 m section as it was impossible to distinguish between slides taken above and below 8 m.

Laminaria spp. are abundant at several sites below 8 m. *A. clathratum* is also common in this zone and shallower at Cape Neddick on the nearshore open coast. The abundance of *D. aculeata* at the Star and White Island sites is much higher than it was in the 1970s and '80s. The zonation of canopy species prior to 1990 tended to separate populations of *Laminaria* spp. and *A. clathratum* above and below the 10 m thermocline, respectively (Witman 1985; Martin *et al.* 1988; Ojeda and Dearborn 1989; Mathieson *et al.* 1991). It now appears that *C. fragile* ssp. *tomentosoides* has become the dominant canopy species at the Isles of Shoals under diverse conditions. The pattern is consistent to at least 7 m, and the presence of young

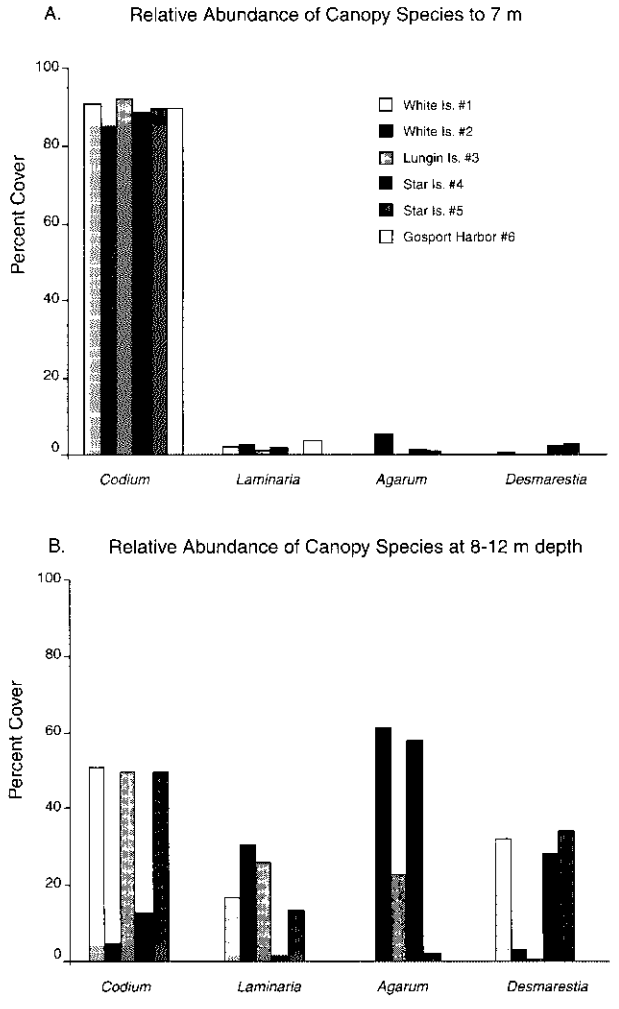

Figure 2. The relative abundance of canopy species at six Isles of Shoals sites. The data were obtained from counts made from more than 30 randomly taken photographic slides at each site. The relative abundance of canopy species from 0 to -7 m is shown in A, while the relative abundance of canopy species from -8 to -12 m is shown in Figure B.

plants among established adults suggests that it will persist for some time.

The saccoglossan gastropod, *Placida dendritica*, was initially found during 1996 occurring in very high numbers on large *C. fragile* ssp. *tomentosoides* plants in Gosport Harbor. Several hundred individuals were observed along with numerous egg masses on portions of plants that were covered by other branches of the same plants. The slugs were aggregated on branches shaded from direct view in most cases. The portions of the plants occupied by the *P. dendritica* were lighter in color and often broke when the plant was lifted. *P. dendritica* feeds by puncturing cell walls and pumping out the cytoplasm. Subsequent visits to the Gosport site have showed no decline in *C. fragile* ssp. *tomentosoides* density in spite of these high populations of a specialized herbivore. *P. dendritica* remains common in Gosport Harbor, particularly on large, old *C. fragile* ssp. *tomentosoides* plants and detached fragments accumulated in depressions. By contrast, it is rare in exposed sites, even after intense searching, which suggests it is better adapted to quiet conditions. *P. dendritica* is most often aggregated at the junctions of branches and appears to weaken the plant by its feeding, potentially increasing fragmentation and dispersal (Fralick and Mathieson 1972; Prince 1987,1988).

Membranipora membranacea

Colonies of the ectoproct bryozoan *M. membranacea* were first observed on *Laminaria* spp. at the Isles of Shoals during the summer of 1987 (Berman *et al.* 1992). During its initial occurrence (1988 and 1989), about 51% of *Laminaria* spp. had colonies of *M. membranacea*, while only 14% of *A. clathratum* populations were colonized by the bryozoan (Berman *et al.* 1992). Lambert *et al.* (1992) described *M. membranacea* overgrowing kelps at Cape Neddick, Maine as well as near Cape Cod, Massachusetts, and within the Damariscotta Estuary of Maine. Heavy overgrowth of *L. saccharina* blades by bryozoans increased fragility and blade loss, leading to kelp declines.

M. membranacea had spread to the northeastern portion of the Gulf of Maine by 1993 (Harris, pers. obs.). Colony size on kelps within these northern waters was not as large nor was overgrowth as extensive as within the southern portions of the Gulf of Maine. Populations of *A. clathratum* appeared to increase in abundance within shallower depths during the early 1990s, while the occurrence of *M. membranacea* on *A. clathratum* and other algae increased. By 1996, *M. membranacea* could be found growing on a wide range of flat-bladed algae as well as terete forms such as *Chordaria flagelliformis*, *Desmarestia aculeata*, *C. fragile* ssp. *tomentosoides*, and *Ascophyllum nodosum* (Harris and Tyrrell, in prep.). *D. aculeata* populations have also exhibited increased abundance below 8 m at Cape Neddick and the Isles of Shoals in the 1990s. Neither Witman (1985) nor Martin *et al.* (1988) previously recorded this brown alga as a common subtidal taxon below 12 m at Star Island; however, Harris and Tyrrell (in prep.) found that it was the numerically dominant canopy species in 1996 and it remains so today. As shown in Figure

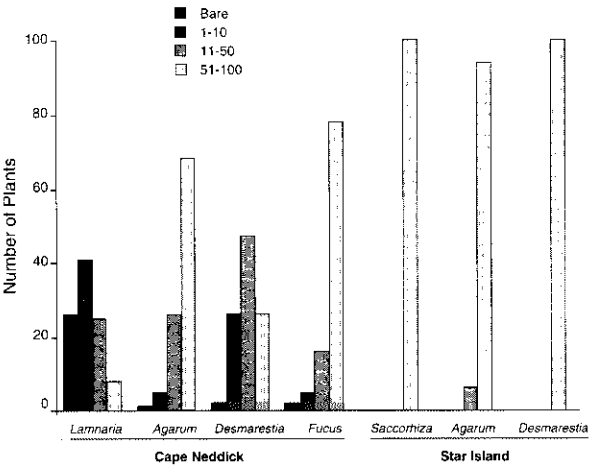

Figure 3. The relative abundance of four categories of percent cover for *M. membranacea* on canopy seaweeds made by direct counts at two sites: Cape Neddick, Maine and the outer side (station 5) of Star Island, Isles of Shoals. The categories are 0% *M. membranacea*, 1 to 10%, 11 to 50% and 51 to 100%. The algal species observed were *Laminaria saccharina*, *Agarum clathratum*, *Desmarestia aculeata*, *Fucus distichus* ssp. *evanescens*, and *Saccorhiza dermatodea*.

2, *D. aculeata* is common between 8 and 12 m at three of the six sites sampled.

Figure 3 documents the abundance of *M. membranacea* populations on canopy seaweed populations at Cape Neddick and Star Island. Two important points should be noted. Foremost, *A. clathratum* and *D. aculeata* now have higher percent coverage by *M. membranacea* than does *Laminaria* spp. The kelp *Saccorhiza dermatodea* appears to be a special case, for it endures total coverage by *M. membranacea* and then clears itself by sloughing the outer cell coating on the blade without apparent damage to the plant (Harris, pers. obs.). The second point represents a new observation, as this is the first time that *L. saccharina* blades have shown substantial new growth after settlement and growth of *M. membranacea* in the fall season when the bryozoan percent cover is usually at its maximum. At Cape Neddick, less than 10% of *L. saccharina* blades had 50% or more cover while almost 70% of *A. clathratum* and 25% of *D. aculeata* had 50% or more bryozoan cover. Ninety percent of *A. clathratum* and *D. aculeata* populations at Star Island had 50% or more bryozoan coverage. The values presented in Figure 3 are based on direct counts (Harris, pers. obs.). A survey of the values recorded from the slides used in Figure 2 gave the following estimates of 50% or more bryozoan coverage: *Laminaria* spp. – 16.3 to 66.7%; *A. clathratum* – 91.2 to 100%; *D. aculeata* – 79.7 to 100%. The results are for the five stations outside of Gosport Harbor and -8 to -12 m. In all cases, the most conspicuous feature was the area of bare *L. saccharina* blade below the zone of *M. membranacea* cover.

Upon its arrival in the Gulf of Maine, *M. membranacea* was free of predators. By contrast, in Europe and on the west coast of the United States, several dorid species readily feed upon *M. membranacea* (Thompson and Brown 1984; Harvell 1986; Behrens 1991). The dorid *Onchidoris muricata* is common in the Gulf of Maine (Harris 1973), and while it is reported to feed on *M. membranacea* in Europe, it was primarily associated with other bryozoans, particularly *Electra pilosa*, within the Gulf of Maine. Until 1997, the only time *O. muricata* were found feeding on *M. membranacea* was in the spring as older adults, *i.e.*, after other food sources had been depleted. It was predictable that sooner or later, some members of the species would produce veligers that accepted *M. membranacea* as a site for metamorphosis and juvenile growth. In the fall of 1997, numerous juvenile *O. muricata* were initially found on *M. membranacea*. The densities of recruiting *O. muricata* were even more conspicuous in the fall of 1998, at which time nudibranchs were found associated with *M. membranacea* as far north as Bar Harbor, Maine. Figure 4 summarizes the occurrence of *O. muricata* associated with *M. membranacea* colonies on different canopy species. The sampling was limited to canopy plants with either no *M. membranacea* or with at least 10% cover of the bryozoan. *A. clathratum* with *M. membranacea*

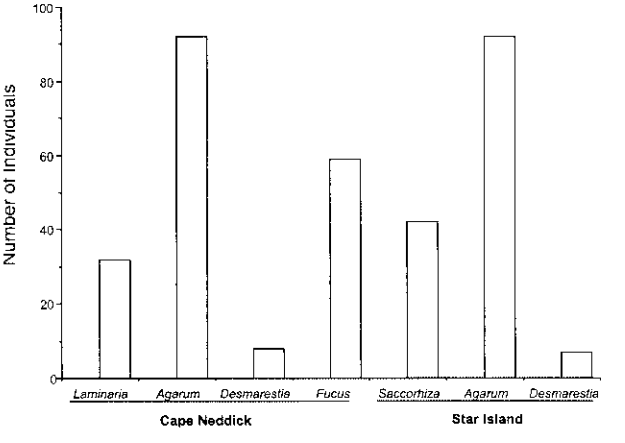

Figure 4. A comparison of the presence of the nudibranch *Onchidoris muricata* on *M. membranacea* on different canopy species. Only plants with at least 10% cover of *M. membranacea* were used in the counts.

had the highest nudibranch association, while it was lowest for *M. membranacea* associated with *D. aculeata*; the relative percent association for these two algae was almost identical for both Star Island and Cape Neddick. At Cape Neddick, the density of nudibranchs per plant was also highest on *A. clathratum*, with the mean number of nudibranchs per blade with 10% or more cover as follows: *A. clathratum* – 6.1; *Fucus distichus* ssp. *evanescens* – 3.8; *L. saccharina* – 3.7 and *D. aculeata* – 0.64.

The unexpected occurrence of three separate groups of *O. muricata* laying egg masses on 4 December 1998 should be noted, as well as the occurrence of newly settled juveniles less than 1.0 mm in length. *O. muricata* is well known to have an annual life cycle, with its young appearing in late summer and early fall and reproduction occurring in the spring with the adults dying soon after egg laying (Thompson and Brown 1984; Clark 1975; Todd 1987). The switch to recognizing a new food source was expected, since the species feeds on *M. membranacea* in other parts of its range; however, the breakdown of its annual reproductive cycle is new.

Discussion

Any evaluation of the role of introduced species in an ecosystem must be placed within the context of multiple human impacts that have been occurring for centuries (Carlton 1998). Ecologists are now recognizing the pervasiveness of human interaction with the environment (Vitousek *et al.* 1997). Fish stocks have been declining in the Gulf of Maine and elsewhere for as long as Europeans have been fishing these waters, but the number of studies relating to this impact are few (Witman and Sebens 1992; Parsons 1996; Botsford *et al.* 1997). Physical alteration of benthic communities by fishing gear is even more recent (Watling and Norse 1998). Little work has been done on how increasing water temperatures in the Gulf of Maine may influence community composition (Harris *et al.* 1998; Mathieson *et al.* 1998). The frequency of introductions and the level of impacts appear to be increasing at a time when parallel pressures from removal of natural populations and habitat modifications within many ecosystems are growing (Laws 1985; Parsons 1996; Vitousek *et al.* 1997; Botsford *et al.* 1997). Three major areas of interest have developed regarding introduced species: (1) rates of spread (Carlton and Scanlon 1985; Grosholz 1996), (2) impacts on established communities (Griffiths *et al.* 1992; Cohen *et al.* 1995; Vitousek *et al.* 1996), and (3) the roles they will assume in the new system (Hedgepeth 1980; Le Roux *et al.* 1990; Carroll and Dingle 1996).

The present study focuses on two of the more conspicuous and recent additions to the Gulf of Maine: *Codium fragile* ssp. *tomentosoides* and *Membranipora membranacea*. Both of these taxa are now prominent members of southern Gulf of Maine communities and their roles within these communities have certainly not reached equilibrium. Further, neither species appears to be following the patterns that might have been predicted from reviewing previous studies from other regions or from the Gulf of Maine. For example, *C. fragile* ssp. *tomentosoides* has previously been known to colonize and dominate protected habitats (Fralick and Mathieson 1973; Carlton and Scanlon 1985; Trowbridge 1996). The initial populations at the Isles of Shoals were in protected Gosport Harbor (Prince 1987; Prince and LeBlanc 1992; Figure 1 and Table 1). However, this pattern began to change in 1990, when a few small plants were observed at several locations outside of the Harbor, such as on the south side of Star Island (Figure 1). Subsequently, populations of *C. fragile* ssp. *tomentosoides* expanded to several shallow subtidal habitats around the Isles of Shoals during the next few years and they are now the dominant canopy species in shallow zones at all exposures (Figure 2). The mechanism by which this shift in habitat breadth occurred is unknown, but *C. fragile* ssp. *tomentosoides* was not the only species undergoing a rapid expansion at this time (Harris and Tyrrell, in prep.). The introduced tunicate *Botrylloides* sp. was also aggressively colonizing exposed subtidal habitats, and *M. membranacea* was beginning to be observed on a wider variety of algal morphologies than previously documented (Berman *et al.* 1992). Carlton and Scanlon (1985) reported that Malinowski (1974) found that Boothbay Harbor populations grew better at lower temperatures than those from Long Island Sound, suggesting that adaptation by natural selection was occurring. It is likely that Boothbay Harbor was the source population for the Isles of Shoals *C. fragile* ssp. *tomentosoides*, given the southward currents prevalent in the Gulf of Maine coastal zone (Brooks 1985). Harris *et al.* (1998) reported a pattern of increasing summer water temperatures in the southern Gulf of Maine that may have influenced changes in sea star populations and community structure. The

changing environmental conditions and community instability due to overfishing of urchins may have provided an ecological vacuum that *C. fragile* ssp. *tomentosoides*, preadapted to Gulf of Maine conditions, could take advantage of to occupy an expanded range of exposures. The fact that *C. fragile* ssp. *tomentosoides* populations have been less effective at colonizing nearshore exposed habitats suggests that further work is needed to understand the mechanisms involved.

The spread of *M. membranacea* throughout the Gulf of Maine and into new habitats has been much faster than for *C. fragile* ssp. *tomentosoides*. Reports for Europe (Ebling *et al.* 1948; Sloane *et al.* 1957; Ryland 1970; Hayward and Ryland 1990) and the Pacific coast of the United States (Yoshioka 1982; Harvell 1986; Grunbaum 1997) consistently describe *M. membranacea* as inhabiting flat-bladed kelps and *Fucus* spp. Berman *et al.* (1992) and Lambert *et al.* (1992) also emphasized the association of *M. membranacea* with the kelp *Laminaria*. However, more recent observations indicate that the bryozoan has expanded its habitat selection to include even terete, aborescent algae, such as *D. aculeata* (Harris and Tyrrell, in prep.; Figure 3). As is shown in Figure 3, *M. membranacea* may actually have a higher percentage of association with *A. clathratum* than with *Laminaria* spp. (Berman *et al.* 1992). *M. membranacea* can also grow on other red and green algae, including *C. fragile* ssp. *tomentosoides*. When *M. membranacea* first appeared in the Gulf of Maine, *Laminaria* spp. were seldom covered with sessile fauna (Berman *et al.* 1992). The heavy overgrowth by *M. membranacea* appeared to have a significant negative impact on *Laminaria* spp. populations (Lambert *et al.* 1992; Harris and Tyrrell, in prep.). The stress of overgrowth of *Laminaria* spp. populations by *M. membranacea* may have facilitated the expansion of *C. fragile* ssp. *tomentosoides* populations into exposed habitats at the Isles of Shoals (Figure 2). *C. fragile* ssp. *tomentosoides* colonized and replaced *L. saccharina* as the dominant canopy species at the site at White Island, which never had an urchin barrens (Figure 2). *L. saccharina* appears to be adapting to competition with *C. fragile* ssp. *tomentosoides*, or rather it seems to be shifting its center of abundance below the *C. fragile* ssp. *tomentosoides* zone (Figure 2, site 2). *L. saccharina* also appears to be adjusting its growth to minimize *M. membranacea* overgrowth (Figure 3). One result of the shift in the association of the bryozoan with *L. saccharina* appears to be a closer association with *A. clathratum* and *D. aculeata* (Figure 3). What factor led to shifts in substrate selection for *M. membranacea*? The bryozoan is dormant through the winter and is susceptible to sloughing off via intercalary kelp growth at the base of the blades. The success of colonies occupying the slower growing, more stable *A. clathratum* as well as the adaptation to grow around the holes in *A. clathratum*'s blades may have opened the way to colonize perennial, cylindrical substrates such as *D. aculeata*.

The acquisition of predators on *C. fragile* ssp. *tomentosoides* and *M. membranacea* was predictable since both have these nudibranch predators in other regions. For example, *P. dendritica* is common in salt marsh environments where it feeds primarily on the siphonaceous green alga *Bryopsis plumosa*, which is a close relative of *C. fragile* ssp. *tomentosoides* (Bleakney 1996; Harris, pers. obs.). *O. muricata* was commonly associated with the bryozoan *E. pilosa* (Harris 1973). The finding of high numbers of each predator on its respective introduced host has been a recent development. Few observations of *C. fragile* ssp. *tomentosoides* populations in Gosport Harbor were made before 1996 so the period of buildup for *P. dendritica* populations is unknown. However, *M. membranacea* populations were observed regularly and the appearance of newly settled *O. muricata* juveniles on this colonial bryozoan became noticeable in fall of 1997. One year later, it had become very common. Trowbridge (1992) documented a negative impact of *P. dendritica* on the distribution of *Codium setchellii* in Oregon. *C. fragile* ssp. *tomentosoides* plants in Gosport Harbor having large numbers of *P. dendritica* showed bleached thalli and ragged branches when the saccoglossans were aggregated. *P. dendritica* predation can cause fragmentation of *C. fragile* ssp. *tomentosoides* (Trowbridge 1992), potentially increasing the plant's dispersal as these fragments are capable of attaching to new substrates (Fralick and Mathieson 1973; Carlton and Scanlon 1985).

O. muricata has an annual life history that begins with settlement of juveniles in the late summer/fall followed by growth in the winter and reproduction in the spring after which the adults die (Todd 1987, Bleakney 1996). Thus, *O. muricata* feeds and grows while *M. membranacea* is dormant in the winter. The results presented in Figure 4, as well as the density of *O. muricata* per plant presented in the results, suggest that *M. membranacea* occupying *D. aculeata* may suf-

fer the lowest predation pressure. This may be due to the inability of the broad-footed nudibranch to maneuver efficiently on the narrow and highly branched fronds of *D. aculeata*. The net result of high predation on bryozoan colonies occupying flat-bladed algae may have increased selection towards *D. aculeata* as described above.

In summary, the two introduced species described in this report, the green alga *Codium fragile* ssp. *tomentosoides* and the bryozoan *Membranipora membranacea*, are functioning within a system that has undergone significant changes in community structure and the relative abundances of a number of species (Harris *et al.* 1998; Harris and Tyrrell, in prep.). The changing ecology of *C. fragile* ssp. *tomentosoides* and *M. membranacea* must be viewed in the context of an ecosystem in flux. That is, the instability and changes occurring provide an environment that favors shifts in the roles played by a species in its interactions with other taxa. It is likely that the conditions described here will continue to change for some time and only continued studies will answer the questions of how these introduced species will fit into the diverse environments occurring within the Gulf of Maine. The rates at which changes in their ecology have occurred suggests that each species represents an interesting subject for the study of natural selection.

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Using Niche Theory to Understand Invasion Success: A Case Study of the Asian Shore Crab, *Hemigrapsus sanguineus*

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INTRODUCTION

Ecological niche theory (*e.g.*, Hutchinson 1957) provides a foundation for understanding the role of a species in a community. Niche theory can therefore be used to address questions of why some exotic organisms succeed in fitting into communities with which they have shared no evolutionary history. Based on a wealth of experimental and theoretical literature, a series of niche-based models was developed into a framework for studying the invasive success of exotic species (Lohrer 2000). This framework was created to provide testable hypotheses characterizing alternative modes of successful invasion by exotic species.

CASE STUDY

Here we report the findings of a case study which served as the initial application of the framework. *Hemigrapsus sanguineus*, a grapsid shore crab indigenous to Japan and other western Pacific regions, was recently introduced to the Atlantic coast of North America (McDermott 1991). First noted in southern New Jersey in 1988 (Williams and McDermott 1990), the crab now ranges from North Carolina to New Hampshire (McDermott 1998a; A.H. Hines and M.C. Tyrrell, pers. comm.). *H. sanguineus* is frequently the most abundant brachyuran in a variety of rocky intertidal habitats in southern New England (Lohrer 2000), with maximum densities of >100 crabs m^{-2} in several localities. Unfortunately, basic ecological parameters of *H. sanguineus* are still largely undescribed in its native and invaded habitats (Kikuchi *et al.* 1981; but see Fukui 1988; Lohrer and Whitlatch 1997; McDermott 1998a, 1998b; Gerard *et al.* 1999; Lohrer *et al.* 2000, Lohrer *et al.* in press). Table 1 summarizes a comparative analysis of *H. sanguineus* in Tanabe Bay, Japan and Long Island Sound, USA. Objectives were to understand how this Asian crab successfully invaded the northeastern coast of North America as well as to provide specific predictions of future ecological impacts in New England.

Several factors listed below may have contributed to the successful invasion of *H. sanguineus* into North America. (1) Physical and climatological features of northeastern Asia and northeastern North America are similar. (2) *H. sanguineus* is tolerant of a wide range of physical conditions and has opportunistic feeding habits. (3) The large reproductive capacity of *H. sanguineus* (>40,000 eggs $brood^{-1}$, several broods $year^{-1}$ in large females; Fukui 1988) is not limited by rhizocephalan parasites in the invaded range as it is in its native range (4) Intertidal areas of cobble and boulder (apparently suitable for *H. sanguineus*) are common in parts of the invaded range (particularly at latitudes >40° N). (5) Other crab species are not likely to compete with or restrict the niche of *H. sanguineus* in the invaded range; the region has

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Table 1. Observations pertaining to the successful invasion of the northeastern coast of North America by the Asian shore crab, *Hemigrapsus sanguineus*. Table is based on literature reports and data collection in Tanabe Bay, Japan and Long Island Sound, USA.

	Northwestern Pacific (Asia)	Northwestern Atlantic (USA)	Notes	References
Latitudinal Range of *H. sanguineus*	≈20° N to 50° N	≈36° N to 42° N	Current range in USA is well within the observed range in Asia and annual air and water temperature fluctuations are similar in both regions	Sakai 1976; Takahashi *et al.* 1985; McDermott 1998b
Diversity of crabs co-occurring with *H. sanguineus*	High (≈20 spp.)[A]	Low (≈5 spp.)[B]	There are many other grapsids which co-occur with *H. sanguineus* in Asia, but *H. sanguineus* is the only grapsid in northeastern USA rocky intertidal areas	[A]Sakai 1976; Fukui and Wada 1983; Fukui 1988; [B]Williams 1984
H. sanguineus abundance	Common (mean of 5 to 10 crabs m^{-2})	Abundant to very abundant (mean of 20 or more crabs m^{-2})		Lohrer *et al.* in press; Lohrer 2000
H. sanguineus habitat utilization	Moderately exposed cobble and boulder shores[A]	Moderately exposed cobble and boulder shores[B]	Apparent association with larger-sized stones and >60% cover by all stones[C], less abundant when stones are buried sediment, rare/absent in low-salinity, in marsh and soft-sediment habitats in Asia and North America[D]	[A]Kikuchi *et al.* 1981; Fukui and Wada 1983; ; Takahashi *et al.* 1985; [B]Lohrer and Whitlatch 1997; McDermott 1998b; Gerard *et al.* 1999; [C]Lohrer *et al.* 2000; [D]Lohrer *et al.* in press
H. sanguineus vertical distribution	Found throughout intertidal zone[A], associated with high structural complexity areas[B]	Found throughout intertidal zone[A], associated with high structural complexity areas[B]	*H. sanguineus* is not exclusively an "upper intertidal" species[C]. No crabs were found below the level of maximum low tide (i.e., subtidally) in Tanabe Bay or Long Island Sound[D]	[A]Lohrer and Whitlatch 1997; Lohrer *et al.* in press; [B]Lohrer *et al.* 2000 [C]McDermott 1992; Lafferty and Kuris 1996; [D]Lohrer, pers. obs.
H. sanguineus diet	Omnivorous, ≈40:60 animal:plant matter	Omnivorous, ≈40:60 animal:plant matter	Judging from gut contents, male and female crabs consume small-sized calcified invertebrates (snails <4 mm shell height, mussels <10 mm shell length); annelids and arthropods also consumed; red and green algae dominated algal matter found in stomachs; barnacles only found in guts from North American *H. sanguineus* population	Lohrer and Whitlatch 1997; Lohrer *et al.* in press
Parasitism by rhizocephalans (parasitic castrators)	Low % parasitism in Tanabe Bay, reports of heavy parasitism elsewhere in Japan	Parasites absent in invaded range		Takahashi and Matsuura 1994; Lützen and Takahashi 1997; Takahashi *et al.* 1997; A.M. Kuris, pers. comm.; Lohrer, pers. obs.

low species richness in rocky intertidal areas and *H. sanguineus* is distantly related to the resident crabs in this habitat type (Sakai 1976; Williams 1984). (6) Comparing *H. sanguineus* to other crabs in the rocky intertidal, measurements of niche overlap (along 3 niche dimensions: habitat utilization, crab size, food habits) were low (25-62%) in Long Island Sound (Lohrer *et al.* in press). (7) Roughly seven years following its establishment in Long Island Sound, no restriction of the niche of *H. sanguineus* is apparent relative to Tanabe Bay (Lohrer *et al.* in press).

Most likely, *H. sanguineus* did not invade by competitively displacing resident crabs from rocky intertidal habitats in Long Island Sound, but rather, it appears to occupy a habitat type which is marginal to and/or underutilized by other resident species. The impact of *H. sanguineus* will likely be restricted to rocky intertidal habitats where its abundance is greatest. At a larger scale, its rate of spread and impact will likely be greater north of 40° latitude where appropriate rocky intertidal habitat is more common than on the mid-Atlantic coast of the U.S. *H. sanguineus* will likely impact local populations through predation and grazing (rather than by competition). Furthermore, gut content data and the relatively small size of *H. sanguineus* indicate a potential threat to small-sized prey. For example, *H. sanguineus* is incapable of consuming the largest *Mytilus edulis*, but will readily consume mussels <10 mm shell length. In addition, *Carcinus maenas* recruits and juveniles (<35 mm carapace width) are known to reside intertidally and their densities in rocky intertidal habitats of Long Island Sound have been declining concurrent with increases in *H. sanguineus* (Lohrer 2000). The biological composition of rocky intertidal areas could be altered by the extreme densities of *H. sanguineus* if this invader preys significantly on small organisms in this habitat type.

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Geographical Distributions and Organism-Habitat Associations of Shallow-Water Introduced Marine Fauna in New England

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ABSTRACT: In the past several decades, a number of benthic invertebrates have successfully invaded New England coastal waters and are frequently found as dominant members of these habitats. These species include several ascidians (*Botrylloides diagensis, Styela clava, Ascidiella aspersa, Diplosoma macdonaldi*), an encrusting bryozoan (*Membranipora membranacea*), and the Western Pacific shore crab (*Hemigrapsus sanguineus*). In order to better understand their potential impact on resident fauna, establish current distributions of the species, and assess habitat types where the species do and not occur, we conducted a broad-scale survey of coastal habitats ranging from Connecticut to Maine in 1997 and 1998. Our findings indicate that *Botrylloides* and *Styela* are found throughout most of New England; particularly in tidal inlets and embayments. *Diplosoma* and *Ascidiella* appear more restricted to southern New England, whereas *Membranipora* is most commonly found north of Cape Cod. *Hemigrapsus sanguineus* has spread rapidly throughout southern New England and was found at several sites in Massachusetts Bay, and showing high habitat overlap with the invasive green crab *Carcinus maenas*.

Key words: New England, invasive ascidian, invasive crab, range, survey

INTRODUCTION

In recent years we have seen the environmental devastation that can result from the inadvertent introduction into a region of non-native or exotic species such as the zebra mussel (*Dreissena polymorpha*). Valuable resources have been invested in efforts to control or eradicate such pest species. For the most part these efforts have resulted only in temporary solutions. There is a severe lack of knowledge about the general ecology of invading species. This knowledge is needed both to estimate future dangers and to prepare management approaches that will enable us to react intelligently to the inevitable new introductions. The need for such information is extremely critical for the marine environment. There has been little or no research directed at identifying how such exotic marine species invade, yet the "barrier-free" ocean environment places marine communities at great risk.

In the past 25 years several species have invaded the New England coast and have spread from Connecticut to Maine. These include several species of ascidians (*Botrylloides diagensis, Styela clava, Ascidiella aspersa, Diplosoma macdonaldi*), an encrusting bryozoan (*Membranipora membranacea*), and the Western Pacific shore crab (*Hemigrapsus sanguineus*). We have been studying these species (*e.g.*, Osman and Whitlatch, this volume; Lohrer *et al.*, this volume) in order to determine and test future strategies for the management of introduced species. Our research has generally focused on three inter-related problems: (1) determining the extent to which newly introduced species had invaded coastal New England and whether they were restricted to specific habitats, (2) examining the differences between native and introduced species in how their populations expand into new areas, and (3) delineating the interactions between invading species and the native community.

In order to better understand the potential impact of these invaders on resident fauna, we conducted a broad-scale regional survey in the summers of 1997 and 1998. The primary goal of the surveys was to establish the current distributions of the

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introduced species and assess habitat types where the species occur and do not occur.

Materials and Methods

In 1997 we concentrated on areas from Groton, Connecticut southwest to Greenwich, Connecticut and in 1998 we surveyed the coast from Groton northeast to Bar Harbor, Maine. In the two years, we sampled at 35 locations along the coastline. At most of these sites samples were taken in a variety of habitats including harbors, exposed points, headlands and coves, mussel beds, marshes, and eelgrass beds. Sampled substrates included pilings and floating docks in marinas, natural rock faces and boulders, jetties, fronds of macroscopic algae, eelgrass blades, shells of living and dead molluscs, and debris. Since our primary objective was to examine broad-scale distribution patterns, we did not quantify the abundance of the invaders.

Results

The regional distribution patterns that we found for the four ascidians and the bryozoan *Membranipora membranacea* are shown in Table 1. *Botrylloides* was the most broadly distributed of the five fouling species. We found it as far north as Penobscot Bay, Maine and as far south as Bridgeport, Connecticut. In addition we have had unconfirmed reports from colleagues that this species may have been found as far north as Prince Edward Island, Canada and as far south as Chesapeake Bay. The distribution of *Styela* was almost as extensive. We found this species from Casco Bay, Maine to east of the Connecticut River in Long Island Sound. Both *Ascidiella* and *Diplosoma* had much narrower distributions. As with *Styela*, neither was found west of the Connecticut River. *Ascidiella* was found as far north as Buzzards Bay, Massachusetts and *Diplosoma* was not found north of Narragansett Bay, Rhode Island. Finally, *Membranipora* was found more commonly north of Cape Cod, usually on fronds of *Laminaria* and other subtidal seaweeds (*e.g.*, Berman *et al.* 1992).

In Table 2, the distribution of Western Pacific shore crab, *Hemigrapsus sanguineus,* is contrasted with that of the green crab, *Carcinus maenas*, which was introduced to New England early in the 19th century (Grosholz and Ruiz 1996). *Hemigrapsus* was found as far north as Cape Cod Bay and because it was first found in New Jersey, we know it extends far to the south of New England. Reports from colleagues indicate recent sightings of *Hemigrapsus* along the New Hampshire shoreline. This range overlaps completely with the green crab which is known to occur from New Jersey to Canada (Grosholz and Ruiz 1996) and was found at our most northern most stations.

Discussion

Overall, the distributions reflected in Tables 1 and 2 demonstrate the continued but sporadic spread of these exotic species. Of the ascidians, those species that have been in New England the longest, *Botrylloides* and *Styela*, have spread much further than more recent arrivals. In addition, *Hemigrapsus,* which has relatively long-lived larvae and is motile as an adult, has also spread rapidly. The patchy distribution of all the species is also not indicative of a slow spread or diffusion from the initial invasion site. Rather, the pattern is suggestive of a process of establishing persistent local populations from which larval colonists are released and occasionally transported to other distant sites.

The general habitat patterns recorded in our survey are very similar to the local patterns we observed in eastern Long Island Sound over the past ten years. Sheltered areas such as harbors had a fairly rich ascidian fauna, while the exotic species were generally missing from open coasts and exposed headlands. Salt marshes and the low salinity parts of estuaries also seemed to be poor habitats for these species. For example, in Long Island Sound *Styela* was not found to the west of the Connecticut River. In addition, a sheltered site at the mouth of this large river had no ascidians. It is possible that the large amount of freshwater entering Long Island Sound at the Connecticut River may act as a partial barrier and slow the spread of these species to the southwest. Our findings indicate that *Botrylloides* and *Styela* are found throughout most of New England, particularly in tidal inlets and embayments. In contrast, we only found *Diplosoma* and *Ascidiella* in southern New England shallow subtidal habitats. L. Harris (pers. comm.) has noted extensive populations of *Diplosoma* off the coast of New Hamsphire. From our studies in Long Island Sound over the past decade, this species displays remarkable spatial and temporal variability in its abundance, and our "one shot" survey may simply have failed to record the presence of the species north of Cape Cod. In general, colonial ascidians appeared to be more common

Table 1. Presence of fouling ascideans at surveyed sites along the New England coast. Sites are arranged from the Northeast to the Southwest and grouped by water body or coastal feature. Floats include floating docks within harbors. Long Island Sound was surveyed in 1997 and the remaining sites in 1998.

Location	State	Type	Habitat	*Botrylloides*	*Diplosoma*	*Styela*	*Ascidiella*	*Membranipora*	Native Ascidians
Mt. Desert Island									
Northeast Harbor	ME	Harbor	Floats						
Bass Harbor Coast	ME	Coast	Bedrock						X
Northeast Harbor	ME	Cove	Rocks						X
Penobscot Bay									
Ellsworth	ME	Estuary	Floats						
Castine	ME	Harbor	Floats	X				X	
Belfast	ME	Harbor	Floats	X				X	
Casco Bay									
Boothbay Harbor	ME	Harbor	Floats	X		X			X
Cape Elizabeth – Open	ME	Coast	Bedrock	X					
Cape Elizabeth – Cove	ME	Coast	Bedrock	X					
Cape Porpoise	ME	Harbor	Floats	X				X	X
Bigelow Bight									
Kennebunkport	ME	Harbor	Floats	X		X			X
York Harbor	ME	Harbor	Floats	X				X	
Rye Harbor	NH	Estuary	Floats	X					X
Massachusetts Bay									
Gloucester – Rockport	MA	Harbor	Floats						
Cape Cod Bay									
Plymouth Harbor	MA	Harbor	Floats	X		X		X	X
Manomet Point	MA	Coast	Rocks	X					X
Sagamore	MA	Harbor	Floats	X					X
Barnstable Harbor	MA	Harbor	Floats	X		X			X
Rock Harbor	MA	Estuary	Floats						X
Pleasant Bay									
Chatham	MA	Harbor	Floats	X					X
Vineyard Sound									
Hyannis	MA	Harbor	Floats	X		X			X
Woods Hole	MA	Harbor	Floats	X	X	X			X
Buzzards Bay/ RI Sound									
West Falmouth	MA	Harbor	Floats	X		X			X
Westport	MA	Harbor	Floats	X					X
Sakonnet Point	RI	Coast	Bedrock	X		X			
Narragansett Bay									
Tiverton	RI	Harbor	Floats	X		X			X
Jamestown	RI	Harbor	Floats	X	X	X	X		X
Beavertail Point	RI	Coast	Bedrock						
Long Island Sound									
Groton	CT	Coast	Rocks	X	X	X	X		X
Niantic	CT	Harbor	Rocks	X					X
Saybrook	CT	Estuary	Floats						
Hammonassett Point	CT	Coast	Rocks						
Sachem Head	CT	Coast	Rocks						
New Haven	CT	Harbor	Piles						X
Bridgeport	CT	Harbor	Piles	X					X

along exposed coastlines north of Cape Cod than in southern New England. Also, *Botrylloides* was frequently found to inhabit the intertidal zone in northern New England, whereas it is uncommon to find intertidal populations in southern New England. Based on recruitment to floating docks, local populations of the encrusting invaders are well established throughout coastal New England. While *Membranipora* is more commonly found north of Cape Cod, *Hemigraspsus* has rapidly spread throughout southern New England and was found at several sites in Massachusetts Bay.

Table 2. Presence of the invading crabs, *Carcinus maenas* and *Hemigrapsus sanguineus*, at sites surveyed along the New England coast. Long Island Sound was surveyed in 1997 and the remaining sites in 1998.

Location	State	Type	Habitat	*Carcinus*	*Hemigrapsus*
Mt. Desert Island					
Bass Harbor Coast	ME	Coast	Bedrock	X	
Northeast Harbor	ME	Cove	Rocks	X	
Penobscot Bay					
Camden	ME	Estuary	Rocks		
Casco Bay					
Cape Elizabeth:					
Open	ME	Coast	Bedrock		
Cove	ME	Coast	Bedrock	X	
Cape Porpoise	ME	Harbor	Rocks	X	
Cape Cod Bay					
Plymouth Harbor	MA	Harbor	Shore	X	
Manomet Point	MA	Coast	Rocks	X	X
Vineyard Sound					
Woods Hole	MA	Harbor	Rocks	X	
Martha's Vineyard	MA	Harbor	Rocks	X	X
Buzzards Bay/ RI Sound					
Horseneck Beach	MA	Harbor	Rocks	X	X
Sakonnet Point	RI	Coast	Bedrock		
Narragansett Bay					
Beavertail Point	RI	Coast	Bedrock		
Narragansett	RI	Coast	Rocks		X
Point Judith	RI	Coast	Rocks	X	X
Block Island Sound					
Matunuck	RI	Coast	Rocks	X	X
Weekapaug	RI	Coast	Rocks		X
Long Island Sound					
Stonington	CT	Coast	Rocks	X	X
Groton	CT	Coast	Rocks	X	X
Niantic	CT	Harbor	Rocks	X	X
Hammonassett Pt	CT	Coast	Rocks	X	X
New Haven	CT	Coast	Rocks	X	X
Milford	CT	Coast	Rocks	X	X
Greenwich Point	CT	Coast	Rocks	X	X

Our present work, coupled with previous studies, has indicated that one of the invading species (*Botrylloides*) is resistant to native predators but the others are not (*e.g.*, Osman and Whitlatch 1995). In the absence of predators, the invading species can reduce the abundance of similar native species and permanently change the nature of local communities (Whitlatch *et al.* 1995). Our surveys along 500 km of coast in New England have confirmed that those habitats without these predators had the highest densities of several of the introduced ascidian species. We have also recently experimentally demonstrated that more diverse, species-rich fouling assemblages appear more resistant to these invaders (Stachowicz *et al.* 1999), thereby providing a compelling reason for the perservation of global marine biodiversity. Continued studies are underway on: (1) What makes some species particularly good invaders? and (2) Why are some communities more easily invaded than others? The long-term goal of our work is to develop a predictive framework for the study and management of invasions in coastal habitats.

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Climate Effects on the Geography of Nonindigenous Peracaridan Crustacean Introductions in Estuaries

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ABSTRACT: **Global relations between climate and the diversity of peracaridan crustacean introductions in estuaries reveal where management resources may be concentrated for maximum limitation of nonindigenous species and their impacts. Nearly all non-indigenous species (NIS) in estuaries of the northern hemisphere are from the western sides of the Pacific or the Atlantic oceans. In contrast, few native northeast Pacific species have invaded western ocean estuaries. Peracaridan NIS diversity declines also in the northeast Pacific between 38° and 60° north latitude with nearly all NIS occurring in Puget Sound, Washington or Prince William Sound, Alaska occurring also in San Francisco Bay, California. These regional and global patterns of introductions coincide closely with variations in climate of the northern hemisphere between 25° and 60° N. Annual sea surface temperatures vary less in the northeast Pacific and northeast Atlantic relative to temperatures of western ocean coasts. Low salinities occur in the coldest months of the northeast Pacific and the northeast Atlantic below 50° N. while low salinities occur in the warmest months on the western sides of these oceans. Assuming introductions occur only in the range of climate conditions in which species are adapted, the proliferation of introductions in the northeast Pacific may result, from the diversity of climates that encompass the relatively mild northeast Pacific maritime climate. Similarly, species native to the northeast Pacific transported to other regions may be less likely to survive because of the broader range of climate conditions they must endure there. The south to north decline of northeast Pacific NIS also coincides with fewer introductions establishing where greater annual variations in temperature and lower summer salinities occur. The patterns of NIS diversity and assumptions of risk for introductions may vary among latitudes with greater ecological importance of individual introductions at high latitudes.**

Keywords: introduced species; northeast Pacific; Atlantic; range; estuary; climate; risk analysis; crustacea; peracarida; biogeography; *Mya arenaria*.

INTRODUCTION

Major objectives of invasion ecology are to discover which nonindigenous species (NIS) can be introduced, where, and the factors that control their dispersal and probable survival once introduced. The diversity of mechanisms that disperse NIS among estuaries are rapidly being discovered (*e.g.*, Carlton 1979a, 1985, 1996b; Kelly 1993; Carlton and Geller 1993; Cangelosi 2000; Cohen 1998; Frey *et al.* 1999; Draheim and Olson 2000; Ruiz *et al.* 1997a, 1997b; Smith *et al.* 1999a; Miller and Chapman 2000; Moy 2000; Thresher 2000) while the processes regulating NIS survival and diversity among estuaries remain poorly resolved. The distributions of NIS reveal how survival varies with dispersal and thus the interactions of dispersal and ecology. Interpreting the geography NIS distributions is thus a critical part of the search for factors controlling NIS invasions.

Nonindigenous species are diverse and abundant in estuaries of the northeastern Pacific, including San Francisco Bay, California (Carlton and Geller 1993; Cohen and Carlton 1995, 1997; Ruiz *et al.* 1997a, 1997b) and in Europe (Leppäkoski 1994; Leppäkoski and Olenin 2000; Eno *et al.* 1997). Most eastern ocean NIS are endemic to western ocean coasts (Cohen and Carlton 1995; Leppäkoski and Olenin 2000) and eastern ocean NIS diversity declines with increasing latitude (Carlton 1979a; Cohen *et al.* 1998; Mills *et al.* 2000). Neither geographical pattern results entirely from the mechanisms of dispersal or from interactions between native and nonindigenous species. Additional processes, including climate and control of NIS distributions, warrant consideration.

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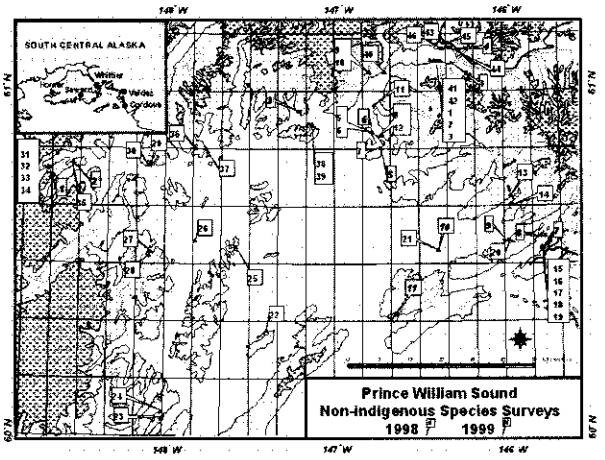

Figure 1. Prince William Sound, Alaska and south central Alaska rapid assessment sites with open boxes indicating all 46 sampling sites of 1998, inset indicating the five port areas of the sound in 1999. (Latitudes, longitudes and site descriptions reported in Chapman 2000, appendix table 1).

Most chemical, biological, and hydrological processes of estuaries that limit abundances and distributions of estuarine organisms are also controlled by, or closely correlated with, salinity and temperature (*e.g.*, Kinne 1963; Southward 1969; Green 1971; Ebbesmeyer *et al.* 1991; Cohen and Carlton 1995, 1998; Chapman 1998; Thompson 1998). Long-term seasonal variations of salinity and temperature are poorly known in most estuaries. However salinity and temperature are controlled largely by precipitation and air temperature. Seasonal and geographical patterns of precipitation and air temperature are well known (*e.g.*, Ebbesmeyer *et al.* 1991; Cayan 1993; Nichols *et al.* 1986). Therefore, NIS diversity can be compared with geographical patterns of precipitation and air temperature to infer whether climate affects the probability of NIS invasions in estuaries.

Methods

The diversities of marine and estuarine peracaridan crustaceans of the northern hemisphere are used as a sample of the global NIS diversity to compare with variations in climate. Crustacea comprise approximately 25% of the 250 NIS reported from San Francisco Bay (Cohen and Carlton 1995; J. T. Carlton, pers. comm.) where they are the most diverse NIS taxon. The majority of crustacean NIS are peracaridans. Peracaridans are small, short-lived and consist primarily of mysids, amphipods, isopods, tanaidaceans and cumaceans. Nonindigenous peracaridans are prominent in most North Pacific and North Atlantic marine and estuarine communities (Bowman *et al.* 1981; Chapman 1988, 1998; Chapman and Carlton 1991, 1994; Mees and Fockedey 1993; Leppäkoski 1994; Cohen and Carlton 1995, 1997; Eno *et al.* 1997; Toft *et al.* 1999) diverse, ubiquitous and relatively well resolved taxonomically (*e.g.*, Barnard 1975; Barnard and Barnard 1983a; 1983b, Barnard and Karaman 1990a, 1990b; Chapman 1988; Cohen and Carlton 1995; Staude 1997). Peracarida also develop directly, without larval dispersal stages or unique life history traits that complicate identifications and interpretations of their geographical distributions.

North-south NIS diversity

North to south variation in northeast Pacific NIS diversity is assessed primarily from rapid assessment field surveys (Mills *et al.* 2000) of the native and introduced peracaridan crustaceans in Prince William Sound, Seward and Homer, Alaska (60°00' – 61°00' N) Puget Sound, Washington (47°10' – 49°00' N) and San Francisco Bay, California (37°30' - 38°10' N). Collections were made from three sites in Port Valdez in early spring of 1997, 46 sites throughout Prince William Sound in June 1998, and from 23 sites in Prince William Sound, Seward and Homer in 1999 (Ruiz and Hines 1997; 2000) (Figure 1). Twenty-six sites were surveyed in Puget Sound, Washington in September 1998 (Cohen *et al.* 1998; Mills *et al.* 2000). San Francisco Bay was surveyed in early fall, late spring or summer of 1993, 1994, 1996 and 1997 at 25 regular sites plus several irregular sites (Cohen 1998; Cohen and Carlton 1995, 1997, 1998). The three regions are suitable for comparison because they have all received and have been interconnected by significant aquaculture and shipping activities that are vectors of NIS dispersal in at least the last century and are of similar areas and environmental diversities.

Each site was sampled by hand scrapings, cores, or dredge as necessary to remove biological communities or substratums from accessible floats, intertidal pilings, rocks and mudflats. These samples were washed on an 0.5 mm mesh sieve directly or decanted onto the sieve and washed following vigorous sloshing in buckets of seawater, to suspend organisms from the removed substratum. Harbor float, rock and piling substratums were sampled most intensely in all three survey areas but other habitats were sampled extensively as available. Organisms were picked directly from substratums during sample collection, from the sieves on which the substratums were

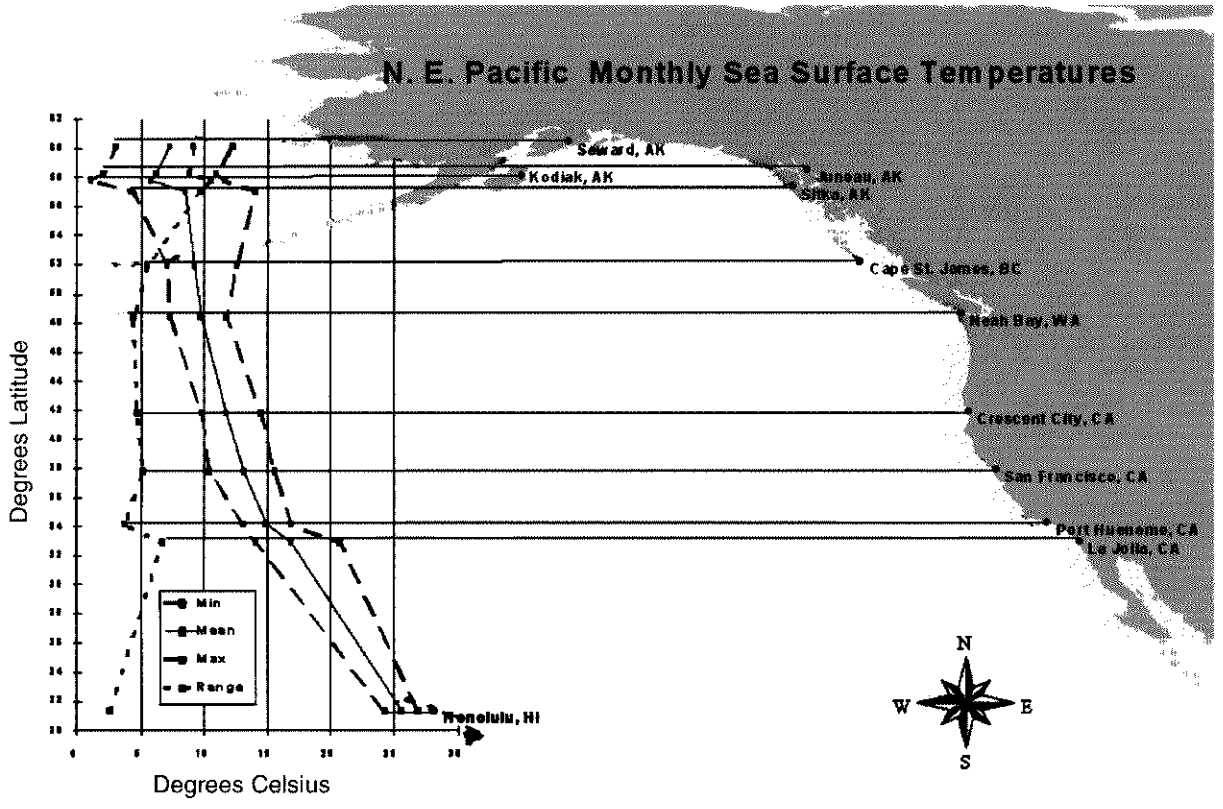

Figure 2. Sea surface temperature monthly average minimum, mean, maximum and range of northeast Pacific coastal waters estimated over an approximately 100 years up to 1986 (Cayan 1991).

washed or from voucher samples of substratums examined under a stereomicroscope. All collected organisms were fixed in 10% formalin and then transferred to 70% ETOH for long-term preservation. All specimens were identified to lowest possible taxonomic category.

Voucher specimens will be deposited in the Los Angeles County Museum, the California Academy of Sciences and the Smithsonian National Museum of Natural History. The precise locality records and notes for each collection site are available from the author. Temperature and salinity was measured at each collection site. Surface salinties ranged between 0 and 33‰ in all three survey areas. Surface water temperatures ranged between 8 and 20°C in Prince William Sound, between 10 and 21°C in Puget Sound and between 12 and 30°C in San Francisco Bay. San Francisco Bay is a well-mixed estuary. Low surface salinities and vertical stratification in temperature were apparent in both sounds during the surveys.

All species from the three surveys were collected and examined directly by the author and thus are likely to provide a more standardized sample of diversity. The origins of species are inferred from previously published records or herein using the criteria for nonindigenous species provided by Carlton (1979) and Chapman and Carlton (1991, 1994). The criteria used for cyptogenic species (species that are not clearly native or introduced) are adopted from Carlton (1996a). Only populations of species that have been moved by human activities to new locations, that are reproductive there, and that satisfy the criteria for nonindigenous species (Carlton 1979a; Chapman and Carlton 1991, 1994) are considered to be introduced.

East-west NIS diversity

East to west variation of NIS diversity in the northern hemisphere is assessed from common peracaridan introductions that are documented either in the literature or by personal observations. Poorly documented introductions, introductions not examined directly, cryptogenic species or species introduced from outside of the North Atlantic or North Pacific, are not included in the east to west survey. Examples

Table 1. The 106 peracaridan crustaceans identified as nonindigenous, cryptogenic or native, and the records per species collected from San Francisco Bay, California, (**bold**), Puget Sound, Washington (**underlined and bold** or underlined and light) or south central Alaska and Prince William Sound (*light only*). *Gnorimosphaeroma lutea* was collected from San Francisco Bay and Prince William Sound only. No species were collected only in Puget Sound.

Nonindigenous	Records	Cryptogenic	Records	Native	Records
Mysidacea		Tanaidacea		Mysidacea	
Acanthomysis aspersa	1	**Leptochelia dubia**	3	**Mysis littoralis**	1
Tanaidacea		Cumacea		Isopoda	
Tanais stanfordi	2	**Cumella vulgaris**	3	Dynamenella glabra	2
Isopoda		Gammaridea		**Gnorimosphaeroma lutea**	2
Asellus sp.	1	**Ampithoe lacertosa**	3	**Gnorimosphaeroma oregonensis**	3
Dynoides dentisinus	1	**Dulichia sp.**	1	Ianiropsis kincaidi	2
Eurylana arcuata	1	**Hyale plumulosa**	3	**Idotea montereyensis**	3
Ianiropsis serricatus	1	**Ischyrocerus sp.**	2	Idotea obscura	1
Limnoira quadripunctata	1	**Jassa staudei**	3	**Idotea resecata**	2
Limnoria tripunctata	2	Monocorophium carlottensis	2	**Idotea wosenskii**	3
Munna upbiquita	1	**Pontogeneia rostrata**	3	Ligia pallasi	1
Paranthura sp.	1	Caprellidea		Limnoria lignorum	2
Sphaeroma quoyanum	1	Caprella depranochir	2	Cumacea	
Synidotea laevidorsalis	1	**Caprella laeviuscula**	3	Diastylis alaskensis	1
Cumacea		**Caprella penantus**	1	Diastylis sp.	1
Nippoleucon hinumensis	2	**Caprella verrucosa**	2	Lamprops beringi	1
Gammaridea		**Tritella sp.**	1	Lamprops quadriplicata	2
Ampelisca abdita	1			Gammaridea	
Ampithoe valida	2			**Allorchestes angusta**	3
Crangonyx sp.	1			Americorophium brevis	2
Eochelidium sp.	2			Americorophium salmonis	2
Gammarus daiberi	1			**Americorophium spinicorne**	2
Grandidierella japonica	2			Ampithoe dalli	2
Hyallela azteca	1			Ampithoe kussakini	1
Jassa marmorata	2			Ampithoe sectimanus	1
Laticorophium baconi	2			Ampithoe simularis	2
Leucothoe alata	1			**Anisogammarus pugettensis**	3
Melita nitida	2			**Aoroides columbiae**	3
Melita sp.	1			Aoroides intermedius	2
Monocorophium acherusicum	2			Calliopius carinatus	2
Monocorophium insidiosum	2			Calliopius pacificus	2
Monocorophium oaklandense	1			**Eogammarus confervicolus**	3
Monocorophium uenoi	1			Eogammarus oclairi	1
Orchestia enigmatica	1			Gammaridae n. gen. n. sp.	1
Paradexamine sp.	1			**Gnathopleustes pugettensis**	2
Parapleustes derzhavini	1			**Hyale frequens**	3
Senothoe valida	2			Lagunogammarus setosus	2
Sinocorophium heteroceratum	1			Locustogammarus locustoides	1
Caprellidea				Megamoera subtener	2
Caprella acanthogaster	2			**Microdeutopus schmitti**	1
Caprella bidentata	1			Najna n. sp.	1
Caprella californica	2			Paracalliopiella pratti	2
Caprella equilibra	2			**Parallorchestes ochotensis**	3
				Paramoera bousfieldi	2
				Paramoera mohri	1
				Parampithoe humeralis	2
				Parampithoe mea	1
				Photis brevis	2
				Pontogeneia inermis	3
				Pontogeneia ivanovi	1
				Pontoporeia femorata	2
				Spinulogammarus succarinatus	1
				Trasorchestia traskiana	3
				Caprellidea	
				Caprella alaskena	1
				Caprella gracilior	1
				Caprella irregularis	2
				Metacaprella kennerlyi	2
Total Species	38		14		54
Records/Species	1.4		2.1		1.8

of excluded species include the amphipod *Chelicorophium curvispinum* (Sars, 1895), which spread from the Black and Caspian Sea to northern Europe (Eno *et al.* 1997), the introduced mysid *Acanthomysis bowmani* Modlin and Orsi, 1997 in San Francisco Bay that has unknown origins, and many northern NIS that are native to the southern hemisphere.

Climate

Long-term climate conditions in the northeast Pacific, including San Francisco Bay, Puget Sound and Prince William Sound are inferred from monthly average climate time series data for the Pacific Ocean and western Americas (Cayan *et al.* 1991). These data extend over approximately 100 years up to 1986. Global records of sea surface temperature and precipitation minus evaporation (http://www.cdc.noaa.gov/ 1998) are used for comparisons of temperature among ocean regions. The term "western ocean" is used in reference to the Pacific Ocean bordering the east Asian coast and the Atlantic Ocean bordering the eastern North American coast. The term "eastern ocean" refers to the ocean areas bordering the west coasts of Europe and North Africa and the west coast of North America.

Results

North-South NIS Diversity and Climate

The maximum, minimum, mean and range of monthly sea surface temperatures of the eastern Pacific vary by 5°C or less between 28° and 52° N latitude (Figure 2). The 11 to 14°C maximum average monthly temperatures of Seward, Kodiak and Sitka, Alaska (between 58 and 60° N) overlap the Neah Bay, Washington maximum surface temperatures at 48° N and are similar to the 13°C average sea surface temperatures adjacent to San Francisco at 38° N (Figure 2). Nonindigenous species likely to survive in south central Alaska could include those that reproduce within Puget Sound in summer temperatures or in San Francisco at average temperatures (Figure 2).

Of the 106 peracaridan crustacean species identified from the surveys of Prince William Sound, Puget Sound and San Francisco Bay, 54 are native, 14 are cryptogenic and 38 are introduced (Table 1). Eight prominent species in benthic or fouling communities recovered in the survey are cryptogenic (Table 1). The tanaidacean *Leptochelia dubia* (Kroyer, 1842) is cosmopolitan (Miller 1975; Ishimaru 1985). The cumacean *Cumella vulgaris* Hart, 1930 occurs in Asia (Lomakina

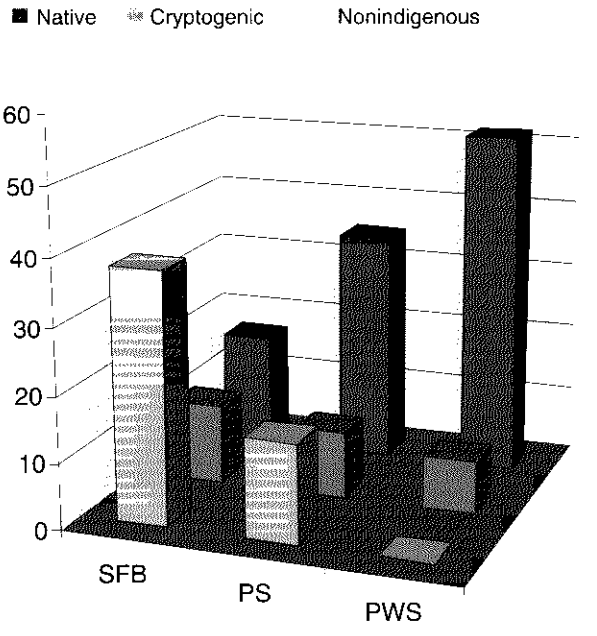

Figure 3. Native, cryptogenic, nonindigenous and total species in samples from San Francisco Bay, California, Puget Sound, Washington and Price William Sound, Alaska (SFB, PS and PWS, respectively).

1958) as well as the many habitats of the eastern Pacific. The amphipod *Monocorophium carlottensis* Bousfield and Hoover, 1997 is not clearly distinguished from the nonindigenous species *Monocorophium acherusicum* and *Monocorophium insidiosum* (Ruiz and Hines 1997). The amphipod *Hyale plumulosa* (Stimpson, 1857) is reported also from the western Atlantic (Bousfield 1973). The amphipod *Jassa staudei* Conlan 1990 is extremely similar to the cosmopolitan *Jassa marmorata* Holmes, 1903. The amphipod *Pontogeneia rostrata* Gurjanova, 1938 is reported from the eastern and western Pacific (Gurjanova 1938, 1951; Barnard 1962, 1964). The caprellid *Caprella depranochir* Mayer, 1880 is reported from the eastern and western Pacific (Arimoto 1976, Kozloff and Price 1997).

The diversity of species collected is nearly the same between San Francisco, Puget Sound and Prince William Sound (66 to 60 and 59, respectively). Of the 54 native species collected in Alaska, 52 (96%) were collected also in San Francisco Bay or Puget Sound (Table 1). The common pool of native species and the similar species diversities collected among the three areas both indicate that the habitat selection, collection and sample processing methods of

the rapid assessment surveys were consistent between the three areas. Little variation in NIS diversity among these three sample sets therefore results from sample biases.

From San Francisco Bay north to Puget Sound and Prince William Sound, 38, 15 and 0, NIS were found respectively (Figure 3, $X^2 > 27.01$; $P < 0.0001$; $df = 2$) while the frequencies of cryptogenic species are nearly the same at 11, 10 and 7, respectively ($X^2 = 2.0$; $P > 0.73$; $df = 2$). In contrast, the frequencies of native species increase to the north from 17 to 35 and 52, respectively (Figure 3, $X^2 > 27.01$; $P < 0.0001$; $df = 2$). All NIS and all but two of the cryptogenic species at any site also occur in San Francisco Bay. In contrast, only 17 of the 54 native species were recovered from San Francisco Bay (Table 1, Figure 3). Most of the successful NIS introductions to northeast Pacific estuaries are thus adapted to lower latitude climates than are the native species. The massive numbers and broad diversities of NIS in ballast water arriving in Alaska from California, Oregon and Washington (Hines and Ruiz 1998; 2000) are predominantly warm water species, which are inappropriate indicators of the risk of introducing cold water NIS to Alaska.

East-West NIS Diversity and Climate

NIS diversity varies similarly with climate throughout the northern hemisphere. Peracaridan NIS diversity varies among the four major ocean coasts of the northern hemisphere ($X^2 = 17.27$, $P = 0.001$ $df = 3$) with five times as many introductions occurring on eastern ocean coasts as on western ocean coasts (Table 2; $X^2 = 16.05$, $P = 0.001$ $df = 1$). Except for the gammaridean amphipods *Orchestia gammarella* (Pallas, 1766) and *Corophium volutator* (Pallas, 1766) in the marshes and tidal mudflats of the Bay of Fundy, nonindigenous peracaridan species diversities and population abundances in western Atlantic estuaries are low. Similarly, of the 28 introduced peracaridan species examined (Table 2) only 2–3 are introduced to the northwest Pacific, compared to 20–23 in the northeastern Pacific and 10–12 in the northeast Atlantic (Table 2). None of the common northeast Pacific peracaridans are introduced to other areas of the world. Only 2 of the northeast Atlantic species are clearly introduced to other regions compared to 13 from the northwest Pacific and 14 from the northwest Atlantic (Table 2). Remarkably, 5–9 of these 28 NIS (Table 2) have been

Table 2. The east and west destinations and sources of common introduced peracaridan crustaceans of the Northwest Pacific, Northeast Pacific, Northwest Atlantic and Northeast Atlantic (NWP, NEP, NWA and NEA, respectively), their native, introduced, or probable introduced status (N, I, and I?, respectively) and the numbered reference sources.

	NWP	NEP	NWA	NEA	Sources
Mysidacea					
Acanthomysis aspera	N	I			1,2
Cumacea					
Nippoleucon hinumensis	N	I			1,3
Isopoda					
Asellus communis		I?	N	I	4
Caecidotea racovitzai		I	N		7
Dynoides dentisinus	N	I			1
Ianiropsis serricatus	N	I			1,8
Paranthura sp.	N	I			1
Synidotea laevidorsalis	N	I	I	I	1,5
Amphipoda					
Ampelisca abdita		I	N		1,6,10
Ampithoe valida		I	N		1,9,10
Apocorophium lacustre			N	I	13,14
Caprella acanthogaster	N	I	I		1,10,11
Corophium sp.	N			I?	15,16
Corophium volutator			I	N	13,14,17
Crangonyx floridanus		I	N		7
Crangonyx pseudogracilis			N	I	18
Gammarus daiberi		I	N		1,7
Gammarus tigrinus			N	I	13,14,18,19
Grandidierella japonica	N	I	I		10,20,21
Jassa marmorata	I	I	N	I	10,22,23
Leucothoe alata	N	I			1,24
Melita nitida	I?	I	N		1,6,10,25
Microdeutopus gryllotalpa		I	N	I	13,26
Moncorophium uenoi	N	I			9,10
Monocorophium acherusicum	I	I	N	I	1,9,10
Monocorophium insidiosum	I	I	N	I	1,9,10
Orchestia gammarella			I?	N	13,14,19
Parapleustes derzhavini	N	I			1,6,10
Sinocorophium heteroceratum	N	I			1,12
Total Natives (Exports)	13	0	14	2	
Total NIS (Imports)	1-3	21-23	2-3	10-12	

N = native; I = introduced; I? = incompletely resolved but probable introduction (1) Cohen and Carlton 1995; (2) Modlin and Orsi 1997; (3) Watling 1991; (4) Williams 1972; (5) Mees and Fockedey 1993, Chapman and Carlton 1991, 1994; (6) Chapman 1988; (7) Toft *et al.* 1999; (8) Kussakin 1988; (9) Barnard 1975; (10) Carlton 1979a; (11) Platvoet *et al.* 1995; (12) Chapman and Cole, in prep.; (13) Bousfield 1973; (14) Lincoln 1979; (15) Hirayama 1984, 1986; (16) Janta 1995; (17) Chapman and Smith, in prep.; (18) Costello 1993; (19) Watling 1979; (20) Chapman and Dorman 1975; (21) Smith *et al.* 1999b; (22) Conlan 1990; (23) Mills *et al.* 2000; (24) Nagata 1965a-d; (25) Hirayama 1986; (26) Chapman and Miller, in prep.

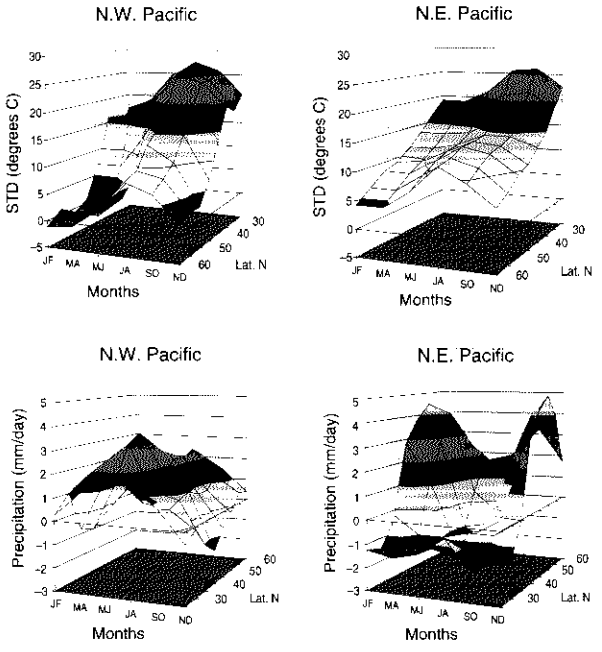

Figure 4. Northwest and northeast Pacific sea surface temperature in degrees Celsius and bimonthly precipitation minus evaporation in mm/day at ten degree latitude intervals for the bimonthly periods of Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec. (Note: axes of latitude are reversed between graphs of temperature and precipitation.)

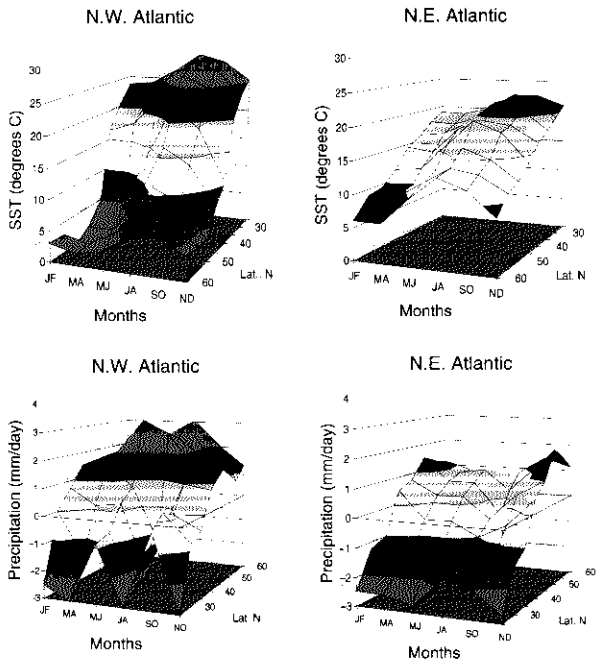

Figure 5. Northwest and northeast Atlantic sea surface temperature in degrees Celsius and bimonthly precipitation minus evaporation in mm/day at ten degree latitude intervals for the bimonthly periods of Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct and Nov-Dec. (Note: axes of latitude are reversed between graphs of temperature and precipitation.)

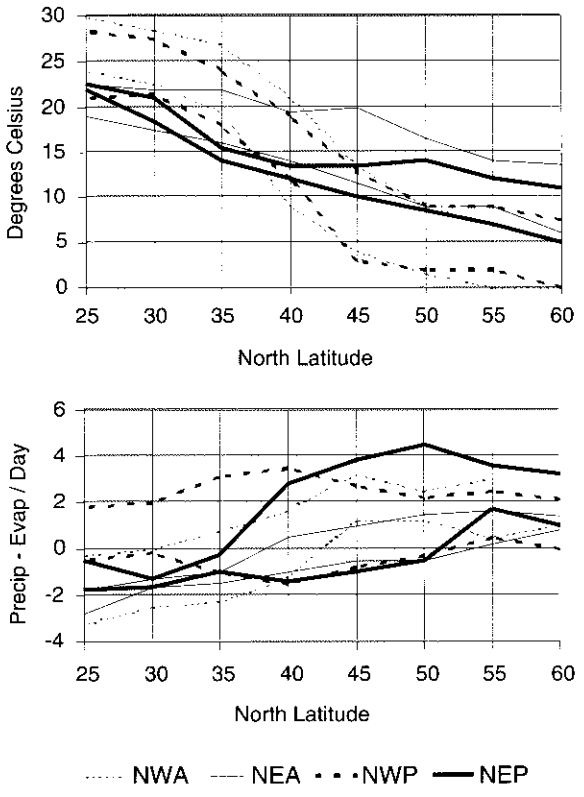

Figure 6. Maximum and minimum monthly sea surface temperature in degrees Celsius (A) and monthly precipitation minus evaporation in mm d-1 (B) at ten degree latitude intervals for the northwest (dashed lines) and northeast (solid lines) Atlantic (thin lines) and Pacific (thick lines). Northeast Pacific, northwest Pacific, northeast Atlantic and northwest Atlantic are NEP, NWP, NEA and NWA, respectively.

introduced to two coasts and 4 have been introduced to 3 coasts.

Sea surface temperatures of eastern ocean coasts are more constant than on western ocean coasts between 25° and 50° N latitude (Figures 4 and 5). Eastern ocean populations between 32° and 50° N are exposed to temperature ranges that span only latitudes 40° to 42° N in western oceans (Figure 6). Selection for broad temperature tolerance ranges is therefore less for eastern ocean species than for western ocean species. Eastern oceans below 50° N are thus broad thermal targets for western ocean species while western ocean coasts are narrow thermal targets for eastern ocean species.

Precipitation and salinity also vary from east to west in patterns consistent with the south to north pattern between San Francisco to Alaska. The broadest ranges of precipitation occur in the eastern Pacific north of 35° N. (Figure 4). The narrowest ranges of

precipitation and negative net precipitation (desert conditions) occur in the eastern Pacific and Atlantic south of 35° N. (Figure 4). Desert conditions do not occur at low latitudes in the northwest Pacific and occur in the northwest Atlantic only below 30° N (Figure 4). The latitudinal range and area of low salinity estuaries is therefore less in eastern oceans than in western oceans.

The seasonal patterns of precipitation (Figures 4 and 5) also differ consistently between eastern and western oceans. More precipitation occurs in western oceans during summer when temperatures are greatest while most precipitation occurs in eastern oceans in winter when temperatures are low (Figures 4 and 5). Where snow-melt is not important, and in the absence of major water impoundments, the salinity-temperature patterns of eastern and western ocean estuaries are out of synchrony. In high latitude regions, such as Alaska, runoff varies most with snow-melt. Salinity in Alaskan estuaries is lower in warm seasons in correspondence with western ocean climates.

The ranks of climates from least to most similar based on overall temperature variation and seasonal precipitation (Figures 4, 5 and 6) are, northeast Pacific, northeast Atlantic, northwest Pacific and northwest Atlantic (NEP, NEA, NWP and NWA, respectively). The ranks of NIS invaders of these regions (Imports, Table 2) are from highest to lowest: NEP, NEA, NWP and NWA. The ranks of native species that have been introduced to other regions (Exports, Table 2) are from lowest to highest: NEP, NEA, and NWP and NWA. The east and west variations in NIS imports and exports are thus correlated with climate variation (Kendall coefficient of concordance ($W = 1.0$; $X^2 = 8.2$; $P < 0.02$; $df = 2$) (Siegal 1956) in a similar pattern to the south to north pattern of NIS between San Francisco Bay and Prince William Sound.

Discussion

Climate effects and processes controlled by climate superimpose over all other processes controlling the distributions of NIS if they are the dominant factors controlling NIS diversity and geography. The climate hypothesis assumes that adaptations of NIS must equal or exceed the stresses in the invaded areas to become established; that invasions from all areas of the world superimpose upon and are superimposed on the distributions of NIS from similar climates and; that climate creates NIS distributions largely independent of particular life history or taxonomic origins or dispersal pathways of the invaders. Major predictions are possible if these assumptions are true, including: (1) NIS from only a narrow range of western ocean latitudes and climate conditions readily become established in eastern ocean climates; (2) eastern ocean invaders, being poorly adapted to broad ranges of climate variation, readily invade only a narrow range of western ocean latitudes and climates; (3) NIS diversities decline north and south of 42° N due to increasing incompatibilities of mid latitude climates across the Atlantic and Pacific Oceans between 25° and 60° N; (4) the large seasonal weather variations of latitudes above 50° N favor western ocean NIS over eastern ocean NIS. The south to north and west to east declines in NIS diversity observed here largely corroborate all four specific predictions of the climate hypothesis.

These geographical patterns of diversity do not appear to be biased or obscured by insufficient taxonomic resolution, sampling biases or uneven human mechanisms of dispersal. Likely sources of overlooked nonindigenous peracaridans are among the cryptogenic species discovered in the eastern Pacific surveys (Table 1). Cryptogenic species can prove to be nonindigenous after further investigation (Carlton 1996a). However, 7 of the 8 cryptogenic peracaridans of Prince William Sound (Figure 3) occur also in Puget Sound and San Francisco Bay (Table 1). Disproportionate numbers of NIS are therefore unlikely to be discovered where NIS diversity presently appears to be low. The great overlap of native and nonindigenous species discovered among the three rapid assessment areas indicates that the taxonomy and sampling procedures are sufficiently consistent and comprehensive.

The declining west to east NIS diversity (Table 2) is not a likely sampling artifact or an artifact unique to peracaridan crustaceans. West to east declines in NIS diversity are noted among other taxa (Cohen and Carlton 1995; Eno *et al.* 1997; Leppäkoski and Olenin 2000). These distributions are probably stable since diverse estuarine taxa have been introduced extensively over the last 500 years (Carlton 1992, 1999; Carlton and Hodder 1995). The south to north decline in NIS diversity in the northeast Pacific must also be an old and stable pattern, not exclusive to peracaridans and atypical of native species. A majority of native southeast Alaskan intertidal organisms

(O'Claire and O'Claire 1998) occur also in Washington (Kozloff and Price 1997) and central California (Smith and Carlton 1975). The NIS introductions in the northeast Pacific arrived with dispersal mechanisms that have operated for hundreds of years (Carlton 1979a; Cohen and Carlton 1995; Carlton and Hodder 1995; Cohen *et al.* 1998; Hines and Ruiz 2000; Mills *et al.* 2000).

The lack of cold-water peracaridan NIS in Alaska also does not result from unequal passive dispersal mechanisms. Dispersal of species associated with ship fouling and solid ballast has been active between Alaska and Asia since Alaska was a territory of Russia. Port Valdez is the third largest ballast water port in the U.S. and has already received diverse, abundant populations of Asian species in ballast water shipments (Hines and Ruiz and 2000). Moreover, aquaculture activities (Foster 1991; Carlton 1979a, b) have been an important vector for potential introductions to Alaska for many decades.

The absence of unique, cold water NIS in Puget Sound and all other northeast Pacific estuaries north of San Francisco Bay is more likely due to incompatible receiving areas than to insufficient vectors from donor regions. Unique, cold water NIS are lacking also in Puget Sound. Yet, NIS dispersal vectors to Puget Sound are even more active than NIS dispersal mechanism to Alaska. Mechanisms to transport all taxa of NIS into Puget Sound and the Georgia Strait from nearly all latitudes by international ballast water traffic (Levings *et al.* 1998), aquarium, live seafood, aquaculture or ship fouling (Elston 1997; Smith 1998; Levins *et al.* 1998) have been operating for at least 200 years (Mills 2000). Although similar broad sources of transport mechanisms move NIS to San Francisco Bay (Carlton 1979b), all peracaridan NIS of Puget Sound occur also in San Francisco Bay (Table 1). The warm water origins of northeast Pacific NIS are thus unlikely results of unequal dispersal vectors.

The west to east declines of NIS diversity (Table 2) are consistent with climate predictions 1 and 2. Sea surface temperature maximums and averages in eastern oceans above 42° N exceed temperature maximums and averages of western oceans at the same latitudes by 5° to 7°C (Figure 6). Higher summer temperatures in high latitude eastern ocean estuaries may prevent invasions of western ocean species from similar latitudes (consistent with prediction 3). Peracaridan NIS cannot be used to test prediction 4 (western ocean NIS favored by the more variable high latitude climates) since they were not found in Alaska. However, most NIS of San Francisco Bay and Puget Sound occur in shallow, warm, brackish environments. Invasions of eastern oceans by cold-water western ocean species therefore appear to be limited in the northeast Pacific above 42° N and prevented above 52° N by high summer temperatures (Figure 6).

The seasonal salinity variations of all estuaries north of 50° N may coincide despite great differences in precipitation patterns between eastern and western ocean regions (Figures 4 and 5). Precipitation at these latitudes occurs predominantly as winter snow in eastern oceans and does not enter estuaries until spring snowmelt. In contrast, freshwater enters western ocean estuaries directly from runoff during spring and summer rains. The seasonal salinity differences between eastern and western ocean estuaries are therefore greatest below 50° N. The great diversity of NIS in San Francisco Bay compared to other eastern Pacific estuaries could result from nearly complete water impoundments and diversions that create low summer salinity conditions atypical of eastern oceans and more closely resembling eastern ocean estuaries.

Above 38° N, adaptations of native eastern ocean species to high summer salinities and narrow temperature ranges must prevent their establishment in western ocean estuaries. The entire sea surface temperature range of the eastern ocean coasts between 25° and 60° N is overlapped by the temperature ranges of western ocean coasts between 37° and 42° N (Figure 6). Thus, the regions of western oceans with suitable temperature ranges for all eastern ocean species are small while the regions of eastern ocean estuaries with suitable temperature ranges for some western ocean species are large.

Temperature limitation of western ocean NIS in the eastern Pacific is evident beyond biogeographical correlations. The Pacific oyster *Crassostrea gigas* (Thunberg, 1795) is native to Asia and cultured commercially as far north as Hokkaido, Japan (Quayle 1969) at 44° N. The minimum monthly sea surface temperatures of Prince William Sound match western Pacific minimum sea surface temperatures as far south as 44° N (Figure 6). From figure 6, lethal low temperatures for *C. gigas* or other western ocean NIS from 44° N, or even slightly farther south, would not be predicted in Prince William Sound and south central Alaska. However, maximum northeast Pacific sea surface temperatures at 60° N match western Atlantic

and western Pacific maximums only as far south as 48° N (Figure 6). *Crassostrea* grows from spat but require temperatures greater than 15° C to spawn (Barrett 1963). The climate model thus predicts that *C. gigas* can survive in Alaska but cannot reproduce there. In correspondence with these predictions, even the 11-14° C summer temperatures in the most protected bays and estuaries of Prince William Sound and south central Alaska (Hines and Ruiz 2000) are too low for *C. gigas* to reproduce (Foster 1991).

Conclusions

The mosaic of peracaridan introductions in the northern hemisphere indicates that NIS establish only in climates within their evolved physiological tolerances. This relationship extends beyond physiology, peracaridan crustaceans and beyond the northern hemisphere (Carlton 1987; Ruiz *et al.* 1997b; Edgar *et al.* 1999; Hewitt *et al.* 1999). The overall correlation between NIS diversity and climate allows predictions of invasion risks among regions of the world. For instance, baring Arctic Ocean passages of ballast water from the north Atlantic, global climate changes, or NIS transport from high latitudes of the southern hemisphere, there are no diverse sources of species that are adapted to climates similar to those of Alaska. The present mechanisms for introducing species to Alaska are thus unlikely to cause large diversities of NIS to become established. West to east, the risks of introducing high diversities of NIS from the western ocean climates to mild eastern ocean climates are high while the risk of such introductions in the reverse direction are low. The relative importance of different biodiversities within climate types or east and west ocean coasts and the different target areas or habitats into which species can be introduced in creating this pattern are unknown.

The exceptional NIS introductions should be examined to determine whether variation in NIS diversity with climate is a sufficient metric for ecosystem risk assessments. Among these exceptions are the amphipods *Corophium volutator* (Pallas, 1766) and *Orchestia gammarellus* (Pallas, 1766) reported here (Table 2). The introductions of these species from Europe to eastern North America are exceptions to the western ocean to eastern ocean pattern of introductions. *Corophium volutator* is confined in North America to the Bay of Fundy while *Orchestia gammarellus* is confined in North America to the Bay of Fundy and the outer coasts north to Newfoundland (Bousfield 1973; Watling 1979). The climates of these areas are isolated from the Gulf Current and have narrower temperature ranges than areas either to the north or south (Bousfield 1973). The particular successes of these two peracaridans and other European species such as the green crab *Carcinus maenas* (Linnaeus, 1758) and the European littorine snail *Littorina littorea* (Linnaeus, 1758) may result from the closer match of the climate of this region with northern European climates. The cumulative impacts of these few high density NIS are large in the Gulf of Maine and adjacent areas relative to the low overall diversity of NIS.

The relations between NIS diversity and NIS impacts are not resolved in estuarine ecosystems. Impacts per NIS, for example, may be particularly high in Alaska. The seven cryptogenic peracaridans found in Alaska are abundant over broad ecological conditions within south central Alaska (Chapman 2000). These species, if introduced, indicate a low diversity of NIS can increase to ecologically significant densities in Alaskan waters. The high potential for single species to produce massive impacts (*e.g.*, Barber 1997; Nichols *et al.* 1990) may reduce the value of NIS diversity surveys as metrics for ecological risk assessments.

The soft shell clam, *Mya arenaria* Linnaeus, 1758, an introduction from the east coast of the United States to the eastern Pacific, may be such an exceptional species in Alaska. Hines and Ruiz (2000) report 24 NIS in marine and estuarine ecosystems of Alaska (Chapter 8). Twenty-three of these species are poorly resolved, species complexes preventing definitive identification, cryptogenic (Carlton 1996a) or are not marine or estuarine. *Mya arenaria* is the only marine or estuarine member of the 24 NIS that satisfies a significant number of the criteria for introduced species (Carlton 1979a, Chapman and Carlton 1991, 1994; Eno *et al.* 1997). *Mya arenaria* is extremely widespread in south central Alaska and occurs in great abundance in some areas (Feder and Paul 1974; Foster 1991; Hines and Ruiz 2000) and is likely to be ecologically important. The occurrence of *M. arenaria* in Alaska is consistent with prediction 4 (western ocean NIS favored by the more variable high latitude climates).

If *M. arenaria*, the most clearly documented introduction, proves to be native to Prince William Sound, the cryptogenic species in the Sound are less likely to be introduced. In this case, the ecological

and economic risks of introducing low diversities of estuarine species to Alaska via ballast water or by other mechanisms would be small. If *M. arenaria* is introduced or any of the present cryptogenic species prove to be introduced to south central Alaska however, there is little evidence that any of the other cryptogenic species in Alaska are not also introduced. The rapid NIS assessment surveys, focused on NIS diversity rather than particular species, data from these surveys are, in this latter case, inappropriate for assessing ecological risk. These risks could be large.

The most parsimonious hypothesis is that *M arenaria* is nonindigenous in south central Alaska. Assumptions of risk for NIS in Alaskan waters therefore should be that introductions of even a few species into the region would pose large and unacceptable ecological and economic risks. However, "Perhaps more confusion has surrounded the history of this common soft-shell clam on the Pacific coast than any other introduced species" (Carlton 1979a, p. 526). Native populations of *M. arenaria* occur in the Bering Sea (Carlton 1979a, p. 529). The nonindigenous status of *M. arenaria* is of critical concern for the risk analysis. Its origins in Alaska and should be more clearly resolved.

Global relations between NIS diversity and climate may indicate where to concentrate limited resources to provide the greatest control of NIS impacts. Managers can possibly use climate models to predict which NIS vectors transport the most NIS invasions from the western sides of oceans. The climate model may also reveal management options to limit NIS introductions in eastern ocean estuaries. Water diversions could create or eliminate western ocean salinity patterns in eastern ocean estuaries to prevent or control introductions of western ocean species. The climate model may allow predictions of changing NIS distributions with global warming. The value of NIS risk assessments can be increased with greater resolution of the overall global patterns of NIS diversity and abundances in all regions. These data are critical in the search for, the relationships between NIS diversity and impacts, the most threatened ecosystems and the major sources, rates of introduction and vectors of NIS. These general patterns of invasion occur also in the southern hemisphere and in all taxa if the climate model is valid.

Effects of climate on introductions of nonindigenous estuarine species are clearly indicated by these results. Implicit predictions of this crude model are that the same NIS discovered at higher latitudes of eastern oceans occur also between 28° and 37° N due to eastern ocean temperatures that are low relative to western ocean temperatures at these latitudes (Figure 6). Western ocean NIS invading eastern Pacific bays and estuaries of Mexico are predicted to spread north or to arrive by secondary dispersal from the north. Similarly, NIS invading Canada and Washington bays and estuaries are predicted to spread south or colonize from the south if the climate model is valid.

The international cooperation is critically needed to limit further introductions and deal with their proliferation. These data indicate that similar nonindigenous estuarine species threaten to invade eastern Pacific estuaries and coastal marine waters of Mexico, the United States and Canada. The United States provides the largest target and thus has the greatest potential to benefit and the greatest responsibility for action. More extensive surveys, including more coasts, more taxa and greater ranges of latitudes than are covered here are needed to develop, test and refine a better model of climate effects on NIS.

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Assessing the Risk of Nonindigenous Species Invasion in a High-Latitude Ecosystem: Ballast Water Treatment Facility in Port Valdez, Alaska

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ABSTRACT: **Ballast-mediated invasions of nonindigenous species in coastal ecosystems remain a global issue receiving widespread attention. Presently, mid-ocean exchange of ballast water is the only feasible management practice available to limit transfer of coastal organisms in ballast water, and evaluation of alternative treatment methods is urgently needed. As part of a three-year study on the risks of invasion in a high-latitude ecosystem, we measured the effect of a Ballast Water Treatment Facility on biological organisms associated with nonsegregated (oily) ballast water of oil tankers arriving at Port Valdez, Alaska. Nonsegregated ballast water comprised nearly 50% (approximately 20 million mt) of the water released each year into Port Valdez, the third largest volume for a U. S. port. Samples of nonsegregated ballast water were collected from four successive stages of treatment, and analyzed for occurrence, density, and viability of planktonic organisms. Of the 23 morphologically distinct taxa present in the samples, discoid diatoms and nematodes dominated in percent occurrence and density. Densities of all taxa, with the exception of nematodes, decreased from initial to final stages of treatment. Compared to segregated ballast water, the abundance, diversity, and viability of planktonic organisms in arriving ballast water was very low, even before treatment, and remained relatively low throughout all treatment stages. These findings suggest that conditions in the nonsegregated ballast water and/or the Ballast Water Treatment Facility were not favorable for high survivorship of most biological organisms. Thus, nonsegregated ballast water did not appear to be an important source of potential species introduction. However, although it readily handles large volumes of ballast water, the treatment facility was designed to remove petroleum products and does not appear to be a good system for removing organisms from ships' ballast water.**

Key words: ballast water, ship, plankton, nonindigenous species, Alaska

INTRODUCTION

Ballast-mediated invasions of coastal ecosystems by nonindigenous species remain a global problem receiving widespread attention (Carlton 1989; Ruiz *et al.* 1998). Despite the recent increase of information on biological invasions by nonindigenous species (NIS) in temperate marine ecosystems, our understanding of the frequency, patterns, and impacts of NIS invasion in high latitude, cold waters remains unclear. Over a 3-yr period, we conducted a study to assess the risk of NIS invasion in Prince William Sound, Alaska (Ruiz and Hines 1997; Hines *et al.* 1998; Hines *et al.* 2000). Prince William Sound, located on the south-central coast of Alaska, encompasses several glacially fed embayments and fjords, including Port Valdez. Port Valdez serves as the terminus of the 800-mi Trans-Alaska Pipeline and experiences high volumes of commercial ship traffic, primarily oil tankers, which load crude oil from the pipeline.

Tankers arriving at the Valdez Marine Terminal transport ballast water originating primarily from domestic ports of the west coast of North America. In addition, oil companies recently gained permission from the U.S. Congress to conduct foreign sales of Alaska crude oil, thus opening up trade routes between several Asian ports and Port Valdez. These tankers transport two types of ballast water: (1) segregated ballast water, which is carried in dedicated ballast water tanks; and (2) nonsegregated or "oily" ballast water, which is carried in cargo tanks also used for crude oil. Prince William Sound and Port Valdez receive approximately 20 million mt of segregated ballast water each year, the third largest annual volume of ballast water in the United States (Ruiz and Hines 1997; Hines and Ruiz 2000). While segregated ballast water is discharged directly into the surrounding coastal waters, nonsegregated ballast water is

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treated at a shore-side Ballast Water Treatment Facility at the Valdez Marine Terminal. The purpose of this facility is to remove residual petroleum compounds prior to release into Port Valdez.

Given that ballast water is a major vector for introductions of NIS into coastal ecosystems (Carlton and Geller 1993), management practices are urgently needed to reduce the quantities of planktonic organisms in ballast water (National Research Council 1996; Greenman *et al.* 1997). Presently, mid-ocean exchange of ballast water is the only feasible management practice available to limit transfer of coastal organisms in ballast water, and evaluation of alternative treatment methods is a high management priority (National Research Council 1996; Greenman *et al.* 1997). Our research in Prince William Sound has focused on five major objectives: (1) to provide a detailed search for, and analysis of, existing and potential NIS from field collections, museum vouchers, and literature; (2) to assess abundance and viability of organisms transported in ballast water, tank sediment, ship hulls, and sea chests; (3) to measure survivorship of plankton collected from segregated ballast water when exposed to temperatures and salinities characteristic of Port Valdez; (4) to analyze ballast water delivery patterns and management practices of arriving vessels; and (5) to calculate efficacy of ballast water exchanges. During 1997, we sampled segregated and nonsegregated ballast water from several tankers to describe the plankton communities transported into Prince William Sound and Port Valdez. Prior to this research, the effect of the Ballast Water Treatment Facility on planktonic organisms within the nonsegregated ballast water was unknown. Here, we report the characteristics of the planktonic communities associated with nonsegregated ballast water and the effects of the Ballast Water Treatment Facility on these organisms.

Methods

During the period from 23 May to 6 June 1997, samples of the discharged nonsegregated ballast water were collected from four successive stages of the Ballast Water Treatment Facility at the Valdez Marine Terminal operated by Alyeska Pipeline Service Company (Figure 1). The four stages are (1) Chicksan Arms, which link vessels to the treatment facility; (2) 90s tanks, which allow oils to separate from the ballast water through differential settling; (3) Dissolved Air Filtration (DAF) facility, which injects microbubbles of air following the addition of a polymer, causing residual petroleum compounds to adhere to the foam and volatilize; and (4) Biological Treatment (BT) tanks, which culture microbes that metabolize petroleum compounds and remove any residual oil chemicals before discharge into Port Valdez. Obtaining samples from the Chicksan Arms allowed for characterization of the plankton community in the nonsegregated ballast water of each vessel before entering the treatment process. We attempted to sample the same parcel of water as it passed through the stages of treatment, but, in several cases, water from more than one ship was mixed in the 90s tanks and subsequent stages of the treatment process. As ballast water passed through the treatment facility, it often became mixed to varying degrees with water from other ships, and samples were therefore composed of composited water. Thus, nonsegregated ballast water was sampled distinctly from 11 tankers as it was pumped into the treatment facility, while samples of water from subsequent treatment stages were derived from water comingled to varying degrees from these tankers (Table 1). Five additional ships arriving during the sampling period were boarded to determine quantities of ballast water on board, and included one ship from a nearby, local port containing no ballast water.

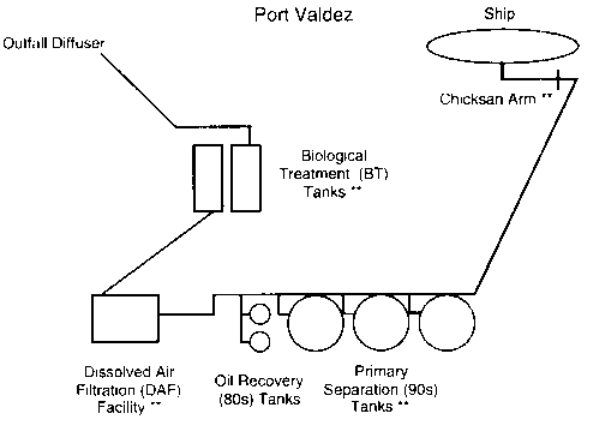


Figure 1. Diagram of the Ballast Water Treatment Facility in Port Valdez, Alaska. Asterisks (**) indicate the four locations of sample collections in the treatment process: Chicksan Arm, 90s Tanks, DAF Tanks, and BT Tanks. The 80s Tanks are not a treatment stage: they receive oil that is separated from the ballast water as it stands in the 90s Tanks.

For the first three stages, duplicate samples of 0.06 m^3 (50 gal) of water were collected from spigots specifically designed for taking water from the piping system of the treatment facility. Each sample was

Table 1. Characteristics of all ballast water arriving at Port Valdez, Alaska, for each of 16 oil tankers. Shown are the sources and volumes of segregated and nonsegregated ballast water, percent of total ballast water (BW) that is nonsegregated, and date of arrival for each vessel. Mean and standard error (S.E.) are also given for the three volume characteristics. EXCH. indicates ships that conducted some open ocean ballast water exchange.

Vessel	Date of Arrival	Segregated BW (m^3)	Segregated BW Source	Nonsegregated BW (m^3)	Nonsegregated BW Source	Nonsegregated BW (%)
ARCO SPIRIT	23-05-97	27421	Long Beach, CA	55415	Long Beach, CA	67
ARCO ANCHORAGE	25-05-97	21529	Cherry Pt., WA	29174	Cherry Pt., WA	58
SR *BATON ROUGE*	26-05-97	32234	Anacortes, WA	14415	Anacortes, WA	31
SR *LONG BEACH* (EXCH.)	26-05-97	28306	San Francisco, CA	36786	37-47N 122-34 W/ 38-01N 124-19W	57
POTOMAC TRADER	27-05-97	10528	Cook Inlet, AK	0	none	0
CHEVRON MISSISSIPPI	27-05-97	19764	Anacortes, WA	2594	Anacortes, WA	12
	28-05-97	21504	Cherry Pt., WA	28636	Cherry Pt., WA	57
OS *WASHINGTON*	28-05-97	508	Richmond, CA	43680	25 %–Richmond, CA 75%–100 mi offshore	99
SR *NORTH SLOPE*	02-06-97	48192	Portland, OR	50363	Portland, OR	51
ARCO JUNEAU	02-06-97	22579	Cherry Pt., WA	34293	Cherry Pt., WA	60
BT *ALASKA*	02-06-97	57498	San Francisco, CA	14669	San Francisco, CA	20
SR *SAN FRANCISCO*	02-06-97	30241	Anacortes, WA	11383	Anacortes, WA	27
ARCO INDEPENDENCE	03-06-97	29832	Long Beach, CA	60305	Long Beach, CA	67
OMI COLUMBIA	03-06-97	16701	Barber's Pt., HI	50168	Barber's Pt., HI	75
PRINCE WILLIAM SOUND	04-06-97	26202	45-40N 156-50E/ 40-27N 134-39E	16963	Yosu, Korea	39
SR *BENICIA* (EXCH.)	06-06-97	22706	Benecia, CA	29692	Benecia, CA	57
Mean		25984		29909		49
S.E.		3317		4693		0.06

poured through an 80-µm mesh plankton net, and rinsed to ensure that any plankton retained on the net would be collected within the cod-end jar. Duplicate samples (0.32 m^3) were also collected at the BT tanks using an 80-µm mesh plankton net with a 30-cm diameter that was pulled vertically through a 4.5-m water column. Temperature and salinity of each sample were measured with a calibrated alcohol thermometer and refractometer, respectively.

Following collection, each sample was examined using a microscope (10–40X zoom magnification) for qualitative measures of abundance and viability of plankton. For each morphologically distinct taxon, we estimated qualitative abundance as rare (present, but <10 individuals), common (10–100 individuals), and abundant (>100 individuals). The percentage of live individuals was evaluated according to morphological integrity and movement. Samples were preserved in 5% buffered formalin solution immediately after live analysis, and transported to our laboratory at the Smithsonian Environmental Research Center for further quantitative analysis.

For quantitative analysis, samples were concentrated on an 80-µm mesh sieve, transferred to a glass finger bowl, and examined under a microscope (10–40X zoom magnification). All morphologically distinct organisms were identified to the lowest possible taxonomic level. Taxa in abundances of fewer than 100 individuals per sample were counted within the whole sample. For abundant taxa (>100 individuals per sample), samples were split with a Folsom plankton splitter (1/8 to 1/32 splits), and two subsamples were counted. Counts from split samples were adjusted to standard sample size. Statistical analysis employed Kruskal-Wallis tests to determine statistically significant differences in densities of planktonic organisms among stages of the ballast water treatment process.

Results

Of the 11 ships sampled, nonsegregated ballast water accounted for an average 29,909 mt per ship (approximately 49% of the total ballast water) (Table 1). The sources of the water included predominantly

Table 2. Characteristics of nonsegregated ballast water sampled at four stages of the Ballast Water Treatment Facility in Valdez, Alaska. Shown are the date, source, temperature, and salinity of water sampled at each stage. Temperature and salinity measures represent means of two consecutive samples. Multiple sources indicate comingling of water at that stage.

Treatment	Date	Vessel Source(s)	Temperature (°C)	Salinity (ppt)
CHICKSAN ARMS	24-05-97	*ARCO SPIRIT*	12.0	32.0
	25-05-97	*ARCO ANCHORAGE*	10.8	29.0
	27-05-97	SR *LONG BEACH*	12.0	32.0
	27-05-97	SR *BATON ROUGE*	10.0	28.0
	27-05-97	*CHEVRON MISSISSIPPI*	11.0	31.0
	28-05-97	*ARCO FAIRBANKS*	11.0	29.5
	02-06-97	SR *NORTH SLOPE*	14.0	13.0
	02-06-97	SR *SAN FRANCISCO*	11.0	31.0
	02-06-97	BT *ALASKA*	11.5	31.0
	03-06-97	*ARCO INDEPENDENCE*	13.0	32.0
	03-06-97	*OMI COLUMBIA*	15.0	35.0
90s TANKS	24-05-97	*ARCO SPIRIT*	12.0	32.0
	25-05-97	*ARCO ANCHORAGE*	12.0	30.0
	27-05-97	SR *LONG BEACH*/SR *BATON ROUGE*	12.0	32.0
	28-05-97	*CHEVRON MISSISSIPPI*	14.5	30.0
	02-06-97	SR *NORTH SLOPE*/BT *ALASKA*	10.0	13.0
	03-06-97	SR *SAN FRANCISCO*/BT *ALASKA*	12.0	32.0
	03-06-97	*ARCO INDEPENDENCE*	12.0	32.0
	04-06-97	*OMI COLUMBIA*/*ARCO INDEPENDENCE*	13.0	34.0
DAF TANKS	24-05-97	*ARCO SPIRIT*	12.0	32.0
	25-05-97	*ARCO ANCHORAGE*	13.0	30.0
	28-05-97	SR *LONG BEACH*/SR *BATON ROUGE*	14.0	31.0
	28-05-97	*CHEVRON MISSISSIPPI*/*OS WASHINGTON*	13.0	30.0
	02-06-97	SR *NORTH SLOPE*/BT *ALASKA*	12.0	12.5
	03-06-97	SR *SAN FRANCISCO*/BT *ALASKA*	13.0	32.0
	03-06-97	*ARCO INDEPENDENCE*	13.0	30.0
	04-06-97	*OMI COLUMBIA*/*ARCO INDEPENDENCE*	14.0	34.0
BT TANKS	25-05-97	*ARCO SPIRIT*	12.0	32.0
	26-05-97	*ARCO ANCHORAGE*	12.8	33.0
	28-05-97	SR *LONG BEACH*/SR *BATON ROUGE*	15.0	31.0
	01-06-97	*CHEVRON MISSISSIPPI*	14.0	30.0
	03-06-97	SR *NORTH SLOPE*/BT *ALASKA*	13.0	23.5
	03-06-97	SR *SAN FRANCISCO*/BT *ALASKA*	13.0	32.0
	03-06-97	*ARCO INDEPENDENCE*	13.0	29.0
	04-06-97	*OMI COLUMBIA*/*ARCO INDEPENDENCE*	14.0	32.0

domestic but also foreign ports (Table 1). Temperature and salinity of the water varied little throughout the successive ballast water treatment stages, averaging 11.5°C and 31 ppt, respectively (Table 2). Nonsegregated ballast water coming off the tankers often contained globs of crude oil as well as lighter petroleum components as residuals in the cargo tanks. At all stages of treatment, the water had a distinct odor of petroleum, and an element of the treatment process was designed to drive off volatile petroleum components. Residence time of the water passing through the treatment facility averaged about 24 hr, ranging from about 12 to 36 hr.

Nonsegregated ballast water contained planktonic

communities, which were characterized by relatively low diversity and abundance (Table 3). A total of 23 morphologically distinct taxa were identified among 70 samples collected (Table 3). Occurrence of individual taxa ranged from 0 to 94% of the samples. Although the samples were dominated by various stages of copepods, discoid diatoms, and nematodes, relative abundance of taxa changed with sequential stages of treatment. Percent occurrence of discoid diatoms remained relatively constant among samples from sequential stages of treatment. While the occurrence of copepods declined with treatment sequence, the percent of nematodes increased.

Numerically dominant taxa were discoid diatoms and nematodes (Table 4, Figure 2A). The density of discoid diatoms differed significantly among treatments (Kruskal-Wallis, H=10.722, P=0.013). Densities of discoid diatoms increased 3- to 8-fold at intermediate treatment stages, and decreased 4-fold in the final treatment stages compared to initial samples from the Chicksan Arms. Densities of nematodes also differed significantly among treatments (Kruskal-Wallis, H=28.051, P=0.0001). Nematodes increased greatly between the Chicksan Arms (16.7 individuals/m^3) and the BT tanks (992 individuals/m^3). Similar significant differences among treatments were found for densities of other taxa combined (Kruskal-Wallis, H=7.8578, P=0.0490). These densities averaged 1450 individuals/m^3 in samples from Chicksan Arms, and decreased with treatment stage to an average of 174 individuals/m^3 in the BT tanks.

Viability of organisms in the samples was rather low, particularly in the initial stages of treatment (Figure 2b). Nonsegregated ballast water sampled from the Chicksan Arms before passing to the treatment facility frequently contained plankton that was in poor condition or dead and in various stages of decomposition. Nematodes and diatoms exhibited an increase in viability in the BT tanks (35% and 40%, respectively), while the viability of other taxa remained low throughout all treatment stages.

Table 3. Percent occurrence of organisms in each taxonomic group that occurred in samples collected from each of the four treatment stages of the Ballast Water Treatment Facility in Valdez, Alaska. Percentage calculated as the number of samples containing the specified taxa divided by total number of samples (indicated in parentheses). Counts do not distinguish between live and dead organisms (see text for discussion).

Taxon	Chicksan Arms (n=22)	90s Tanks (n=16)	DAF Tanks (n=16)	BT Tanks (n=16)
Dinoflagellates				
Ceratium	18.2	43.8	25.0	25.0
Peridinium	36.4	18.8	12.5	25.0
Diatoms				
Chain forming	18.2	18.8	31.3	6.3
Discoid	81.8	87.5	81.3	93.8
Protozoans				
Foraminifera	59.1	37.5	43.8	81.3
Tintinnids	31.8	25.0	18.8	56.3
Rotifers	0.0	6.3	0.0	12.5
Nematodes	36.4	37.5	93.8	93.8
Platyhelminthes				
Turbellarians	9.1	0.0	0.0	0.0
Arthropods				
Amphipoda	4.5	0.0	0.0	0.0
Barnacle Nauplii	45.5	25.0	6.3	25.0
Barnacle Cyprids	18.2	6.3	0.0	0.0
Copepods				
Calanoida	54.5	43.8	6.3	6.3
Cyclopoida	27.3	31.3	18.8	12.5
Harpacticoida	31.8	6.3	0.0	12.5
Poecilistoma	13.6	0.0	0.0	0.0
Nauplii	45.5	37.5	18.8	12.5
Copepodites	50.0	43.8	12.5	6.3
Cumacea	4.5	0.0	0.0	0.0
Mite	9.1	0.0	0.0	0.0
Molluscs				
Bivalvia	18.2	6.3	0.0	0.0
Gastropoda	13.6	0.0	0.0	0.0
Eggs	50.0	37.5	12.5	25.0

Discussion

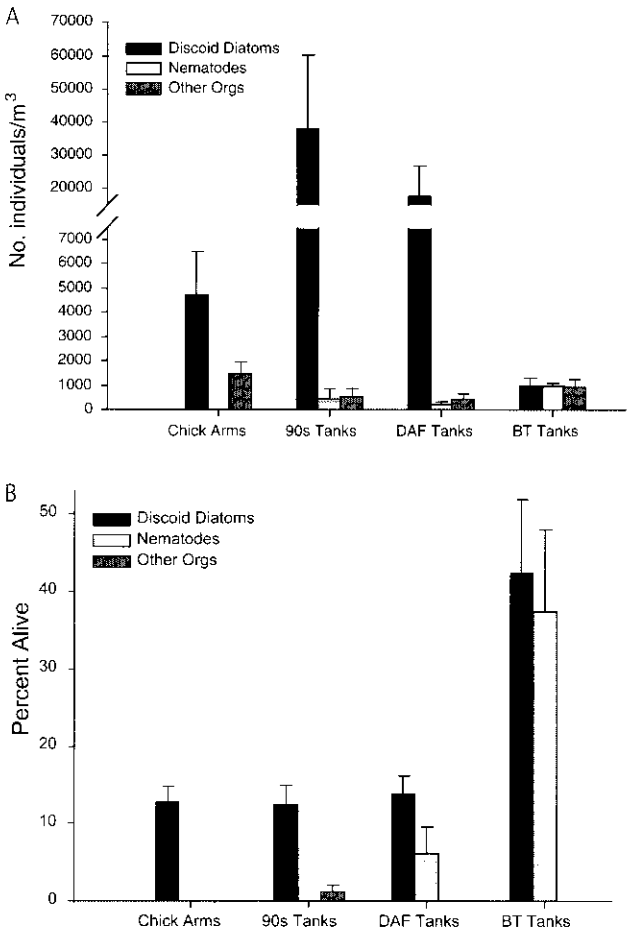
The abundance and viability of planktonic organisms in the nonsegregated ballast water discharged into the Ballast Water Treatment Facility of the Valdez Marine Terminal were relatively low, particularly when compared to the abundant and species-rich plankton communities found in segregated ballast water of the same ships (Ruiz and Hines 1997). Plankton in segregated ballast water arriving at Port Valdez during the study period included hundreds of morphologically distinct taxa from virtually all major groups of planktonic invertebrates and algae; most of these were very active and healthy in appearance (Ruiz and Hines 1997).

In most cases, densities of the 23 taxa in the nonsegregated ballast water decreased throughout the

Table 4. Density of organisms (number/m^3) in each taxonomic group that occurred in samples collected from the Ballast Water Treatment Facility in Valdez, Alaska. Shown are the means and standard errors obtained from preserved samples for each of the four locations (with indicated samples sizes). Counts do not distinguish between live and dead organisms (see text for discussion).

Taxon	Treatment							
	Chicksan Arms (n=16)		90s Tanks (n=9)		DAF Tanks (n=6)		BT Tanks (n=12)	
	Avg Density	Std Error	Avg Density	Std Error	Avg Density	Std Error	Avg Density	Std Error
Dinoflagellates								
Ceratium	14.6	8.7	235.2	206.3	169.4	105.2	1.0	0.4
Peridinium	267.7	172.0	7.4	4.0	5.6	3.5	1.8	1.1
Diatoms								
Discoid	4714.6	1795.5	37996.3	22160.1	17686.1	9326.4	969.3	328.9
Chain diatoms	12.5	7.2	5.6	3.9	5.6	3.5	0.0	0.0
Protozoans								
Tintinnid	59.4	38.5	55.6	47.4	72.2	40.1	5.5	2.6
Foraminifera	604.2	312.2	72.2	38.9	111.1	75.0	134.4	41.9
Rotifers	0.0	0.0	1.9	1.9	0.0	0.0	26.5	26.2
Nematodes	16.7	5.5	463.0	385.6	244.4	93.3	992.4	112.3
Platyhelminthes								
Turbellarians	3.1	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Arthropods								
Amphipods	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Barnacle Nauplii	124.0	56.1	5.6	3.9	2.8	2.8	1.3	0.6
Barnacle Cyprid	20.8	17.6	1.9	1.9	0.0	0.0	0.0	0.0
Copepods								
Calanoida	127.1	69.6	35.2	29.1	0.0	0.0	1.0	1.0
Cyclopoida	61.5	39.8	14.8	7.1	25.0	14.1	1.3	1.1
Harpacticoida	16.7	6.1	5.6	5.6	0.0	0.0	0.5	0.4
Poecilistoma	14.6	9.7	0.0	0.0	0.0	0.0	0.0	0.0
Nauplii	38.5	12.0	29.6	16.1	19.4	10.9	0.5	0.4
Copepodite	65.6	19.0	50.0	31.8	19.4	16.3	0.3	0.3
Cumacea	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Mite	3.1	2.3	0.0	0.0	0.0	0.0	0.0	0.0
Molluscs								
Bivalvia	14.6	10.4	3.7	3.7	0.0	0.0	0.0	0.0
Gastropoda	3.1	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Eggs	116.7	36.5	64.8	24.9	0.0	0.0	6.3	4.9
Total	6301.0	2258.6	39048.1	22611.8	18361.1	9580.3	2142.3	403.3

sequential stages of treatment, except for an increase in abundance of nematodes in the final stages. The source of this increase in nematodes remains unclear. One possibility is that they originated in low numbers from the nonsegregated ballast water, and then flourished under conditions within the BT tanks. Alternatively, nematodes, which as cysts are easily dispersed by wind, could have colonized the BT tanks if conditions were favorable for population growth. Despite the increase, the viability of nematodes, discoid diatoms, and other combined taxa in the BT tanks appeared low. We did not assess the conditions in the nonsegregated ballast water that may have contributed to the low survival of the plankton. Clearly, the nonsegregated ballast water contained high levels of petroleum products. In addition, oxygen levels in the tanks may have been low, because ship operations are required to reduce oxygen concentrations in air space overlying the ballast water below critical levels for combustion. In contrast, oxygen levels in segregated ballast water were consistently high. In any case, the conditions of the



Figures 2.A) Total density of organisms and 2.B) viability (% live) in samples collected from the Ballast Water Treatment Facility in Valdez, Alaska. Shown are means (+/- S.E.) for diatoms, nematodes, and all other combined taxa at the four successive stages of treatment.

nonsegregated ballast water were not favorable for nearly all plankton.

The Alyeska Ballast Water Treatment Facility at the Valdez Marine Terminal provides both useful insights and an inappropriate model for the design of shore-side facilities for removal of plankton from ballast water. The Valdez facility does demonstrate the engineering capability to receive large volumes of ballast water pumped off ships and to process it through multiple stages of treatment. However, this plant does not provide a good alternative management system for reducing NIS in segregated ballast water. The Alyeska facility was designed for removal of petrochemicals from ballast water and not for removal of plankton. It appears unlikely that the plant's treatment process produced the great reduction of plankton abundance and diversity in the nonsegregated ballast water. Further, the facility probably would not tolerate the addition of large volumes of segregated ballast water, because the important last biological treatment stage of the process uses microorganisms that depend upon a supply of petrochemicals to sustain large standing stocks of bacteria to attack the oil. Ultimately, a variety of other treatment processes need to be evaluated for reducing the risk of introducing alien species transported in ballast water.

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Larval Experience Can Influence Invasion Potential for Benthic Marine Invertebrates

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ABSTRACT: Marine invertebrate larvae may be transported large distances in ship ballast water and then discharged into remote locations. Planktotrophic larvae are likely to be food-limited during ballast transport, whereas competent larvae, whether they are feeding or nonfeeding, may delay their metamorphosis until after they are discharged into the new habitat. In laboratory studies, even a few days of nutritional stress during larval development can result in significantly reduced juvenile growth rates, as in the gastropod *Crepidula fornicata*. Similarly, prolonged larval life can reduce size at metamorphosis (as in the crab *Chasmagnathus granulata*), rates of postsettlement survivorship (as in the polychaete *Capitella* sp. I), rates of postmetamorphic growth (as in the barnacle *Balanus amphitrite* and the polychaete *Hydroides elegans*), or rates of colony development (as in several bryozoan species). Interspecific differences in the sensitivity of larvae to these and other sublethal stresses may play important roles in determining the competitive ability of transported individuals and their likelihood of successfully invading a new location. The following questions must be asked: 1) To what extent are planktotrophic larvae of different species food limited during ballast water transport? 2) To what extent do the larvae of particular species delay their metamorphosis during ballast water transport? 3) To what extent does postmetamorphic fitness decline over time for particular species during ballast water transport? 4) Why are some species more sensitive than others to the postmetamorphic consequences of sublethal stresses experienced as larvae?

Key words: ballast water, delayed metamorphosis, invasion, invertebrate, larva

INTRODUCTION

The frequency with which marine organisms successfully invade new areas–and become long-lasting, reproductive members of those new communities–is apparently increasing in many regions of the world (Carlton 1996; Ruiz *et al.* 1997; Cohen and Carlton 1998). Furthermore, a large percentage of the invasions accomplished to date appear to have been mediated by the transport of larval stages from place to place in ship ballast water (Carlton 1996; McGinn 1999). A single ship can carry about 150,000 mt of ballast water on a single voyage (Ruiz *et al.* 1997). More than 56 million mt of ballast water are discharged each year into U.S. ports alone (Ruiz *et al.* 1997; McGinn 1999), creating the potential for introducing hundreds or thousands of species that would not normally visit those areas, or at least not very often or in large numbers.

Although many successful invasions by benthic marine animals have been documented, it is clear that many other introductions do not succeed. Larvae of many species must have been introduced to particular areas many times over the past several decades, without having successfully established new populations (Ruiz *et al.* 1997).

In general, we do not yet understand why some species have been more successful than others at establishing expanding, reproducing populations after their introduction to new areas (Carlton and Geller 1993; Carlton 1996; Ruiz *et al.*1997). How do species that successfully invade new areas differ from those that are transported to new places but fail to become established? To a large extent, the likelihood of a successful invasion must depend on changes in the availability of transportable individuals of any particular species in a donor population and the numbers of individuals that end up in ballast water. It must also depend on the travel time between ports and how that affects larval survival, the frequency with which ships travel between any two particular ports, and how hospitable the new environment is to the transported individuals at the time of their release (Carlton 1996; Ruiz *et al.* 1997). In this article I suggest one additional possibility that has been largely overlooked: the potential role of the transport

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process itself in determining the fitness of transported individuals.

Life Cycles of Marine Benthic Marine Invertebrates

Typically, the life cycles of benthic marine invertebrates include a free-living larval stage that may or may not be able to feed in the plankton (Thorson 1950). At some point in development the larva becomes competent to metamorphose. Metamorphosis can then be triggered by specific environmental factors, which in many species are chemical cues associated with appropriate habitat for the juvenile (Thorson 1950; Burke 1983; Pechenik 1990). In the absence of those cues the larval stage is prolonged, and we can say that metamorphosis is being delayed or postponed (Pechenik *et al.* 1998). The capacity for delaying metamorphosis varies considerably among species, ranging from minutes or hours in some to many months in others (Pechenik 1990). The important point is that throughout much or all of the delay period, the larvae retain the ability to metamorphose if appropriate cues are encountered. The ability of competent larvae to delay metamorphosis has been demonstrated in a wide range of animals (reviewed by Pechenik 1990), including sponges (*e.g.*, Wilson 1935; Maldonado and Young 1999), cnidarians (*e.g.*, Mathews 1917; Chia and Spaulding 1972; Chia and Bickell 1978; Fitt *et al.* 1987; Richmond 1985), bivalved molluscs (*e.g.*, Bayne 1965; Pechenik *et al.* 1990), gastropod molluscs (Scheltema 1961, 1986; Hadfield 1977; Kempf 1981; Pechenik 1980, 1984a), polychaete annelids (Wilson 1948; Butman *et al.* 1988; Pawlik 1988; Pechenik and Cerulli 1991; Bryan *et al.*1998) decapod crustaceans (O'Connor 1991; Harvey 1996; Weber and Epifanio 1996; O'Connor and Judge 1997; Fitzgerald *et al.*1998; Gebauer *et al.*1999; O'Connor and Gregg 1998; Strasser and Felder 1998), barnacles (Pechenik *et al.*1993), echinoids (Burke 1983; Highsmith and Emlet, 1986), asteroids (Birkeland 1971; Barker 1977), bryozoans (Nielsen 1981; Woollacott *et al.* 1989; Wendt 1996,1998), brachiopods (Zimmer 1996), and even some tropical fishes (Victor 1986; Cowen 1991; McCormick 1999). The phenomenon is widespread, and presumed to be adaptive in promoting the eventual placement of individuals into habitats that will best support juvenile survival, growth, and reproductive success (Thorson 1950).

Although we commonly think of larval and juvenile lives as being largely independent–separated by what is often a dramatic metamorphosis that reshapes the animal morphologically, physiologically, and ecologically–larval and postlarval lives may not, in fact, be as independent as commonly thought: certain subtle larval experiences can influence how well juveniles perform after metamorphosis. As used here, "performance" refers to rates of survival, growth, maturation, and other factors likely to influence fitness. In particular, some larval experiences during ballast transport may at least partially determine the competitive ability of individuals that metamorphose successfully after they have been released into a new environment.

The Effects of Larval Experience on Postlarval Performance

Two sorts of larval experiences have been shown to influence postmetamorphic performance: delayed metamorphosis and nutritional stress. The effects of delayed metamorphosis were first demonstrated for nonfeeding bryozoan larvae in preliminary observations by Nielsen (1981) and then documented in greater detail by Woollacott *et al.* (1989). Larvae of *Bugula stolonifera* and *B. neritina* are competent to metamorphose within minutes of being released from the parent zooid, but will keep swimming for at least 24 hr in the absence of triggering substratum and in the presence of bright light (Woollacott *et al.* 1989; Wendt 1996,1998). Prolonging larval swimming in the laboratory by as few as 6-8 hr (at 20°C) resulted in significantly slower rates of colony development for several bryozoan species (Woollacott *et al.* 1989; Orellana and Cancino 1991; Wendt 1996). More recently, Wendt (1998) showed that when young colonies of *B. neritina* were transplanted to the field, the effects of delayed metamorphosis on colony growth and development persisted for at least two weeks. In addition, delaying metamorphosis increased the time to reproductive activity and reduced the numbers of brood chambers per colony in these transplants. Thus, increasing the swimming time of bryozoan larvae by only 6-24 hr has the potential to diminish the competitive ability and reproductive fitness of colonies in the field.

Similarly, delaying metamorphosis of barnacle cyprids (*Balanus amphitrite*) by as few as 3 days (at about 28°C) significantly reduced juvenile growth rates, even though it had no effect on metamorphic

success *per se* (Pechenik *et al.* 1993). Longer swimming periods were also associated with lower survival and slower growth rate in the demosponge *Sigmadocia caerulea* (Maldonado and Young 1999), lower post-settlement survival in the polychaete worm *Capitella* sp. I (Pechenik and Cerulli 1991), reduced survival and growth rates in the calcareous tubeworm *Hydroides elegans* (Qian and Pechenik 1996), and possibly reduced tolerance of environmental stresses in the echinoid *Dendraster excentricus* (Highsmith and Emlet 1986). Prolonging larval life of the estuarine crab *Chasmagnathus granulata* resulted in smaller juveniles, potentially increasing vulnerability to predators (Gebauer *et al.* 1999).

Delayed metamorphosis does not always diminish juvenile performance, however. Prolonging larval life in the polychaete *Capitella* sp. I for up to 5 days, for example, had no detectable effect on average juvenile growth rate, time to reproductive maturity, or fecundity (Pechenik and Cerulli 1991). Similarly, delaying the metamorphosis of decapod crustaceans did not prolong the duration of the 1st crab stage for *Uca pugnax* (O'Connor and Gregg 1998) and had no effect on juvenile survival or molt-cycle duration for *Chasmagnathus granulata* (Gebauer *et al.* 1999). Additional studies will be required to determine why the effects of delayed metamorphosis carry over into later life in some species but not others (Pechenik *et al.* 1998).

Short-term nutritional stress during larval life can also reduce juvenile performance. Transferring larvae of the gastropod *Crepidula fornicata* from seawater with a high phytoplankton concentration (18×10^4 cells ml^{-1} of the naked flagellate *Isochrysis galbana*) to either filtered seawater or to seawater of substantially reduced phytoplankton concentration (less than 10% of the control concentration) for several days in the laboratory significantly reduced mean juvenile growth rates (Pechenik *et al.* 1996a, 1996b). When larvae of this species were starved for 3 days, beginning one day after hatching, and then returned to full ration for about another 10 days until they became competent to metamorphose, larval growth rates returned to control levels within 4-6 days. Following metamorphosis, however, mean juvenile growth rates were again significantly below mean control growth rates, despite the complete recovery of the larval growth rates to control levels (Pechenik *et al.* 1998).

Thus, short-term nutritional stress, even if experienced only very early in development, can--through as yet undetermined mechanisms (Pechenik *et al.* 1998)--reduce the juvenile's capacity for growth. If there is an escape in size from predation (*e.g.*, Wahle and Steneck 1992; Sousa 1993; Moksnes *et al.* 1998), then slower-growing juveniles will remain vulnerable to predators for a longer period of time. Recently metamorphosed juveniles are extremely vulnerable to predators (Gosselin and Qian 1997; Hunt and Scheibling 1997); thus, even if juvenile growth rates would eventually recover to those of control individuals that had not experienced nutritional stress or delayed metamorphosis as larvae, fewer of the initially slower growing juveniles may survive to exhibit such a return to normal growth, and to benefit from the larger size.

Application to Transport in Ship Ballast

What are conditions like in ship ballast water? Many of the specific cues that would normally stimulate competent larvae to metamorphose (*e.g.*, chemicals associated with juvenile food sources and adult members of the same species--reviewed by Pechenik 1990; Pawlik 1992) are probably absent in ballast water. If so, the larvae of many species may delay their metamorphosis until they are discharged into a new habitat at the next port. Also, while feeding larvae may be getting something to eat while in ballast water (*e.g.*, bacteria and detritus: Boidron-Métairon 1995), they probably experience some degree of nutritional stress, and quite possibly a great deal of nutritional stress, in the absence of photosynthesis. Degree of nutritional stress could be surmised by monitoring average larval growth rates for particular species during ballast water transport. Studies investigating changes in the physiological condition of larvae during transport in ship ballast water have not yet been reported, but also seem warranted. Clearly, both delays in metamorphosis by competent larvae and the imposition of nutritional stress during larval development can subsequently reduce the ability of juveniles to grow or develop rapidly. That could make juveniles less able to compete for space or food, and may increase the amount of time that they remain maximally vulnerable to predators. If there is an escape in size, it will take these juveniles longer to reach that size. In at least some species, time to reproductive maturity and total reproductive output can also be affected. Moreover, it seems likely that

the larvae of some species will be found to be more sensitive to these stresses than the larvae of other species. Such differential vulnerability may play a role in determining why some species are more successful than others in invading new areas after being transported to those areas in ship ballast.

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The Arrival of the European Green Crab, *Carcinus maenas*, in Oregon Estuaries

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ABSTRACT: **Since its first discovery in Coos Bay, Oregon, in 1997, *Carcinus maenas* has been found in at least eight Oregon estuaries: Coquille, Coos, Alsea, Yaquina, Siletz, Salmon, Netarts and Tillamook. All *C. maenas* found in Coos Bay in 1997 were large crabs, 58-86 mm carapace width (CW) and we estimate that they represent the 1995/1996 year class. Similar-sized crabs were found in the Tillamook, Netarts, Coquille and Yaquina estuaries in 1998. In addition to these large crabs, a new year class appeared in Oregon estuaries in the summer of 1998; it was also observed in Humboldt Bay, California and Willapa Bay and Grays Harbor, Washington and Vancouver Island, British Columbia. These crabs averaged 14 mm CW in June, 27 mm in July, 42 mm in August, and 47 in September. In December 1998, crabs ranged from 35-66 mm CW. This coast-wide colonization event is correlated with unusually strong northward-moving coastal currents off the northern California and Oregon coast from September 1997 to April 1998. Transport of larvae from well-established populations to the south, rather than transport with oysters, appears to be the dominant mechanism for the appearance of this new year class.**

Key words: green crab, *Carcinus maenas*, Oregon, invasion, larval dispersal, El Niño

INTRODUCTION

The European green crab is native to Europe, inhabiting the Baltic and North Seas and, in the Atlantic, ranging from Morocco to Iceland (Crothers 1968). During the last two centuries, viable populations of *Carcinus maenas* became established on the east coast of North America, as well as in South Africa, Australia, and Japan and, most recently, the west coast of North America (Grosholz and Ruiz 1995). *C. maenas* is tolerant of desiccation and wide ranges in temperature and salinity, and is able to survive ocean voyages and plane rides (J. Carlton, pers. comm.). Moreover, free-swimming larvae of *C. maenas* can develop within the ballast tanks of cargo ships. Once released to a new environment, this ecological generalist can subsist on a variety of food organisms, including marsh vegetation, algae, crustaceans, mollusks, and fish (Cohen *et al.* 1995). Under favorable conditions, *C. maenas* reaches sexual maturity within one year (Grosholz and Ruiz 1995). Planktonic larvae can travel on ocean currents, and thus seed new breeding populations elsewhere. Marine scientists, resource managers, and shellfish growers are concerned that this new invader may change marine food webs by displacing native species and preying on commercially important species such as clams, mussels, and oysters.

On the west coast of North America, *C. maenas* became established in San Francisco Bay during the 1980s, rapidly spreading to Bodega Harbor (1993), Monterey Bay (1994), and Humboldt Bay (1995) in California, Coos Bay, Oregon (1997), Willapa Bay, Washington (1998), Grays Harbor, Washington (1998) Morro Bay, California (1998), and Vancouver Island, British Columbia (1999) (Grosholz and Ruiz 1995; Cohen *et al.* 1995; Miller 1996; Richmond 1998; B. Dumbauld, Washington Dept. Fish and Wildlife, pers. comm., E.D. Grosholz, pers. comm.; G. Jamieson, pers. comm.) (Figure 1). This study documents the invasion process in Oregon. Our goal was twofold: (1) to document the distribution of *C. maenas* and (2) to determine the growth rate and reproductive season of *C. maenas* in Oregon estuaries.

MATERIALS AND METHODS

SURVEY

The first reported specimen of *C. maenas* in Oregon was found by Heath Hampel on his oyster ground in Coos Bay in late March 1997 (Richmond

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1998) (Figure 2). After this, we started searching the Coos, Coquille, Umpqua, Yaquina, and Siletz estuaries for green crabs. In 1998, we added the Siuslaw, Alsea, Salmon, Netarts, Tillamook, and Columbia estuaries.

The survey methods we employed were (1) molt searches, (2) shore searches, (3) rock turning, and (4) trapping. During the spring and summer, when crabs grow rapidly, their exuvia tend to collect with other flotsam in the drift line on beaches. A quick search of the drift-line can yield a non-quantitative species list of crabs present in an estuary. The most productive drift-line searches are in sites where the wind blows along the shoreline and where barriers such as jetties and rushes trap the flotsam. Shore searches consist of walking along stretches of beach at low tide and examining potential crab shelters such as the undersides of rocks or logs, marsh bank burrows, and vegetation. For annual comparisons of relative abundance and sizes of crab species in permanent study sites, we collected, identified, sexed, and measured carapace width (CW) of all crabs under standard-size rocks (30-50 cm maximum diam.). Traps

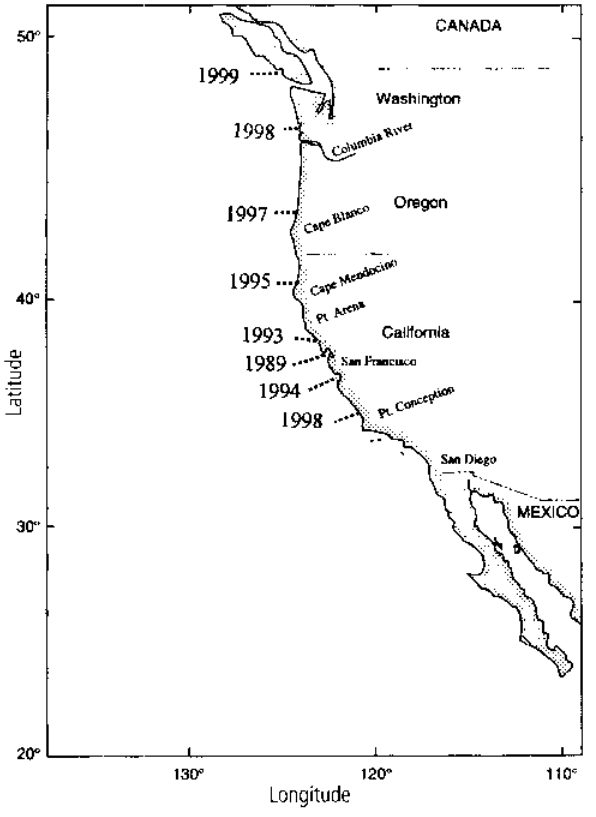

Figure 1. Map of the west coast of North America, showing the spread of *C. maenas* from San Francisco Bay. Dates indicate the year *C. maenas* was first reported.

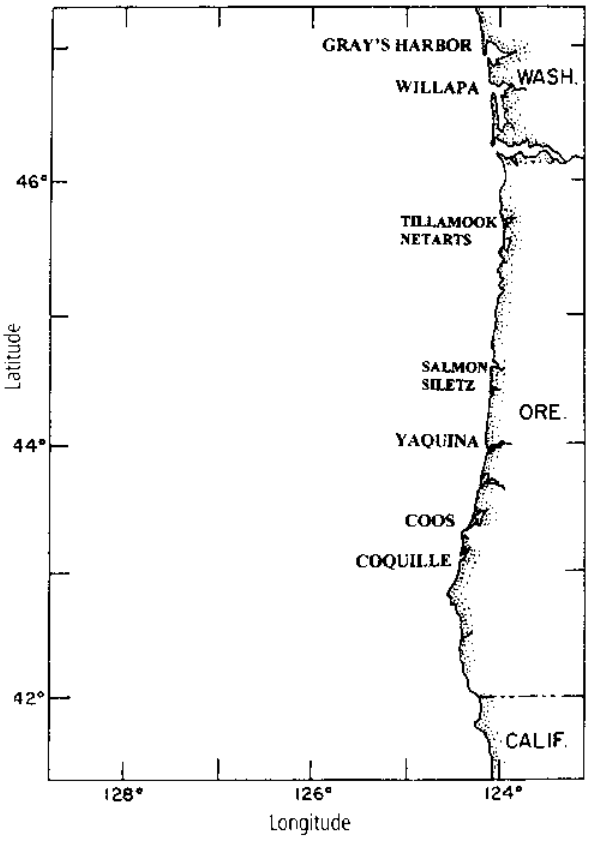

Figure 2. Map of the Oregon coast showing the estuaries in which *C. maenas* has been found. *Carcinus maenas* was not found in the Umpqua, Siuslaw or Columbia.

used were cylindrical crawdad or minnow traps, with 5 mm mesh, 41 cm long, and 23 cm diam. We increased the diameter of the funnel-shaped openings to 9 cm by bending the mesh back. Traps were baited with fish scraps inside a perforated plastic container, weighted down with rocks, and positioned at the upper-mid to lower-high tide level. Traps were typically checked after one high tide, and all organisms inside the trap noted. Crabs were identified to species, sexed, examined for the presence of eggs, and measured to the next lowest mm using vernier calipers.

Molt Increment Study

To confirm field observations on growth, 60 *C. maenas* from various estuaries were reared at Hatfield Marine Science Center in Newport, Oregon, from July 25 to October 15, 1998. Crabs were individually housed in numbered plastic sandwich boxes (5 x 15 x 15 cm) with sides perforated for water circulation. The boxes were kept in tanks (318 x 118 x 30 cm) with flow rates of 5L/min. Crabs were typically

Table 1. Correlation of oyster growing activities and ballast water discharge in Oregon estuaries with the distribution of the new and old year class(es) of *Carcinus maenas*. Estuaries were sampled from spring 1997 to December 1998. An "X" indicates that *C. maenas* was found in an estuary; "M" indicates that only molts were found. We estimate that the new year class settled from the plankton between September 1997 and April 1998, a period of unusually strong northward-flowing El Niño currents. The older year class in Coos Bay most likely settled between fall 1995 and spring 1996.

Estuary	New Year Class	Older Year Class(es)	Oyster Culture	Ballast Water
Tillamook	X	X	yes	no
Netarts	X	M	yes	no
Salmon	X		no	no
Siletz	M		no	no
Yaquina	X	X	yes	yes
Alsea	X		no	no
Coos	X	X	yes	yes
Coquille	X		yes	no

checked for molts twice a week and molted crabs were measured. All crabs were fed a diet of shrimp or crushed mussels. Water temperatures during this time ranged from 11-14°C while salinity varied from 32-35‰.

Results

Survey

In 1997, over 200 hr of shore searching and over 800 trap-hr produced only 8 additional live green crabs in Coos Bay. No *C. maenas* were found by trapping and shore searches in Umpqua, or by rock turning in Yaquina and Siletz. All nine crabs from Coos were large adults ranging in size from 58-86 mm CW. Similar-sized crabs were found in 1998 in the Coquille, Yaquina, Netarts, and Tillamook estuaries (Table 1).

A new year class of *C. maenas* was discovered in seven Oregon estuaries in 1998 (Figure 2, Table 1). Green crabs were not found in Umpqua, Siuslaw, or Columbia. This new year class is characterized by a well-defined size distribution. For example, in Yaquina Bay in September we note a normal distribution with a mean of 47 ± 5 SD mm (Figure 3). No size differences were observed between the males and females. The new year class followed a similar growth pattern in all the bays. Crabs grew very rapidly from June to September and then leveled off

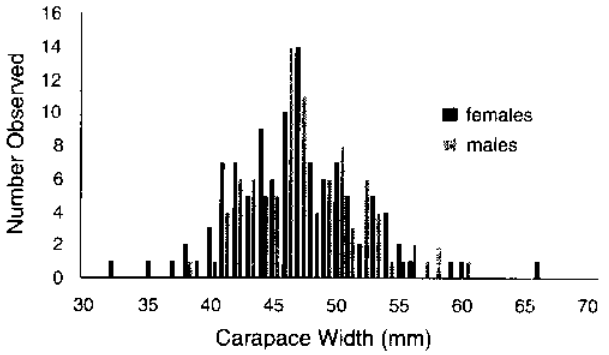

Figure 3. Size-frequency distribution of the new year class of *C. maenas* from Yaquina Bay, sampled between September 7 to 26, 1998. N = 202; mean = 47±5 SD mm CW; solid bars = females; screened bars = males.

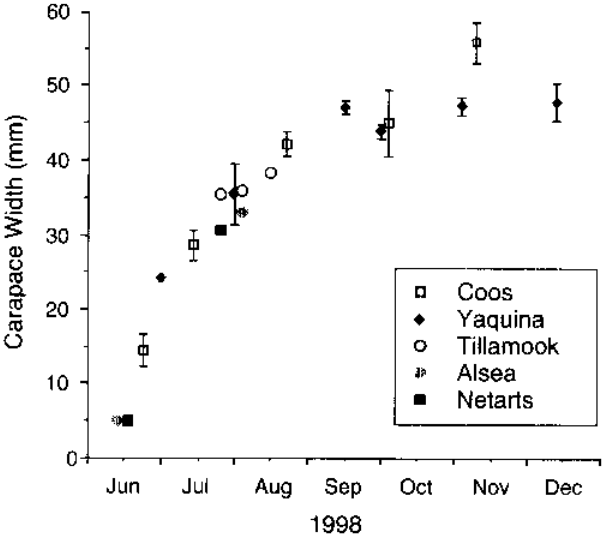

Figure 4. Growth of *C. maenas* in various Oregon estuaries. Mean carapace width (mm) and 95% confidence interval of the mean for Coos Bay and Yaquina Bay samples are indicated. Note that the last sample from Coos Bay was taken while diving and thus may represent the larger crabs of the size spectrum.

(Figure 4). Average CW was 14 mm in June, 27 mm in July, 42 mm in August and 47 mm in September. Note that the last sample in Coos Bay represents a subtidal sample taken while diving and thus may represent the larger crabs of the size spectrum. In November and December 1998, crabs from the new year class ranged from 35-66 mm CW.

This new year class reproduced: gravid females were caught in the Coos, Yaquina and Tillamook estuaries. Seventy-five percent (15/20) of the females caught in Yaquina in early November and held in the laboratory released eggs by early December 1998. Since the life span of green crabs varies from 3-6 yr

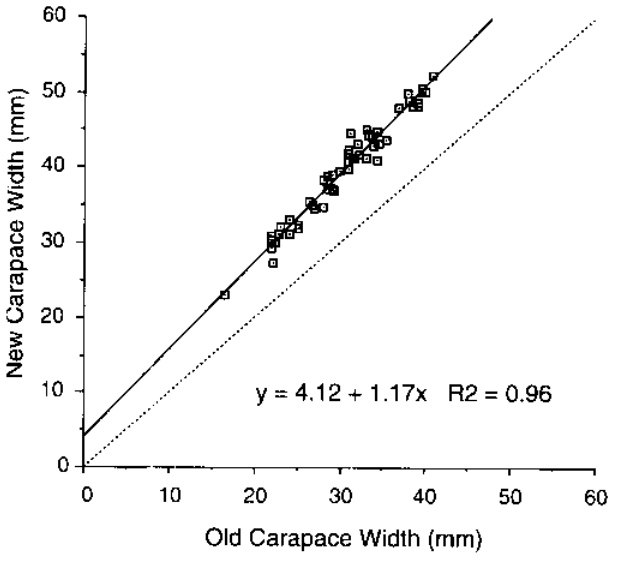

Figure 5. Carapace width (CW) of *C. maenas* before and after molting. Line of no growth is indicated by a stippled line. A 30-mm CW crab typically molts into a 40 mm CW crab.

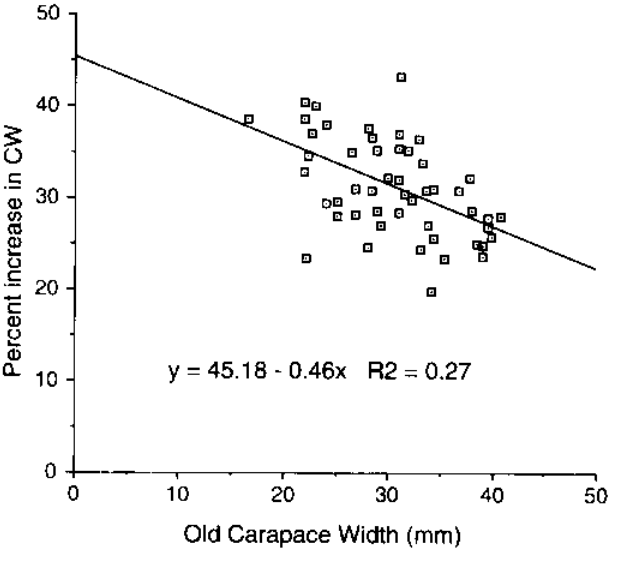

Figure 6. Percent molt increment in *C. maenas* as a function of initial carapace width. A 20-mm crab adds 38%, while a 40-mm crab adds 27% in carapace width.

(Berrill 1982), this strong year class will survive for at least one more reproductive season.

Molt Increment Study

Molt increment data support field growth rates. From the regression of new CW on old CW, we determined that crabs have high growth rates (Figure 5). For example, a 30-mm CW crab will molt into a 40-mm CW crab. From the regression, we can estimate the CW of successive instars: 17, 24, 32, 42, 52, and 65 mm. By plotting percent molt increment against initial size, we see that crabs add between 20-40% CW, depending on size (Figure 6). On average, a 20-mm crab will add 38%, a 30-mm crab 31%, and a 40 mm crab 27% in CW.

Discussion

C. maenas colonized Oregon estuaries on at least two occasions, as inferred by the discovery of at least two distinct size groups of crabs. We estimate that the larger animals (56-86 mm CW) that were found in Coos Bay in 1997 represent the 1995/1996 year class. Only nine live crabs were found in over 1,000 hours of searching and trapping, suggesting that this year class was rare in 1997. Large crabs found in Coquille, Yaquina, Netarts, and Tillamook in 1998 either came from the 1995/1996 or the 1996/1997 year classes. Since only Coos Bay was sampled extensively in 1997, we know nothing about the history of *C. maenas* in these four estuaries. Small crabs sampled in 1998 most likely represent the 1997/1998 year class. Their sizes and growth patterns parallel those of *C. maenas* in its first year of life in Bodega Harbor (Grosholz and Ruiz 1995). The 1997/98 year class of *C. maenas* appeared to be most abundant in the upper-mid and lower-high tide zone of marshy habitats.

The 1997/98 year class exhibits a well-defined size frequency distribution, which did not overlap with the older year class(es) during the summer of 1998. The 1997/98 year class grew at similar rates at all sites in Oregon estuaries sampled. The crabs grew rapidly from an average CW of 17 mm in June to an average of 45 mm CW in September, then leveled off. At the end of 1998, *C. maenas* from the 1997/98 year class ranged in size from 35-66 mm. Similar sizes and growth patterns were observed in Willapa Bay, Washington (B. Dumbauld, pers. comm.). Molt increments for *C. maenas* in Oregon are similar to those in northern Maine or the North Sea (Berrill 1982; d'Udekem d'Acoz 1993).

Three possible mechanisms can be invoked for the appearance of *C. maenas* in Oregon: ballast water discharge, transplantation of oysters, and colonization by planktonic larvae from source populations in California. Ballast water discharge is limited to Coos and Yaquina Bays as these large estuaries are the only ones now receiving cargo ships. Since green crabs were also found in six other estuaries, it is unlikely that ballast water was the primary source of the

green crab introduction. Oyster culture is practiced in all five estuaries in which we found the older year class(es) of *C. maenas*. Since oyster growers frequently import seed oysters and market-size oysters from other growing areas such as Humboldt Bay, California, it is possible that newly settled green crabs could have been introduced along with the oysters. An alternative explanation is that a coast-wide colonization event occurred in which green crab larvae from California entered all the estuaries, but survived only in the most favorable ones—the ones supporting good oyster growth. Based on limited evidence, we speculate that ballast water most likely was not the mechanism for the introduction of the older year class(es) of *C. maenas* and that transportation of oysters and colonization via ocean currents cannot be ruled out.

Larval recruitment from source populations in the south is the most plausible mechanism for the appearance of the 1997/98 class of *C. maenas* in eight Oregon estuaries as well as in Humboldt Bay, (T. W. Miller, pers. comm.), Willapa Bay, and Grays Harbor, (B. Dumbauld, pers. comm.). A few crabs from that same year class were also discovered as far north as Esquimalt Harbor and Barkley Sound on Vancouver Island, British Columbia in the summer of 1999 (G. Jamieson, pers. comm.). Since crabs from the older year class(es) were extremely rare, we feel that any larvae from these crabs alone were not abundant enough to seed Oregon, Washington, and Vancouver Island bays. We suspect that larvae from well-established populations in California were the source. Trapping data for San Francisco Bay indicate that adult green crabs may have been more abundant in 1997 than in previous years (K. Hieb, pers. comm.) suggesting that these crabs could have produced a large larval pool.

The anomalous El Niño ocean conditions from September 1997 to April 1998 were extremely favorable for larval transport from California (Huyer *et al.* 1998). In a typical year, surface currents along the Oregon coast generally are in a southerly direction from April to October and in a northerly direction from October to April. Northward-moving currents typically occur in a narrow, 10 to 20-km strip close to shore at around 10-20 km/da. In 1997/1998, such northward-moving currents were more intense in both duration and magnitude. In 1997 a northward-moving current of 10 km/da was measured off Newport, Oregon as early as September. By November it had intensified over the whole shelf and slope, from Point Arena, Calfornia to Newport, Oregon. The northward currents were especially strong off Crescent City, California and Coos Bay, Oregon measuring >40 km/da. Such strong currents lasted until April 1998. Drifters released off Newport in January 1998 moved north to the west coast of Vancouver Island at a velocity up to 50 km/da (Barth and Smith 1998). These observations support the view that strong northward currents were a coast-wide phenomenon during the fall, winter, and spring of 1997/1998. If we use 55 days as the developmental time for *C. maenas* larvae at 14°C (Dawirs *et al.* 1986), then it is possible for larvae to be transported 2200 km by ocean currents, a distance from San Francisco to the Queen Charlotte Islands. It is feasible that *C. maenas* larvae from the San Francisco Bay area could have seeded Oregon, Washington, and Vancouver Island estuaries between September 1997 and April 1998.

Range expansions of marine fishes and invertebrates are commonly observed during and after an El Niño event (Schoener and Fluharty 1985). For example, permanent populations of the mole crab, *Emerita analoga*, occur from Mexico to Oregon (Hart 1982). During and after El Niño years, this species has been found off Vancouver Island, British Columbia and even on Kodiak Island, Alaska (Butler 1959; Hart 1982). The inference is that larvae from source populations in California and Oregon are transported north by anomalously strong northward moving currents during El Niño years. In subsequent non-El Niño years, these populations die out due to lack of recruitment.

The northern range expansion of *C. maenas* may be similarly limited by El Niño events. While recruitment of *C. maenas* was good after the 1997/1998 El Niño, it was late and poor the following year. The 1998/1999 year class did not enter traps until early September (L. Carr, pers. comm.). These crabs were much rarer and smaller (27-42 mm CW) than the 1997/1998 year class at the same time of year. Weak northward-flowing currents occurred only between November 1998 and February 1999 (A. Huyer and R. L. Smith, pers. comm.), thus limiting the chances for larval recruitment from established populations to the south. Recruitment during 1999, most likely, was localized within each estuary. Females from the 1997/1998 class of *C. maenas* produced viable eggs in Yaquina, Bay, Tillamook Bay

and Willapa Bay (B. Dumbauld, pers. comm.), but these reproductive efforts were insufficient for the parental year class to replace itself. If recruitment remains low in subsequent years, then *C. maenas* in the northern estuaries will decrease in number, until the next El Niño event transports more larvae from California.

Acknowledgements

Many people contributed to this study. The efforts of John Schaefer (Oregon Department of Fish and Wildlife) in locating the first green crabs were crucial in getting this project launched. Todd W. Miller (Oregon State University) kindly supplied the September 1998 trapping data for Yaquina Bay, while John Faudskar (Oregon Sea Grant Extension) provided data for Tillamook Bay. Additional distribution records for live green crabs and molts were provided by Patrick Baker, Chris Brooks, John Johnson, David McGee, J.T. Hesse, J. Schaefer, and students in the Oregon State University Zoology 401 Class. We thank Kelly Chapin Palacios, Kimberly Cleveland, and the students in Zoology 401 for help with sampling as well as Brett Dumbauld for comments on an earlier version of this manuscript. Maps were drawn by Dave Reinert. This research was supported by Oregon Sea Grant.

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The Sabellid Pest of Abalone: The First Eradication of an Established Introduced Marine Bioinvader

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Key words: eradication, introduced marine pest, sabellid, polychaete, abalone

INTRODUCTION

Once a marine invader has become established, the possibility of its subsequent control has been met with little more than fatalism. This fatalism stems from a defeatist attitude that once a pest has become established we can do nothing to eliminate or reduce its impacts, and thus we must learn to live with it. However, as illustrated by the history of the introduced sabellid worm, *Terebrasabella heterouncinata*, in California, such an outlook may not be warranted. A previously unknown species, this sabellid polychaete presumably arrived as a contaminant on imported South African abalone, *Haliotis midae* (Culver *et al.* 1997; Kuris and Culver 1999; Fitzhugh and Rouse 1999). The sabellid infestations caused shell deformation and slowed growth of the cultured abalone, causing substantial economic losses to the industry.

ERADICATION APPROACH

Although initially contained in California abalone mariculture facilities, we detected a well-established sabellid population (a minimum of 2.2 million worms) at an intertidal site near Cayucos, California in 1996 (Culver and Kuris 2000). We considered this pest to be potentially ecologically deleterious, because, having broad host specificity, its potential impacts (*e.g.*, shell deformation, increased mortality) could extend to many native California gastropods (Kuris and Culver 1999; Culver 1999). In fact, moderate to high abundances of these worms substantially impact hosts in culture facilities and natural South African habitats (Oakes and Fields 1996; C. Culver and A. Kuris pers. obs.). Thus, to mitigate the impact of this introduced marine pest at this site and prevent or slow its geographic spread, we proposed an eradication program based on the Kermack-McKendrick epidemiological theory of the threshold of transmission (Culver and Kuris 2000). This theory states that if the density of transmissive stages and the density of highly susceptible hosts are reduced below the replacement transmission rate, successive generations of the pest will damp out (McKendrick 1940; Bailey 1957; Stiven 1964; Stiven 1968).

Specifically, our eradication program included three components: (1) prevention of further release of adult worms from the facility (through screening of facility discharge water), (2) reduction of the adult pest population (through removal of infested animals and shell debris that had been released from the facility), and (3) reduction of the population of the most susceptible native host (through removal of large individuals (> 10 mm shell width) of the abundant black turban snail, *Tegula funebralis*). This three-pronged approach is unique in that it targets not only the pest, but also a component of the pest's life cycle –the host, *T. funebralis*–which is required for continuance of the established population. Importantly, this program was not designed to remove every last individual pest. This was virtually impossible as the sabellid is microscopic and can not be readily detected. Instead, we used a theoretically based strategy to diminish the future success of this population. This eradication program was implemented in July 1997, in collaboration with the associated mariculture facility and the California Department of Fish and Game. Since that time, we have removed over 1.6 million *T. funebralis* and over 2,000 escaped cultured abalone (some live, but mostly empty shells that contained hundreds to thousands of actively reproducing worms per shell).

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Using transect surveys and mark-and-recapture studies, we have monitored the success of the eradication efforts. For over two years, no new infestations have been detected. These results are quite impressive given the rate of infestation documented in September 1996, when 32% of marked sentinel snails became infested within six weeks of being released, with 8% infested within the first two weeks (Culver and Kuris 2000). The lack of sabellid transmission over the last two years indicates that successive generations have not become established. Thus, even though an occasional snail is found with evidence of a previously established sabellid, the population is no longer self-sustaining and has apparently been eradicated.

The success of this eradication program depended on (1) early detection, (2) cooperation between commercial interests, regulatory agencies, and pest control scientists, (3) rapid response (avoidance of analysis paralysis), (4) development of a control strategy with a theoretical basis, (5) persistent efforts beyond the point where the situation had merely improved, and (6) monitoring of eradication efficacy through use of sentinel host experiments. This successful eradication program, the first of its kind against a well-established marine pest, illustrates how vigorous proactive measures can eliminate introduced marine pests. Thus, while prevention of future introductions is the most desirable option, the results of our efforts counter defeatist attitudes and instead support the development and implementation of aggressive coherent strategies for the eradication or control of those non-indigenous marine pests that have already become established.

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Can Biological Control be Developed as a Safe and Effective Mitigation Against Established Introduced Marine Pests?

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ABSTRACT: We define the scientific basis for classical biological control in a marine environment and briefly evaluate the nature of the controversy surrounding its safety. For the natural enemies envisioned for biological control of introduced marine pests, the evaluation of efficacy will be comparable to that of terrestrial insect pest control agents, while assessment of safety will be similar to that of weed pest control agents. Although some are fearful of this approach, the cost of inaction may be very high. Thus, there is a great need for careful scientific analysis of these concerns so that the public can judge the evidence.

Key words: biological control, natural enemy, safety, introduced marine pest

INTRODUCTION

We now have a broad scientific consensus that introduced marine species sometimes become serious environmental pests, and that actions to reduce their rates of movement, entry, and establishment are very necessary. However, after their establishment at a point of entry, we presently lack consensus on the means to control such pests and we have few or no practical tools to prevent their further spread, nor to significantly reduce their impact on native organisms.

Lafferty and Kuris (1996) proposed the use of natural enemies, as developed for the classical scientific biological control of introduced weeds and agricultural insect pests, to significantly reduce the impact of those established marine pests that have the potential to cause great economic and ecological damage. The key issues concerning such an approach are clearly efficacy and safety. Some marine biologists see this as a fruitful avenue for investigation and consider the cost of inaction great enough to assume some risk towards its resolution. Others, however, fear that this approach has substantial potential to cause more harm than benefit. They raise two general arguments. Potential natural enemies are seen as likely to have a disastrous impact on native species. Reference points include cane toads and rabbit calicivirus. For some, its irreversible nature argues against its consideration (*e.g.*, Howarth 1991). Somewhat paradoxically, the other argument is that a biocontrol agent will not be effective because the pest is common where it is native (A. Cohen, E. Grosholz, pers. comm., G. Ruiz and A. Hines, pers. comm. to J. Goddard.) Thus, the natural enemy cannot effectively reduce its density where the pest has been introduced.

SCIENTIFIC CLASSICAL BIOLOGICAL CONTROL USING NATURAL ENEMIES IN THE MARINE ENVIRONMENT

To enable an analysis of this potential methodology, it is essential to carefully define what is meant by this approach. By keeping this in mind, we can avoid spurious arguments. "Scientific" means that the proposed control agent has been carefully evaluated for its potential efficacy and safety. These analytical tools include laboratory experiments, evaluation of natural history, and mathematical models. "Classical" refers to the use of an introduced natural enemy, from the region where the pest was native, against that pest. It does not include the use of introduced natural enemies against native pests, sometimes termed "neoclassical" biological control (Lockwood 1993) that has very different risk factors. Biological "control" implies that the pest will remain, but at reduced densities so that its undesired impact will be decreased or eliminated. "Natural enemies"

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refers to the use of predators, parasites, pathogens, or competitors. Biological control does not include many other "biological" techniques such as sterile males, pheromone traps, biocides, etc.

Insect pest biological control agents are most often tiny hymenopteran parasitoid wasps that are frequently rather host-specific. However, records of parasitization of native species are not uncommon and a few are at high prevalences and likely impact some native insects (Hawkins and Marino 1997). However, little evidence exists for negative impacts of introduced natural enemies on nontarget native species (Roberts 1986; Samways 1988; Waage and Greathead 1988; Greathead 1995; Jervis 1998). In the many examples of damage due to biocontrol listed in Howarth's (1991) influential review, there was only one case where a classical biological control agent, that had been scientifically investigated caused great damage to a probably native organism-the coconut moth in Fiji (Tothill *et al.* 1930). This was the target pest, and it may have been driven to extinction by the tachinid fly biological control agent. All the other listings of significant impacts were due to the release of generalist predators, were largely speculative, or did not stand up to scrutiny of the original reports (Blossey 1995; AMK, unpub.). Greathead 1995 noted that "most of the examples that he [Howarth] cites do not stand up to the scrutiny of the original reports and are largely based on speculation or are totally unsubstantiated."

Application of biological control to introduced marine pests takes its roots from four different fields. (1) Studies of the impact of certain kinds of infectious agents (parasitic castrators and symbiotic egg predators) on fisheries demonstrates that there is a theoretical window of opportunity for such agents to significantly reduce host abundance and that fisheries manage host-parasite dynamics (usually inadvertently) (Kuris and Lafferty 1992). (2) The mechanisms of host population regulation of these agents resemble the use of parasitoids and oligophagous predators in the control of agricultural insect pests; so studies of efficacy borrow from the field of insect pest biological control using such natural enemies. (3) A marine environment in which organisms may become widely distributed, and in which native non-target organisms are often related to the introduced pests, requires a careful evaluation of the safety of potential natural enemies. Because this issue is quite comparable to the safety issues pertinent to weed pest control, studies of host specificity and other safety issues are informed by weed pest biological control studies. (4) Parasitology and epidemiology contribute the physiological and ecological knowledge necessary to evaluate the potential agents for safety and efficacy.

It is worth emphasizing at the outset that although this discussion is focused on the use of biological control, we consider this part of an overall control strategy-an integrated pest management philosophy-in which other control approaches may well be essential elements. These include, but are not limited to, eradication, fisheries, chemical agents, and genetic manipulations. Effective management of pests after they have become established enables scientists, stakeholders, regulatory agencies, and the public to develop a coherent policy on specific pests. Without postestablishment control of pests as a goal, even the study of their impacts lacks essential purpose. It is also crucial to stress that control does not obviate our need to prevent further introductions.

The Biological Control Controversy

As recently as 1987, a blue-ribbon panel of ecologists convened by the U.S. National Academy of Scientists recommended that "biological control can and should become the *primary* [pest control method] in the United States" (NAS 1987). This report stressed "the development of biological control as the foundation of pest control in the United States is the most important challenge we face in making safe and efficient use of our managed ecosystems." Yet, since that time, there has been a flurry of papers opposing biological control, or urging that it be considered only as a *last* resort (*e.g.*, Howarth 1991; Secord and Kareiva 1995; Simberloff and Stiling 1996a, b; Hager and McCoy 1998). Some frequently raised arguments against biological control in general are also pertinent to the possible use of scientific classical biological control in marine habitats.

Most references to bad outcomes (putative control agent did little against the pest and did much damage to native organisms) were for non-scientific projects without safety evaluation, and used generalist predators. The agents involved in these ill-conceived projects were vertebrates such as cane toads, mongooses, and mosquitofish. Those "control" agents are very unlike the parasitoid wasps being considered as efficacy models for marine pest biocontrol or for the parasitic castrators under evaluation for green crab (*Carcinus maenas*) biological control in

California. In some cases, safety was evaluated (predatory land snails introduced to Hawaii and Moorea to control the African giant snail), but regulatory agencies disregarded this information (R. Cowie, pers. comm.). Following the Hawaiian disaster, the subsequent release of these predators in French Polynesia, 20 years later, was egregious and inexcusable from an ecological perspective (M. Hadfield, pers. comm.); particularly since these generalist predators did not provide effective control against the African giant snails (Howarth 1991). This was failed politics, not failed science. That safety issues were brushed aside 30, 50, or 75 years ago does not imply that this will easily happen again in our current environmentally conscious cultural climate. But it does suggest that environmentalists must effectively express their informed concerns as stakeholders in the decision-making process.

The history of the recent use of a calicivirus against introduced rabbits in Australia exemplifies another concern. This was certainly a scientifically evaluated biological control agent. However, it was inadvertently released onto mainland Australia, apparently from a pilot release on an offshore island. The rabbit calicivirus had undergone several levels of safety tests, and in Europe and Asia, where it was native, it had never been detected in anything but a lagomorph. However, it had not yet cleared the last regulatory hurdle of approval for safety when it jumped to the mainland and spread rapidly through rabbit populations. This was an unmitigated public relations disaster for the agency responsible for its study and release (Commonwealth Scientific and Industrial Research Organisation, CSIRO) and for biocontrol in general. However, two years later, the calicivirus has not been detected in any animal other than a rabbit, and its efficacy as a biocontrol agent has been gratifying. Several studies indicate that populations of native marsupial herbivores are increasing in the areas where the rabbits have died off (Drollette 1997). So, the CSIRO safety tests were valid, handling of the safety protocols was conservative, and this biocontrol "disaster story" appears to be an important biocontrol success.

The irreversible nature of the release of a biocontrol agent is a focal point of some of the recent critics of biocontrol (Howarth 1991; Simberloff and Stiling 1996a, b; Secord and Kareiva 1995). They extend this to the evolutionary domain as it is possible that some biological control agents may evolve to use new (native) hosts. These "forever" concerns have led some to propose a "white list" approach to biocontrol agents, requiring "proof" of their safety before they could be approved for use as control agents (Ruesink *et al.* 1995; A. Cohen, pers. comm.). (A white list would include only the organisms proven safe for introduction. A black list includes those organisms known or suspected to be economically or ecologically dangerous.) This, of course, is a formula for never using biological control because the hypothesis of proven safety is untestable. No amount of study can guarantee that anything will *always* be safe.

The evolutionary risk argument is diminished by recent theoretical studies which show, for the type of host-specific control agent being proposed for biological control in marine environments, that selective pressures generally intensify specialization. It is selectively difficult for specialists to evolve towards a generalist phenotype (Kawecki 1998; Hastings and Godfray 1999).

Efficacy and Safety

To consider control, there must be evidence of pest potential for an introduced species. Unless the cost of no action is judged to be considerable, it is unlikely that any society will incur the risk of intervention by biological control or any other means. Several introduced marine organisms have attained sufficient pest status for biological control to have been considered as part of a control strategy. In the discussion below, we will draw a few examples from the european green crab, *C. maenas*, a pest in North America (both coasts) and Tasmania, and the northern Pacific starfish, *Asterias amurensis*, a pest in Tasmania and Victoria. Both are voracious generalist predators, reaching high population densities and spreading rapidly where they were introduced.

The effectiveness of biological control in a marine environment is unknown. Case studies of fisheries and analytical models (Blower and Roughgarden 1987; Kuris and Lafferty 1992) indicate that for parasitic castrators and specialized egg predators there is evidence for population regulation of some marine animals by natural enemies. However, there are concerns, raised from another body of fisheries literature, that the weak relationship between reproductive output and subsequent recruitment indicates that only very prevalent natural enemies acting on host reproduction would be effective con-

trol agents. Continuing development of fisheries models with more realistic recruitment dynamics may resolve this conflict.

Another line of reasoning that sheds doubt on the potential efficacy of natural enemies in the control of introduced marine pests is the observation that they may be abundant where they are native. This is quite evident for both *C. maenas* in Europe and *A. amurensis* in Japan (*e.g.*, Kuris *et al.* 1996). This might suggest that native populations are not under control by native natural enemies. However, where these pests were introduced, they certainly are less parasitized in general and are never parasitized by parasitic castrators (Kuris and Lafferty 1992, Kuris and Gurney 1997; Goggin 1998; Lampo and Bayliss 1996; Calvo-Ugarteburu and McQuaid 1998). There is also evidence to suggest that they may experience release from natural enemies, such as parasites, because they may reach larger sizes and show other evidence of improved performance (M. Torchin *et al.*, in prep.). Thus, although some of these introduced marine pests are abundant where they are native, perhaps the question to be addressed is how much more abundant would they be where they are native, if they lacked important natural enemies such as parasitic castrators?

The safety of natural enemies of introduced marine pests closely resembles safety concerns for weed pest control. Since the pest plants often occur with native plants that are either taxonomically related or ecologically similar (or both), a potential herbivore with low feeding specificity would be a very dangerous introduction. Hence, weed pest biocontrol has a well-developed literature on safety and has generally been regulated with this issue a key concern (Harris 1991; Blossey 1995). Until the recent demonstration of significant damage to native thistle in the biocontrol of the introduced star thistle (Louda *et al.* 1997), the record of safety for weed pest biological control was excellent (Blossey 1995). In this case, safety tests conducted prior to the 1968 release of the control agent had actually predicted its spread to native thistles (Boldt 1997). These native plants were considered abundant, weedy, and of little value. Thirty years later, values have changed–for some, all native species are prized and habitat loss has made some formerly plentiful species less abundant (Boldt 1997; E.S. Delfosse, pers. comm.). There is a substantial literature on protocols, experiments and the evaluation of safety tests (see Blossey 1995). These issues will be very important concerns for marine pest control for they illustrate how conflicting values, and which stakeholders bear the costs and the benefits of control, strongly determine regulatory policies.

Some Safety Guidelines

As a starting point for effective safety testing for natural enemies of introduced marine pests, we propose the following general principles:

1. A standard for a successful infection of a potential natural enemy against a target introduced pest must be defined *a priori* and adhered to throughout the testing protocol. This should include its completed development in the native non-target host, or lack of significant pathogenicity in that host.
2. The designed specificity assay must be statistically powerful. It must have a high likelihood to detect an infection in the non-target host.

Accordingly,

3. The experimental exposure procedure must be logistically reliable. Repeated tests must give similar results.
4. To guard against a false negative error, highly sensitive host life stages must be known and used in experimental exposures. Also, the most competent infective stages of the potential control agent must be known and used in these tests. These conditions for effective safety testing require that considerable research on the infection process be known (or conducted) before comprehensive safety tests are initiated. "Quick and dirty" tests are attractive because they are inexpensive, rapid, and may inform some aspects of host specificity, but they should be avoided because they have a high probability of both types I and II errors.

Finally,

5. A plausible mechanism for the theoretical basis for host specificity of the natural enemy must be proposed and evaluated. Even if safety tests support the contention that a control agent is host-specific, the question will immediately arise as to why this is so. If no mechanism is available and consistent with safety test results, then their reliability will be in doubt.

In general, very high standards for experimentation and analysis of host specificity must be established, rigorously defined, closely adhered to, widely recognized, and critically evaluated. A skeptical

public and politically sensitive, cautious, regulatory agencies will be the final arbiters. Only the best science will be able to pass muster in this climate. And that is as it should be.

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Xenodiversity of the European Brackish Water Seas: the North American Contribution

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ABSTRACT: **The biological diversity of the European brackish water seas, either enclosed (Baltic and Black) or isolated from the world ocean (Caspian and Aral), is by far lower than that of the fully marine water bodies. The ecosystem may consist of only a few components, so that only one or two species represent a functional group. Any new species added quite often means major changes in the structure and functioning of the whole system. The European brackish water seas presently contain a lot of "foreign" biological diversity. We define this as xenodiversity (Gr. *xenos* – strange) to indicate structural and functional diversity caused by nonindigenous species (NIS). Until now, 112 species have been included in a Baltic and Black Sea NIS Database. Of these, 91 species are known from the Baltic, 35 from the Black Sea, and of them 16 NIS of non-European origin are common for both seas. The Atlantic coast of North America has exported more species to Europe than any other donor area due to the successive opening of routes of commerce in the post-Columbian era. At present, there are 33 known NIS of American origin in European brackish-water seas; only 14 of them have been intentionally introduced. Once established, the most successful NIS have spread rapidly, among them some neo-Europeans of American origin. The soft-shell clam, *Mya arenaria*, is thought to have been transported by the Vikings in the 13th century; the barnacle, *Balanus improvisus*, appeared in the mid-1800s; the polychaete, *Marenzelleria viridis*, and the comb jelly, *Mnemiopsis leidyi*, in the 1980s. NIS also contribute to the functional diversity. The most aggressive invaders in enclosed European seas have been capable of altering fundamental ecosystem-level processes. This paper presents an overview on invasive status of the largest European brackish water bodies with special emphasis on the North American component of their xenodiversity.**

Key words: introduced species, biodiversity, marine invasion, Baltic Sea, Black Sea, Caspian Sea, Aral Sea

INTRODUCTION

Enclosed seas are defined as being surrounded by land with a narrow entrance that is at least four times smaller in dimension than the longest axis within the enclosed basin (Healy and Harada 1991). From a biogeographical point of view, these are basins isolated from the ocean by physical and ecological barriers (both continents and fully marine waters) of which the salinity factor is one of the most important. These disjunct ecological islands are sufficiently remote for the rate of natural exchange of species to be extremely slow.

The enclosed brackish-water seas of Europe (Figure 1), the Baltic and Black Seas (in this paper, including the Azov Sea), the Caspian Sea, and the Aral Sea (western Asia) are geologically and biologically young seas. Natural changes are still taking place as their ecosystems are subjected to maturation processes. During their geological past, these seas have changed their areas and shapes, their connections with neighboring seas, and their physical and chemical environments.

Some of the main characteristics of the four brackish-water seas of the western part of Eurasia are presented in Table 1. All of these seas have existed in nearly their recent form since the end of the Ice Age. During the last 50 years they have undergone environmental changes due to a great variety of human activities (for reviews, see Aladin and Potts 1992; Glazovsky 1995; Kaplin 1995; Mandych *et al.* 1995; Leppäkoski and Mihnea 1996). The composition of

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Figure 1. Brackish-waters seas and lakes in western Eurasia; (1) Gulf of Bothnia, (2) Gulf of Finland, (3) Baltic proper, (4) Gulf of Riga, (5) Mediterranean Sea, (6) Adriatic Sea, (7) Black Sea and Sea of Azov, (8) Caspian Sea, (9) Aral Sea.

their biotic communities is regulated largely by temperature and other season-bound changes in the non-living environment, giving rise to seasonal successions. The process of enrichment of their biota with new invading species (spontaneously or by means of passive dispersal) from the adjacent, more saline seas still continues.

Table 1. Some physico-chemical characteristics of the western Eurasian brackish water seas.

	Baltic Sea	Black Sea[1]	Caspian Sea	Aral Sea
Connection with adjacent seas	Danish Straits[2]	Bosporus[2]	Rivers, canal[3]	None
Latitude °N	54 -66	41-46	36-47	43- 47
Major axis length km	1,300	1,150	1,200	600[4]
Surface area 10^3 km^2	412	423	378	67[4]
Water volume 10^3 km^3	21	537	78	1
Catchment area 10^3 km^2	1,730	1,860	3,700	1,800
Maximum depth m	459	2,212	1,025	69[4]
Sill depth m	18	32	–	–
Salinity in central parts (surface) ppt	6-7	17-18	12-13	10->30[4]
Temperature in central parts (surface; summer) °C	14-16	22-26	22-28	25-30

[1] excluding the Azov Sea.

[2] interconnected via the rivers Dnieper and Neman and Oginskij Canal, opened in the 1780s.

[3] Volga-Don canal opened in 1952.

[4] before lowering of the level of the Aral Sea; its area was reduced to 53,000 km^2 and volume to 300 km^3; the salinity increased to 34-35 ppm in the early 1990s (Glazovsky 1995).

Enclosed seas are showing increasing signs of environmental deterioration, raising the question of whether more open, well-flushed coastal areas will become damaged to the same degree (GESAMP 1991; see EMECS '90 (1991) for further reading). Besides the marine eutrophication syndrome (Gomoiu 1992), the introduction of nonindigenous species (NIS) tends to have an increasing effect on the biological integrity of coastal waters all over the world.

Today the biota of these water bodies are exposed to each other, due to the breakdown of geographical barriers by ship traffic (Table 2), leading to an exchange of species. In addition to their contacts by straits with the Mediterranean and the Atlantic, the Baltic, Black, and Caspian Sea basins are connected to each other by canals and rivers. Introduction of NIS by shipping operations (spreading with ballast water) or via canals has resulted in major changes in the near-shore subsystems in these seas, whereas the deep bottoms and the pelagic system seem to have stayed practically free from NIS until the 1980s (Kasymov 1982; Leppäkoski 1984, 1991; Cvetkov and Marinov 1986; Jansson 1994; Gollasch and Dammer 1996; Gomoiu 1998).

A global homogenization of aquatic biota is underway, due to the establishment of NIS, and the effects of these species continue to accrue (Ruiz *et al.* 1997; Cohen and Carlton 1998; Lodge *et al.* 1998). Much of the biological diversity of the enclosed seas is "foreign", especially in their coastal inlets and lagoons. We have defined this as xenodiversity (Gr. *xenos*–strange) to indicate the structural and functional diversity caused by nonindigenous species (Leppäkoski and Olenin 1998).

Estuaries have been especially common sites of invasions, accumulating from tens to hundreds of NIS per estuary; *ca.* 400 NIS are known along the Pacific, Atlantic, and Gulf coasts of the United States (Ruiz *et al.* 1997). Alien species have threatened the Great Lakes for centuries. There have been 139 NIS introduced since the 1800s (Mills *et al.* 1993). Of these, <90 species can be regarded as truly aquatic (*i.e.*, marsh and shore flora excluded); more than 40% of these species are native to Europe. Some of them have appeared to be highly aggressive invaders that have had a heavy impact on the ecology of the Lakes.

In this article we review available documentation of the occurrence of known nonindigenous marine and estuarine species of American origin in enclosed

Table 2. Nonindigenous species of American origin recorded in the Baltic, Black, and Caspian Seas and their coastal ecosystems. In the Aral Sea, *Rhithropanopeus harrisii* is believed to be the only American invader since 1976. Introduction < before or >after (year).

Taxonomic group	Species	Baltic Sea	Black Sea	Caspian Sea
BACILLARIOPHYTA	1. *Coscinodiscus wailesii*	1983	–	–
CORMOPHYTA	2. *Elodea canadensis*	1870	–	–
CNIDARIA Hydrozoa	3. *Gonionimus vertens*	1921	–	–
	4. *Garveia franciscana*	1950	1932	–
CTENOPHORA	5. *Mnemiopsis leidyi*	–	1982	–
ANNELIDA Polychaeta	6. *Marenzelleria* cf. *viridis*	1985	–	–
CRUSTACEA Cirripedia	7. *Balanus eburneus*	–	1936	>1952
	8. *B. improvisus*	1844	1899	>1952
CRUSTACEA Copepoda	9. *Acartia tonsa*	1925	1990	–
	10. *Ameira divagans*	<1974	–	–
CRUSTACEA Amphipoda	11. *Gammarus tigrinus*	1975	–	–
CRUSTACEA Decapoda	12. *Callinectes sapidus*	1951	1967	–
	13. *Orconectes limosus*	1890	–	–
	14. *O. virilis*	1960	–	–
	15. *Rhithropanopeus harrisii*	1951	1932	1957
MOLLUSCA Bivalvia	16. *Crassostrea virginica*	1880	>1900	–
	17. *Ensis americanus*	1981	–	–
	18. *Mya arenaria*	>1200	1966	–
	19. *Petricola pholadiformis*	1931	–	–
MOLLUSCA Gastropoda	20. *Crepidula fornicata*	1940	–	–
	21. *Doridella obscura*	–	1980	–
ENTOPROCTA	22. *Urnatella gracilis*	–	1950	–
PISCES	23. *Gambusia affinis*	–	>1920	–
	24. *Ictalurus melas*	1984	–	–
	25. *I. nebulosus*	1982	–	–
	26. *Lepomis gibbosus*	–	>1890	–
	27. *Oncorhynchus mykiss*	1890	*1970s	–
	28. *Roccus saxatilis*	–	**1970s	–
	29. *Salvelinus fontinalis*	1872	–	–
	30. *S. namaycush*	1959	–	–
AVES	31. *Branta canadensis*	1930	–	–
MAMMALIA	32. *Mustela vison*	1925	–	–
	33. *Ondatra zibethicus*	1920s	>1920	–

* unsuccessful introduction (Zaitsev and Mamaev 1997)

** caught in the early 1970s, no more reports (Zaitsev and Mamaev 1997)

European brackish-water seas (the Baltic, Black, and Caspian Seas) and include some data on their occurrence in the west Asian Aral Sea. This report also includes introductions that failed to persist.

Invasive Status of the European Brackish Water Seas

The Baltic Sea

In Europe, the Baltic and Black Seas (both of them being former post-glacial lakes and thus subjected to spontaneous invasion of fauna and flora in the last 10,000 years) are heavily contaminated by NIS; this is true especially for their coastal lagoons and inlets. These seas are a major destination for thousands of cargo vessels and thus are exposed to NIS that travel as a fouling community on the ships' hulls or in ballast water tanks (Gollasch and Leppäkoski 1999).

For the 91 species known or thought until now to be non-native in the Baltic Sea (including the Kattegat Sea), the exact routes of introduction are in many cases unknown (*e.g.*, Leppäkoski 1994; Jansson 1994; see BMB Working Group 30 (1999) for a species list–in this database all marine and estuarine

species known or believed to have been introduced into the Baltic Sea, either intentionally or unintentionally, are listed). Of the 55 unintentional introductions into the Baltic Sea, with more-or-less known dispersal history, 31 are transoceanic and among these are 14 trans-Atlantic species of American origin (Table 2).

In the Baltic Sea, American species have been introduced unintentionally (58%) rather than intentionally (42%). However, very little is known about the invader status of smaller organisms, such as planktonic algae, copepods, and rotifers, which are transferred in huge numbers in ballast tanks. In the coastal inlets of the northern Baltic Sea, alien benthic animals do not generally dominate over large areas. The only exceptions are the barnacle *Balanus improvisus* (dominant on firm substrates in the upper sublittoral) and, since the early 1990s, the polychaete *Marenzelleria viridis*. Both species are of American origin.

Black Sea

The Black Sea has a long history of unintentional introductions and there is evidence of large-scale biological contamination by NIS in the northwestern and western Black Sea zoobenthic communities. Species introduced into the Black Sea originate predominantly from two donor areas: the North Atlantic and the Indo-Pacific (Zolotarev 1996). About 45% of the NIS in the Black Sea originate from the Northern Atlantic (mainly North America; Figure 2, Table 2) and 35% from the Indo-Pacific (Gomoiu 1998). There is only one intentionally introduced American species in the Black Sea (*Crassostrea virginica;* not established), while non-indigenous fish species of American origin (*Oncorhynchus mykiss, Gambusia affinis, Lepomis gibbosus,* and *Roccus saxatilis*) have spread from their areas of introductions in adjacent fresh waters and into the most diluted parts of the Black Sea, *e.g.*, the Danube delta.

Among the species unintentionally introduced into the Black Sea, listed by Cvetkov and Marinov (1986), are some representatives of the northwest Atlantic (most probably North American) invertebrate fauna. The first known ones are the barnacles, *B. improvisus* and *B. eburneus*, introduced in the late 1800s (Cvetkov and Marinov 1986; Zaitsev and Mamaev 1997). In addition, the decapod *Rhithropanopeus harrisii* appeared in the 1930s, the hydrozoans *Bougainvillia megas* in the 1930s, and the bivalve *Mya arenaria* in the early 1960s. Since then, at least five NIS of different origin have been added to this list (Gomoiu 1998), including two American species: the nudibranch mollusc, *Doridella obscura* (1980), and the ctenophore, *Mnemiopsis leidyi* (1982). Even if the number of NIS in the Black Sea is relatively low, these species have had significant ecological and economic consequences in the area (see below; Gomoiu and Skolka 1996; Leppäkoski and Mihnea 1996; Zolotarev 1996; GESAMP 1997).

Caspian Sea

The Caspian Sea fauna includes at least 16 NIS among the more than 720 native zoobenthic species recorded in the sea (Kasymov 1982; Cheplaga and Tarasov 1997). The first known NIS, the Mediterranean bivalve, *Mytilaster lineatus*, was brought to the Caspian Sea as a fouling organism on the hulls of small ships transferred by trailer from the Black Sea (Bogachev 1928). The introduction of *Mytilaster* during the Civil War (1918-1920s) caused the first anthropogenic crisis in the Caspian Sea because it resulted in the extinction of some endemic Caspian dreissenids (Tarasov 1997). Another crisis was related to the intentional introduction of the polychaete *Nereis diversicolor* and the bivalve *Abra ovata* from the Black and Azov Seas in the late 1950s-early 60s, which also led to elimination (or reduction) of some typical Caspian forms. The benthic community was restructured: *A. ovata* replaced the native cockle *Cerastoderma* on muddy-sediment bottoms. Also, *R. harrisii* vanished from the soft-sediment bottoms (Cheplaga and Tarasov 1997).

The Caspian Sea was connected with the Black and Azov Seas via the rivers Volga and Don, interconnected by a canal between the rivers in 1952. Opening this canal permitted the American invaders *B. improvisus, B. eburneus,* and *R. harrisii* (first found in 1957), among others, to penetrate into the Caspian Sea (Kasymov 1982). In 1976, NIS formed 30–99% of the benthic biomass in most parts of the Caspian. This dominance can be explained by the inability of native fauna to tolerate salinities >13 ppt; there was a slight increase of the salinity in some of the Caspian bays studied by Kasymov (1982).

Features of the globalization process are seen also in this isolated sea. Cheplaga and Tarasov (1997) concluded that "a big part of the Caspian Sea is now in

the process of transforming to a system where species widely distributed in the World Ocean predominate".

Aral Sea

The Aral Lake, called Sea by tradition and formerly the world's fourth largest lake, does not have any connection via waterways to adjacent seas. A major ecological catastrophe is taking place as a result of increasing salinity due to the excessive use of water for irrigation in the Aral drainage basin beginning in the early 1960s (Aladin and Potts 1992; Glazovsky 1995). The level of the sea fell by 16 m from 1960 to 1991, and the surface area was reduced from 67,000 to 53,000 km^2; the water volume decreased by > 60% (Table 1). Drying up the sea resulted in an increase in salinity from 10 ppt up to > 30 ppt. Most of the native fauna of freshwater and low-brackish-water origin have been eliminated and replaced by marine and brackish water NIS, transferred deliberately or accidentally from the Caspian, Black, Azov, and Baltic Seas.

Since 1927, many species (only fish before 1954) have been introduced to the Aral Sea (Glazovsky 1995); altogether 25 NIS are mentioned by Aladin and Potts (1992), among them *N. diversicolor* and *A. ovata*, introduced from the Sea of Azov in the 1960s. The only American species in the Aral fauna is *R. harrisii*, first found in 1976, probably brought in accidentally with the intentional introduction of species from the Caspian Sea or the Azov Sea.

Neo-Europeans of American Origin

In the Baltic and Black Seas, 33 species in total or 30% of all known introductions are of American origin (Figure 2). To focus this paper, we limit our attention to some representatives of American invaders in European seas, with special emphasis on those species that became invasive and have posed significant ecological or economic impacts. Most of these species were introduced unintentionally in the 20th century.

Marenzelleria viridis

Some of the neo-Europeans of American origin in European enclosed seas have appeared to be big and aggressive in comparison to previously dominant organisms in the same habitats. The new inhabitants of the Black Sea are the largest animals in their taxonomic groups (Zolotarev 1996). In the Baltic Sea, the North American spionid polychaete *M. viridis*

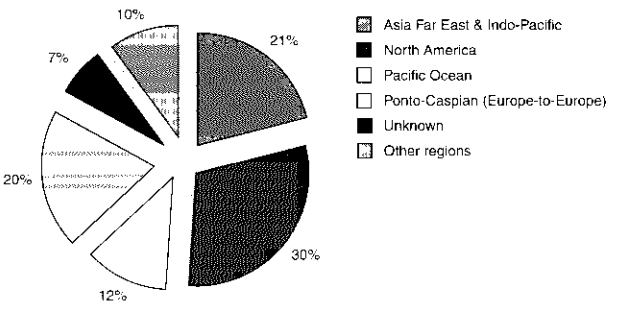

Figure 2. Origin of the Baltic and Black Sea xenodiversity.

burrows down to 40 cm in the sediment and is a giant compared to native burrowing organisms (chironomid larvae and oligochaetes) dwelling in muddy bottoms of the Baltic coastal lagoons (Olenin and Leppäkoski 1999). Conveyed from North America, *M. viridis* was found in Scotland in 1982 and in Germany/the Netherlands in 1983. It was first recorded in the southern Baltic in 1985. After its initial establishment, it successfully colonized, by secondary spread, most of the Baltic Sea and developed into a major faunal element (*e.g.*, Zettler 1996; Bastrop *et al.* 1998). In 1988, it appeared in Polish coastal waters and in 1990 on the southern coast of Finland (Gruszka 1991; Norkko *et al.* 1993). During 1990-1993, *M. viridis* expanded its distribution into the eastern parts of the Gulf of Finland and into the southern part of the Gulf of Bothnia (Stigzelius *et al.* 1997).

The introduction of *M. viridis* into the Baltic Sea has probably occurred via ship transport, as most of the first findings are connected to harbor areas. Although the abundance and biomass of *M. viridis* in open coastal waters is comparatively low, the species seems to have become a permanent member of the macrozoobenthos in the northern Baltic (Stigzelius *et al.* 1997). In the coastal inlets and lagoons of the southern and southeastern Baltic, it developed high densities (up to 5,000-30,000 ind. m^{-2}) and biomasses (up to 400-800 g wwt m^{-2}) in some Polish and German estuaries (Bochert *et al.* 1996; Zettler 1996, 1997a; Zmudzinski *et al.* 1996). For example, in Vistula Lagoon (southeastern Baltic) 95% of the total biomass of bottom fauna consists of *Marenzelleria*, the invasion of which began in the mid-1980s (Zmudzinski 1996).

On the Finnish coast, *M. viridis* has become astonishingly common in some areas. In a benthos study off the town of Rauma (eastern Bothnian Sea),

it was found in 82% of the samples (mean density 290 ind m^{-2}, n = 141) at <15 m depth in 1994; *Potamopyrgus antipodarum* (a gastropod species native to New Zealand) occurred in 21% and *M. arenaria* in 3% of the samples (K. Jumppanen and R. Räisänen, unpubl. data). In the inlets off the town of Turku (inner Archipelago Sea), *M. viridis* was recorded in 17%, *P. antipodarum* in 14%, and *M. arenaria* in 4% of samples at <15 m depth (n = 81) in 1995 (R. Räisänen, unpubl. data; Olenin and Leppäkoski 1999). In contrast to the coastal lagoons of the southern Baltic Sea, the abundance and biomass of the species in the Finnish coastal waters has remained comparatively low (maximum >300 ind m^{-2} and >2 g wwt m^{-2}).

In the coastal waters of northern Germany, *M. viridis*, a euryhaline polychaete, has colonized a wide range of habitats from almost limnic (0.5 ppt) to brackish (up to 10 ppt; Zettler 1996). Off the Polish coast, it has been found from only a few meters down to about 50 m (Zmudzinski *et al.* 1996). The deepest open sea occurrence is known from the southern Bothnian Bay at the depth of 78 m (Stigzelius *et al.* 1997). The depth distribution of *M. viridis* gives an excellent example of brackish water submergence (Remane and Schlieper 1958; Zmudzinski *et al.* 1996): several species known to live on shallow bottoms or only in the intertidal zone in the North Sea have extended their depth range in the Baltic Sea. This phenomenon has been explained as a dependence on salinity stratification, or the absence of predators and competitors in the Baltic. In fact, *M. viridis* is the first NIS in the Baltic to colonize deeper soft bottoms below the halocline; brackish water submergence elucidates the flexibility (plasticity) of NIS in a novel environment (*cf.* Vermeij 1996).

Marenzelleria has created a new food resource for fish. In German coastal inlets, its larval abundance has reached 10^6 ind m^{-3} and the number of newly settled juveniles 270,000 ind. m^{-2} (Bochert *et al.* 1996). Both piscivorous (*Rutilus rutilus, Stizostedon lucioperca,* and *Perca fluviatilis*) and planktivorous (*Clupea harengus*) fish feed temporarily on *M. viridis* or its larvae (Winkler and Debus 1996). This polychaete possesses several attributes of a highly successful colonizer. Its rapid within-basin spread in the Baltic Sea is due to its high fecundity (each female produces 28,000-40,000 eggs), the pelagic phase lasting about four weeks (Bochert *et al.* 1996). Moreover, both the larvae and benthic juveniles are being transported by the anticlockwise currents typical of the Baltic and its Gulfs. Zettler (1997b) did not report any negative consequences for the indigenous macrozoobenthos; on the contrary, there was a positive correlation between *M. viridis* and chironomid larvae.

According to Bastrop *et al.* (1998), there are two species of *Marenzelleria* (*M. viridis* and *M. wireni*) introduced from the northwestern Atlantic to Europe in the 1980s. Based on analysis of mitochondrial 16S rDNA sequences (326-bp segment) of 98 individuals from 17 localities on the North American, North Sea, and Baltic coasts, they were able to separate the locations of origin of the introductions. The individuals from the Baltic Sea probably originated from the Atlantic coast of the United States between Chesapeake Bay and Georgia, and the North Sea populations may stem from the coast region north of Chesapeake Bay to Nova Scotia. Despite their similar morphologies, the two European *Marenzelleria* species may differ ecologically with respect to their preference for habitat salinity.

The recent invasion history of *M. viridis* has been more thoroughly studied than that of any other NIS in the Baltic Sea. The biology and ecology of this extremely invasive species is intensively studied mainly by graduate students in all riparian countries. These studies will provide valuable knowledge of the dynamics and community impacts of an NIS in the soft-bottom subsystem.

MYA ARENARIA

Since the end of the 19th century, it has been understood that the soft-shell clam *M. arenaria* is a late invader and a trans-Atlantic introduction into the coastal waters of Scandinavia (Hessland 1946). Based on multidiciplinary oceanographic, geological, and archaeological evidence, Hessland (1946) was able to show that this species became extinct during the Ice Age and probably did not recolonize European coastal waters before the 16th or 17th century. However, the history of marine introductions from North America to Europe goes back to the pre-Columbian era. The Vikings are thought to have transported *M. arenaria* to Europe in the 13th century (Petersen *et al.* 1992). Although the invasion history of *M. arenaria* in the Baltic Sea is not known, this species is known as one of the most common shallow-water molluscs of western Europe and as a naturalized species in most of the Baltic Sea. Its impact in the Baltic Sea has been benign.

In the Black Sea, *M. arenaria* is one of eight introduced mollusc species (the bivalves *Teredo navalis*, *Paphia philippinarum*, *Cunearca cornea*, *Crassostrea gigas*, and the gastropods *Rapana thomasiana*, *P. antipodarum*, and *D. obscura*). Of these recent introductions, the American *M. arenaria*, the Japanese predacious gastropod *R. thomasiana*, and the Indo-Pacific bivalve *C. cornea*, have markedly altered the structure and function of the benthic community. In places along the Bulgarian coast, the native *Venus gallina* community turned into a *C. cornea* community. Introduced in the early 1980s, *C. cornea* became very numerous: within less than five years it reached maximum biomass values up to 4,300 g m^{-2} (Cvetkov and Marinov 1986).

Introduced possibly from ballast water discharged by oil tankers, and first recorded in the Black Sea in 1966 (Zaitsev and Alexandrov 1998), *M. arenaria* became the dominant benthic species in sediment bottoms down to 30-40 m along the Romanian coast in the 1970s. In some northwestern parts of the Black Sea, its biomass exceeded 1,000 g m^{-2} (Zaitsev and Mamaev 1997). Within 15 years, *M. arenaria* became abundant not only in the northwestern and western Black Sea but also in the Azov Sea. Its Black Sea populations appear to have peaked in 1972 (frequency 72% and average density 58 ind. m^{-2} ; Zolotarev 1996). On the Romanian coast *M. arenaria* made up 60-89% of the total biomass of molluscs in 1976-1979 (Petranu 1997). Flourishing populations of the bivalve *Corbula mediterranea* were reduced by competition by *M. arenaria* (Gomoiu 1981), serving as an example of competitive exclusion. The density of *C. mediterranea* decreased from 21,000 ind m^{-2} in 1965 to only 10 ind m^{-2} in 1991.

The effects of *M. arenaria* on the sublittoral community ecology were many and varied. It acted as an effective biofilter utilizing the increased resources of particulate organic matter, which had increased due to eutrophication. *M. arenaria* also produced a great quantity of planktonic larvae, which formed a new food basis for fish such as turbot, young sturgeons, and gobies (Petranu 1997). Shell deposits along the beaches were dominated by *C. mediterranea* until the early 1970s. In 1977–81, *Mya* shells entered sedimentary processes in huge amounts, and tons of shells were deposited along the Mamaia beach in Romania (Gomoiu 1981). *Mya* shells formed a major source of beach deposits in this area, but also caused considerable changes in the granulometry and chemistry of these beaches and affected their resistance to shore erosion (Gomoiu 1983).

Together with other macrobenthic species, *M. arenaria* was seriously affected by hypoxia, due to exceptional phytoplankton blooms, in the late 1980s. Its numbers decreased from > 6,000 ind m^{-2} (max. biomass 3,000 g wwt m^{-2}) in 1970-1975 to 400 ind m^{-2} (260 g m^{-2}) in 1991 (Tiganus 1992).

Balanus improvisus

B. improvisus, the only barnacle species living in the Baltic proper, is likely to have been introduced from North America. It was first found in the southeastern Baltic in 1844, *i.e.*, 10 years before being described by Darwin, and became common especially in ports (Gislén 1950). The introduction of *B. improvisus* into Europe was so successful that now, not only most of the Baltic, but also the Black Sea (first found in the 1890s; Gomoiu and Skolka 1996) and the Caspian Sea are colonized. It is capable of marked habitat alteration through the construction of dense crusts on hard surfaces and secondary hard substrates.

In dense populations of *B. improvisus*, associated species such as chironomid larvae, ostracods, copepods, and juvenile bivalves increased compared to adjacent sites without crusts (A. Åvall, unpubl. data). The main effect of the barnacle was facilitating settlement of other organisms—as early as 1868 it was observed in a river in southwest Finland among entirely fresh-water organisms (Luther 1950).

Mnemiopsis leidyi

The dramatic increase in biomass of *M. leidyi*, native to the east coast of the Americas from Cape Cod to Brazil, is one of the most spectacular events in the history of species introductions into marine systems and raised much concern in all of the Black Sea countries in recent years. *Mnemiopsis*, probably brought in the ballast water tanks of ships, was first found in the Black Sea in 1982 (Vinogradov *et al.* 1989; Zaika and Sergeyeva 1990; Zaitsev and Alexandrov 1998), but there is uncertainty as to the exact timing of the introduction.

Mnemiopsis feeds on planktonic crustaceans and pelagic fish eggs and larvae (Harbison and Volovik 1994). *Mnemiopsis* is characterized by very high fertility: in a population explosion culminating in the late 1980s in the Black Sea, it developed biomasses >1 kg m^{-2} in the open sea and >5 kg m^{-2} in coastal waters

and had spread all over the Black Sea. The total biomass of *Mnemiopsis* in the Black Sea was estimated at 800 million tons in August-September 1989 (for reviews, see Shuskina and Musayeva 1990; Malyshev and Arkhipov 1992; Kideys and Niermann 1993; Harbison and Volovik 1994; GESAMP 1997). It remained extremely numerous until 1991–1992, in some coastal areas its biomass reached 10–12 kg wwt m^{-2} but did not exceed 1.5–3 kg wwt m^{-2} in the open Black Sea in 1988-1990. Since then its abundance has been reduced to a level 4–6 times lower (Vinogradov *et al.* 1993a).

The zooplankton community of the Black Sea and the Sea of Azov was severely affected by this invasive predator. Predation on mesozooplankton by *Mnemiopsis* appears to have affected the composition of copepodan and cladoceran fauna and reduced populations of *Oithona similis* and *O. minuta* (Konsulov 1998). The numbers of copepods (*e.g.*, species of *Calanus, Paracalanus, Pseudocalanus, Acartia,* and *Oithona*) decreased 15- to 40-fold and the biomass of the jellyfish *Aurelia aurita* to <5% of levels found in the early 1980s (Shuskina and Musayeva 1990; Vinogradov *et al.* 1993b); the chaetognath, *Sagitta* sp., declined more than 10-fold.

In its area of origin, *Mnemiopsis* becomes very abundant during summer and autumn; its heavy grazing pressure on mesozooplankton results in depressed copepod populations in, *e.g.*, the Chesapeake Bay (Wulff and Ulanowicz 1989; GESAMP 1997). *Mnemiopsis* has been thought to represent a "dead end" of food chains in the Black Sea, especially since the decline in the 1970s of the main consumer of gelatinous species, the mackerel (*Scomber scombrus*). Recent studies demonstrate, however, that the scad, *Trachurus trachurus*, feeds on the juvenile forms of *Mnemiopsis* that convert detritus and phytoplankton into fish food and thus act as a "de-eutrophicating agent" (Konsulov 1998).

This comb jelly invasion was generally held responsible for the catastrophic decrease in commercial fisheries in the Black and Azov Seas. Simultaneous with the increase of *Mnemiopsis*, there has been a significant decrease not only in the number of anchovy eggs and larvae (Niermann *et al.* 1994), but also in anchovy, sprat, and horse mackerel stocks and a dramatic decline in pelagic fisheries (Zaitsev 1992; Kideys 1994). Traditional fisheries and fish industries were severely affected during the years of its mass occurrence, the *Mnemiopsis* invasion being the most probable reason for the loss of a $250 million fishery (Ruiz *et al.* 1997).

There is a potential of transport of *Mnemiopsis* into the Baltic although its temperature preferences are very different from those provided by the Baltic environment. Due to several similarities between the Black Sea and the Baltic Sea, the Baltic is obviously more open to immigration of NIS from the Black Sea (and vice versa), than from the more marine European seas (GESAMP 1997).

Other American Crustaceans

R. harrisii has spread from its European bridgehead in the Netherlands (first found in 1874; Turoboyski 1973) into the Black Sea (first recorded in 1932), the Baltic Sea (1951), the Caspian Sea (1957), and the Aral Sea (1976). It became rather common in brackish lagoons on the Polish coast and in the Caspian Sea. The main food item of larval *R. harrisii* in Dead Vistula (an abandoned arm of the Vistula river in Poland) is *B. improvisus* larvae, while adults feed mainly upon *N. diversicolor, Mytilus edulis, Dreissena polymorpha,* and *Cordylophora caspia* (Turoboyski 1973). Three of these five species fed upon are non-native, *i.e.*, whole food chains can be based on NIS in some coastal lagoons. In addition, juvenile crabs use empty carapaces of *B. improvisus* as hiding places. The number of findings of the North American blue crab *Callinectes sapidus* in the Baltic is low. Since 1968, *C. sapidus* has been found in the Black Sea as well but has not become common (Gomoiu and Skolka 1996).

Gammarus tigrinus, a North American brackish (oligohaline) and freshwater amphipod, was brought into northwestern Germany in 1957. The first record from the Baltic Sea is from the German coast in 1975; it has now spread as an epifaunal and infaunal species to Polish estuaries and coastal lakes in the east (Gruszka 1999). The copepod *Acartia tonsa* has been known as a NIS in the Baltic Sea since the 1930s (Leppäkoski 1984). Its presence in plankton samples from the Ukrainian part of the Black Sea in 1990 was reported by Belmonte *et al.* (1994).

Birds and Mammals

Some intentional introductions for hunting purposes of semi-aquatic North American birds and mammals into northern and central Europe have been successful: muskrat (*Ondatra zibethicus*), American mink (*Mustela vison*, an escapee from fur

farms), and Canada goose (*Branta canadensis*). Of these, the muskrat has become common also in southeastern Europe, *e.g.*, the Danube delta. In 1969, over 1 million muskrat skins were taken from the Aral Sea (in the Amu Darya and Syr Darya deltas) (Glazovsky 1995).

Impacts on Man's Uses of the Sea

Not only are biological invasions a major global environmental problem but they are also an economic problem (Cohen and Carlton 1998). In many cases, the impacts of most NIS established in fully marine conditions remain unknown, and the predictability of their direct and indirect effects remains uncertain (Ruiz *et al.* 1997). In the species-poor ecosystems of enclosed brackish seas, the situation is rather different. The number of both native and alien "black list" species is relatively low and thus their impact is easier to assess. We also believe that under such circumstances alien species provide an opportunity to test hypotheses on what characteristics of NIS or habitats are related to successful establishment (*cf.* Lodge *et al.* 1998).

In the Baltic, Caspian, and Aral Seas the impact of NIS of American origin, with a few exceptions, has obviously been benign. As to the fouling species, there were only a few native animal species in the Baltic proper and its major Gulfs: the common mussel *Mytilus trossulus*, the bryozoan, *Electra crustulenta* (whose biofouling potential is low), and the hydroid, *Laomedea loveni*, which foul ship hulls and cause losses in heat exchangers at industrial plants that use cooling water from the sea (Vuorinen *et al.* 1986; Gollasch and Leppäkoski 1999). This was prior to the invasion of *B. improvisus, D. polymorpha*, and *C. caspia* that now are common members of the biofouling community in shallow waters, especially at river mouths and in coastal inlets.

In such cases, the arrival of new species has raised a serious problem for the coastal industries and their use of sea water for different purposes. Some NIS interfere with boating and shipping, even in the low-salinity waters of the Baltic. The extensive use of antifouling paints is due not only to annual filamentous algae, but mostly to the attachment of *B. improvisus* on boat hulls.

Some environmental problems have been caused by alien vertebrate species inhabiting parts of the Baltic coastline, namely *B. canadensis, M. vison,* and *O. zibethicus* (in sheltered coastal inlets only), all of them introduced from North America. The deliberate introduction of mink has caused severe negative impacts on the native communities. Its feeding on the nesting sites has threatened bird populations. Of the American invaders in the Black Sea, the comb jelly *M. leidyi* has caused tremendous changes in the ecosystem and substantial economic losses. Its main impacts on fisheries were identified by GESAMP (1997): (1) predation on fish eggs and larvae, (2) feeding on larvae and adult fish food, thus causing starvation, and (3) accelerating ongoing environmental change due to eutrophication (*e.g.*, anoxia as a result of decaying *Mnemiopsis* on the bottom on the shallow shelf). Prior to the *Mnemiopsis* invasion, there was a change in the Black Sea fisheries from large pelagic predators, abundant in catches from 1969 to 1970, to pelagic plankton feeders that made up 95% of the catch in the early 1990s. The reduced diversity of the catchable stock may have resulted in greater vulnerability of the Black Sea fishery to external impacts (GESAMP 1997).

Discussion and Conclusions

The European history of non-native aquatic species can be divided into three eras: (1) early accidental introductions, (2) a period of experimenting with potentially economically beneficial species, and (3) modern introductions (intentional ones more or less banned, but unintentional ones increasing mainly due to ballast travellers). There are 33 known exotic species of American origin in European brackish-water seas. Only 14 of these have been intentionally introduced; most of the introductions of American species have resulted from trans-Atlantic movement of ballast water.

Once established, several recent NIS of American origin have been able to colonize their preferred habitats on a basin-wide scale (*M. arenaria* and *B. improvisus* in the Baltic and Black Seas, *B. improvisus* in the Caspian Sea, *M. viridis* in the Baltic Sea, *M. leidyi* in the Black Sea, *M. vison* in the northern archipelagos of the Baltic Sea). One of the prerequisites has undoubtedly been the uniformity of the surface water layer in these seas. As soon as the invasion corridor has been opened and a primary establishment has taken place, there are no within-basin barriers to prevent their further spread within the sea.

Several aggressive invaders are known to have pushed the target ecosystems close to the edge of chaos, while many other NIS have established them-

selves without causing any noticeable effects on the indigenous ecosystem. We conducted an analysis of some of the coastal inlets and lagoons of the Baltic Sea (Olenin and Leppäkoski 1999) to assess the ecological impact of NIS by comparing pre-invasion macrozoobenthos data with post-invasion data taken from the same site in the Curonian Lagoon (southeastern Baltic) in which NIS dominate many habitats in terms of number of species, number of individuals, and biomass (Figure 3). The overall number of species was greater following the establishment of NIS. It was shown that NIS in these species-poor native communities manifest their ability of modifying their new habitats in several ways by

- increasing the physical diversity on homogenous sandy and muddy silty bottoms by adding new spatial components into the benthic subsystem (*e.g.*, shells, shell fragments, and burrows), and through this impact create new microhabitats for associated fauna;
- increasing functional diversity;
- increasing bentho-pelagic linkages and thus, the 3-dimensionality of benthic subsystems, or in a broader context, expand the n-dimensional matrix for a species to enter a habitat successfully (*cf.* Carlton 1996);
- broadening the food base of both benthos- and plankton-eating fish and thus modifying the impact of predation on native species; and
- retaining more of the river input of particulate and dissolved nutrients in semi-enclosed coastal systems.

The detrimental (often catastrophic) effects of alien organisms on different human uses of coastal waters and the economy are well known. The seemingly "beneficial" contributions listed above of NIS to the structural and functional diversity must be carefully evaluated in relation to their capacity of competing for space and other resources with indigenous biota and to their negative influence on the settlement of larvae and juveniles of native species.

Were there vacant niches available for the neo-Europeans of American origin or not? There are many examples of "empty" habitats and trophic niches, especially in the Baltic Sea, occupied and utilized by alien species. Some habitats, *e.g.*, secondary hard bottoms (underwater constructions), seem to be rather open to alien fouling organisms everywhere (Leppäkoski 1984). The NIS having most significant effects on coastal waters of the Baltic Sea prior to the

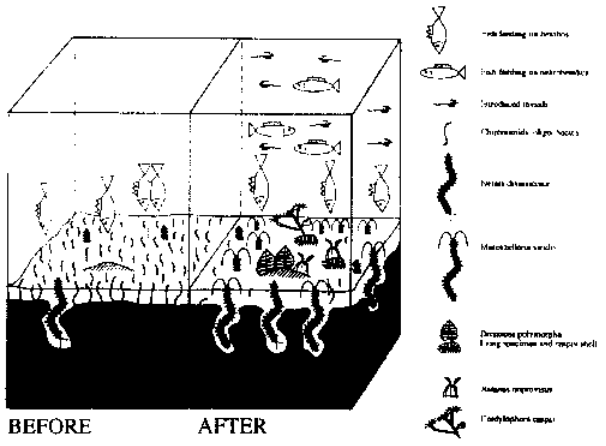

Figure 3. Habitat changes in the benthic and nektobenthic subsystems following the introduction of nonindigenous invertebrates. The model represents a site in the northern part of the Curonian Lagoon, southeastern Baltic Sea (Olenin and Leppäkoski 1999).

invasion of *M. viridis* were, without doubt, *B. improvisus* and the Ponto-Caspian zebra mussel, *D. polymorpha* (in coastal inlets and lagoons only). These species increase the area and volume available for associated macro- and meiofauna, and enhance detritus-based food chains by supplying their habitat with particulate detritus.

The invasion of some successful non-native species has resulted in major changes in community structure and functional biodiversity of the receiving ecosystems of the most intensively inoculated recipient regions. Examples of novelty in functions brought into the Baltic by the American invaders are

- *B. improvisus*—suspension filter feeding in the uppermost hydrolittoral zone;
- *M. viridis*—deep bioturbation of the sediment; this polychaete burrows deeper than most native species, thus increasing the thickness of the populated surface sediment layer and accelerating exchange of nutrients in the water/sediment interface;
- *R. harrisii*—epibenthic invertebrate predator (and scavenger) in the diluted parts of the inlets where native marine decapod crustaceans do not occur;
- *M. arenaria*, the shells of which form a secondary hard substrate available for associated species in the sandy southeastern Baltic Sea coastal zone, and the shells of *B. improvisus* that serve as new microhabitats for small annelids, crustaceans, and chironomids and facilitate colonization of substrate-specific species (*cf.* Olenin and Leppäkoski 1999). Both species create patches of hard substrate for sessile species on uniform soft bottoms.

A similar habitat alteration was observed in the Black Sea, following the introduction of *M. arenaria* in the 1960s, which affected the composition and granulometric structure of shallow-water and seashore deposits (Gomoiu 1983; Leppäkoski 1991).

In conclusion, it is apparent that several introduced species from North America have been able to compete with native species, and in some cases displace them, in the Baltic, Black, and Caspian Seas. This fact gives further evidence of the opportunistic nature and expansive capacity of introduced species, once transported with human aid over physical and ecological barriers into new habitats.

The role of non-native species in ecosystem functions (*e.g.*, nutrient cycling) and their ability to displace native species in the European brackish-water seas is still badly understood. Our results from the Curonian Lagoon (southeastern Baltic), confirm the statement (Crooks 1998) that alteration of habitats is an important effect of exotic species, and that these habitat alterations can have subsequent effects on resident biotic communities.

Once introduced into an ecosystem, dispersal may be easier for aquatic than for terrestrial species as water movements facilitate dispersal and there are fewer dispersal barriers in water (Lodge *et al.* 1998). This hypothesis is clearly supported by our data: within-the-sea dispersal has appeared to be rapid and effective in all the seas compared, as demonstrated by the most successful invaders of American origin. The biogeographical peculiarities of the European brackish-water seas, developed since the last glaciation period, are threatened and the seas are, to an increasing extent, subject to worldwide biological homogenization of the brackish-water fauna.

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Historical and Modern Invasions to Port Phillip Bay, Australia: The Most Invaded Southern Embayment?

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ABSTRACT: **Port Phillip Bay (PPB) is a large (1900 km^2), temperate embayment in southern Victoria, Australia. Extensive bay-wide surveys of PPB have occurred between 1803 and 1963. In 1995/96 the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Centre for Research on Introduced Marine Pests (CRIMP) undertook an intensive evaluation of the region with the aims of developing a comprehensive species list of native and introduced fauna and contrasting previous bay-wide assessments with a current field survey in order to detect new incursions and discern alterations to native communities. Two methods were used to meet these aims: a re-evaluation of regional museum collections and published research in PPB to identify and determine the timing of introductions; and field surveys for introduced benthic (infauna, epifauna, and encrusting) organisms conducted by CRIMP between September 1995 and March 1996. The historic component of PPB invasions groups into four periods based on significant shifts in trade activities: exploration/colonization (pre-1839), immigration (1839-1851), Gold Rush (1852-1860), and modern mechanisms (including aquaculture; 1861-present). Invasions within PPB appear to be increasing, possibly due to an increase in modern shipping traffic and an increase in aquaculture (historically associated with incidental introductions); however, the records of extensive biological surveys suggest that this may in part be an artifact of sampling effort. As expected, the majority of introductions are concentrated around the shipping ports of Geelong and Melbourne. Recent incursions into the region include *Undaria pinnatifida, Codium fragile* ssp. *tomentosoides, Asterias amurensis, Schizoporella unicornis,* and *Pyromaia tuberculata.* Port Phillip Bay is presented as one of the most invaded marine ecosystems in the Southern Hemisphere.**

Key words: survey, introduction mechanism, vector, invasion rate, invasion history, Australia

INTRODUCTION

The threat to biodiversity by introduced species has long been recognized for island systems (Elton 1958). As the only island continent, Australia has developed stringent barrier controls to limit the entry of nonindigenous organisms. This holds true for terrestrial and freshwater systems, and more recently for marine systems. Despite these efforts, Australia has experienced numerous invasions on par with the spectacular international invasions of the Atlantic comb jelly, *Mnemiopsis leidyi*, into the Black Sea, the zebra mussel, *Dreissena polymorpha*, into the North American Great Lakes, and the Asian clam, *Potamocorbula amurensis*, into San Francisco Bay, California. In the late 1980s, the northern Pacific seastar, *Asterias amurensis*, was identified in the Derwent Estuary of Tasmania. *A. amurensis* has attained an estimated population of 28 million and more recently has spread (most likely via coastal shipping) to Port Phillip Bay (PPB) where current population estimates are of similar magnitude (~30 million). Similarly, the edible Japanese kelp, *Undaria pinnatifida*, introduced near Triabunna, Tasmania, in the mid-1980s, has since spread to a region of approximately 100 km along the coast and was discovered in PPB in 1996. Other high-profile invaders include the Mediterranean fanworm, *Sabella spallanzanii*, the Asian alga, *Codium fragile* ssp. *tomentosoides*, and feral settlement of the maricultured Pacific oyster, *Crassostrea gigas*.

In 1995, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Centre for Research on Introduced Marine Pests (CRIMP) began a two-fold effort to determine the extent of invasions in Australian coastal waters. First, the Australian National Introduced Species Port Surveys Program was established in conjunction with the Australian Association of Ports and Marine Authorities (AAPMA), in which 62 international ports of

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first call are being surveyed using a consistent protocol (Hewitt and Martin 1996). Second, the Port Phillip Bay Introduced Species Study was begun to provide a detailed analysis of the invasion history and introduced species status of a major Australian embayment. PPB was selected based on several factors: its long history of use by maritime trade extending back to the early 1800s; extensive surveys and evaluations for the physical and biological characteristics; numerous scientific collaborators in the immediate vicinity; and CSIRO's previous work in the region relating to taxonomy, distribution, and ecology of the biota.

This paper provides a short synopsis of the results of Port Phillip Bay Introduced Species Study (Hewitt *et al.* 1999), which was a collaborative effort involving Victoria, CSIRO, and New Zealand scientists and represents one of the most thorough investigations of the introduced species status of a single embayment in the world, particularly the Southern Hemisphere.

Methods

A review of the vectors for species transfer, historical trade activities, and shipping patterns into PPB was conducted by CRIMP. The bioregion scheme developed by the International Union for the Conservation of Nature (IUCN; Kelleher *et al.* 1995) was used to aid in evaluating species origins and changes in trade route patterns through time. These bioregions are based on marine physical properties (*e.g.*, salinity, temperature, ocean currents) with secondary regard to biological criteria. An additional classification was created for cosmopolitan species (defined as a species whose native distribution was described as being greater than five IUCN bioregions).

Reviews of major groups were commissioned with specific emphasis on those groups for which taxonomic expertise was available in Victoria (Table 1). The taxonomic experts were requested to review the literature and re-evaluate the museum and personal collections with an eye towards introduced and cryptogenic marine and brackish water species. If necessary, additional field collections were encouraged to produce a comprehensive and authoritative review of the introduced and native status of the biota. We made no attempt to direct the experts in the assignment of species to native, introduced, or cryptogenic status. We recommended using as a guide the ten-point criteria of Chapman and Carlton (1991, 1994) to aid in the identification of introduced species and Carlton (1996a) as a guide for cryptogenic species.

Table 1. Taxonomic experts, target groups, and target habitats for the taxonomic review component of the Port Phillip Bay Introduced Species Study.

Taxonomic expert	Organization	Target Group	Target Habitat
J. Lewis	Defense Science and Technology Organisation (DSTO)	Macroalgae	All
M. Lockett[1] & M. Gomon[2]	[1]University of Technology, Sydney, [2]Museum of Victoria	Fish	All
M. Keough[3] & J. Ross[4]	[3]University of Melbourne, [4]CRIMP	Fouling species	Hard substrates
J Watson	Marine Science and Ecology	Hydroids	All
S. Boyd	Museum of Victoria	Molluscs	Soft substrates
R. Wilson	Museum of Victoria	Polychaetes	Soft substrates
G. Poore	Museum of Victoria	Crustaceans	Soft substrates
T. O'Hara	Museum of Victoria	Echinoderms	Soft substrates

A field-sampling program was initiated and carried out by CRIMP (1995-1996) to fill any apparent gaps in the geographic or sampling coverage of previous surveys. This field-sampling program was supplemented by an introduced species survey of the Port of Geelong undertaken by the Marine and Freshwater Resources Institute (MAFRI; Currie *et al.* 1997). Collection methods were consistent with the protocols developed for the Australian National Introduced Species Port Surveys Program (Hewitt and Martin 1996) with the following differences:

- pile scrapings were performed in a qualitative fashion to represent the fouling community across all depths;
- qualitative visual surveys towing divers on a manta board along 100-m transects;
- beach transect cores were collected at depths of 0, 1, 2, 5, and 10 m depth along a transect perpendicular to the beach;
- beam trawl tows were conducted at 1, 2, 5, and 10 m depth parallel to shore for a known duration (5 min) or length (100 m);
- a small version of a CSIRO seamount sled was developed to sample benthic infauna and epifauna at depths of 5, 10, 15, 20, and 25 m depending on site depth;
- no algae were collected or preserved during this survey.

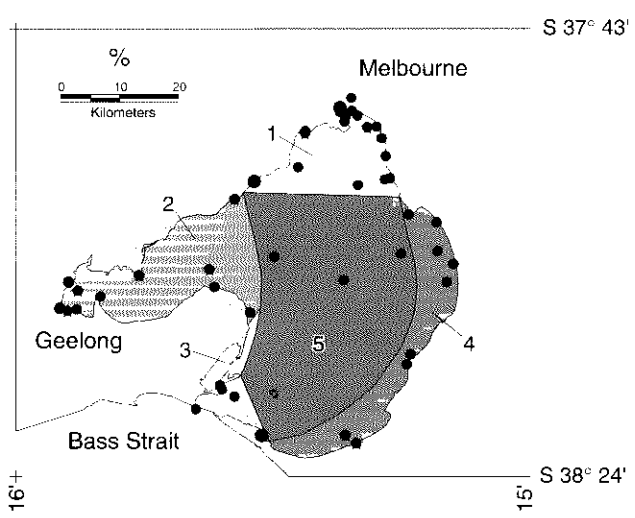

Figure 1. Port Phillip Bay, Australia regions (1–5) and CRIMP sampling locations (for detailed information on locations of specific sampling techniques see Hewitt *et al.* 1999). Note the major shipping ports of Geelong and Melborne and the connection of sampling effort in these areas.

The geographic extent of PPB (1900 km^2) precluded a systematic survey of all locations and habitats; instead, a targeted survey was performed in regions of high historic and modern shipping traffic with an overview of the remaining regions (Figure 1). Consequently, the regions were not sampled with equal effort; port regions (1 and 2) were sampled with much greater intensity. The sampling methods used ensured a comprehensive coverage of both hard and soft habitats.

Survey efficacy was evaluated by comparing the target list developed by taxonomic experts from museum and literature reviews with specimen collections from the CRIMP survey. All specimens from the CRIMP survey were identified to the lowest possible taxinomic unit and then verified by the taxonomic consultants that developed the target list, or against a reference specimen provided for that purpose.

Results

Trading activities in PPB group into four periods (see Campbell and Hewitt 1999a for further details): exploration/colonization (pre-1839), immigration (1839-1851), Gold Rush (1852-1860), and a modern era (1861-present). The history of European influence in Australia is relatively short (Crosby 1986). Despite the long history of aboriginal culture in the region, the history of biological invasions most likely begins with European contact, however the first biological collections did not begin until the early 1840s and no detailed surveys were conducted until the 1860s. Consequently, while the following presentation of trading patterns broken into four periods provides a context to evaluate invasions, biological surveys did not begin until the mid-1800s and consequently an evaluation of the modern period (1861-present) is presented.

Exploration/Colonization (pre–1839)

Sealing and whaling operations were established in the Bass Strait islands by 1796, often using Western Port (the embayment to the immediate east of PPB) as a home base (Shaw 1997). These sealers and whalers were typically from North America, and frequently had contact with Asia (Shaw 1997). British entry into the Port Philip Heads by John Murray of the *Lady Nelson* in 1802 led to the eventual establishment of a convict colony in 1803 (Shillinglaw 1972). Trade during this period was largely with other Australian colonies; however, periodic visits from Great Britain occurred. From 1803 to 1835, only three ocean-going vessels entered the bay (Shaw 1997). By 1839, regular intra- and inter-colony (South Australia, New South Wales, and Tasmania, Australia; New Zealand) trade routes were established and international routes to Great Britain. Due to the East India Company's monopoly of British trade between the Cape of Good Hope and the Straits of Magellan, there was limited direct trade between British colonies until the British Parliament repealed the British/China trade monopoly laws in 1834 (Staples 1966; Bach 1976). During this period, international vessels trading with PPB followed the Admiralty and later the Great Circle routes. Vessels originating in Europe would typically travel to South America (Rio de Janeiro), South Africa, then to Australia (with some exceptions to trade in India).

Immigration (1839–1851)

Free British immigrants arrived in Melbourne in 1839 from Sydney; however, the *David Clarke* arrived from Great Britain later in that year (Strahan 1994). During 1839, 11,500 immigrants arrived at the Point Ormond quarantine station in PPB (Shaw 1997). Pacific trade began in 1840, specifically catering for the demand of Newcastle coal in California (Bach 1976). The repeal of British Navigation laws in 1849

allowed foreign vessels entry into British colonial ports. Simultaneously the signing of the Treaty of Nanking ceded Hong Kong to Great Britain and opened Chinese Ports to British residence and trade (Lubbock 1933).

The Gold Rush (1852–1860)

The announcement of gold in Victoria was made in 1851 (shortly after the Port Phillip District separated from Victoria), despite discovery in 1849. This discovery had much the same effect as the California gold strike: the population swelled from <40,000 to 416,000 in five years (Bach 1976; Wild 1950) with immigrants from all continents often abandoning the vessels to rot. Port facilities expanded to meet the needs of a burgeoning population and new domestic (coastal) and international trade routes were opened.

Modern Shipping (1861– present)

Australian shipping tonnage was 93% British until the early 1900s. As trade became increasingly commercial, more ports of call were added to Conference shipping routes (established routes and cargo). By 1870, the trans-Pacific route went from Melbourne to Honolulu, Vancouver, Seattle, Tacoma, Portland, San Francisco, and Los Angeles before returning to Melbourne (Bach 1976). The opening of the Suez Canal in 1869 and subsequent deepening (1875) led to increasing shipping traffic through the Mediterranean. As has been reviewed elsewhere (Carlton 1985, 1996b), shipping changed considerably between the 1860s and present. The shift from wooden hulled to steel hulled vessels reduced the transport of marine borers. Simultaneously, the shift from dry ballast (rock, cobble, sand) to water ballast (in steel ships) halted the transport of near shore meiofauna and adult benthic encrusting and epifauna while developing the transport of holo-, mero- and tycho-planktonic organisms. The increased speeds of vessels and advent of more effective anti-fouling paints is believed to have significantly reduced the transport of encrusting and fouling organisms in numbers if not diversity.

The first biological surveys and collections of PPB began after the 1840s for flora (Harvey 1847, 1855, 1858-1863, 1869; Sonder 1852, 1853, 1880; Wilson 1886, 1889, 1890, 1892, 1894, 1895) and benthic fauna with surveys by Wilson, Agardh, Carpenter, Hickson, Spencer, Sendy, and Pritchard among others (Anon 1890, 1892, 1894, 1895). After 1895,

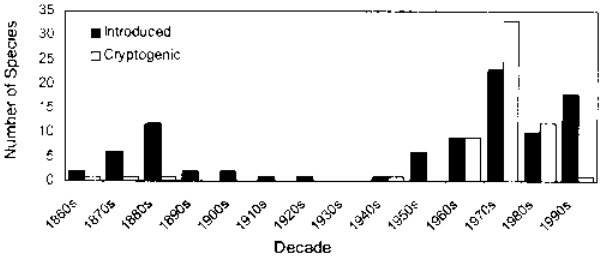

Figure 2. Numbers of introduced and cryptogenic species identified per decade in Port Phillip Bay.

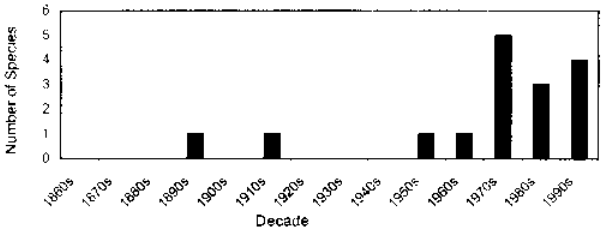

Figure 3. Numbers of identified introduced species from well-known and conspicuous groups (algae, molluscs, and fish) per decade in Port Phillip Bay.

few surveys occurred within the region until the early 1950s. Consequently, despite an increase in the numbers of recognized introduced and cryptogenic species through time (Figure 2) our understanding of the invasion history of PPB is limited by survey intensity. A small subset of well-known and conspicuous groups (*e.g.*, algae, molluscs, fish) that appear to have had a consistent sampling effort provide a mechanism to surmount this difficulty. For these groups a consistent trend towards an increase in introductions post-1950 remains evident (Figure 3).

Survey Results

The taxonomic experts identified a target list of 182 introduced (92), cryptogenic (65), and possibly introduced (*i.e.*, known from Victoria but not PPB; 25) marine and brackish water taxa. These species spanned nine invertebrate phyla and four algal divisions. Numerous taxa (301) held in the Museum of Victoria collections or collected in previous bay-wide surveys have not been provided specific Latin binomials due to a lack of description or because they were not identifiable. In all cases, these taxa were assigned endemic status by the taxonomic experts rather than cryptogenic. No explicit reasoning was provided for this assignation and their status should be considered questionable without further examination.

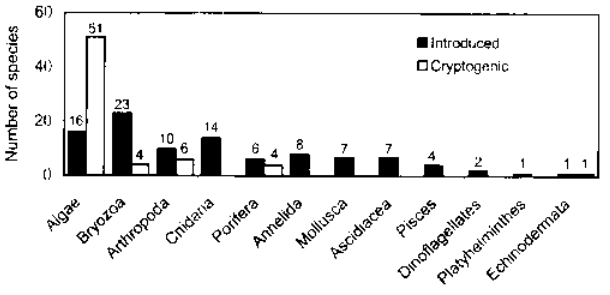

Figure 4. The number of introduced and cryptogenic species in Port Phillip Bay by taxonomic group (does not include unidentified species, see text for explanation).

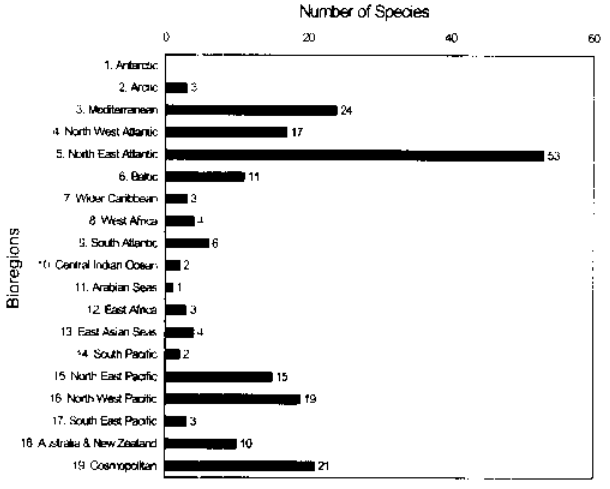

Figure 5. Native bioregions for introduced, cryptogenic and possibly introduced species that have become established in Port Phillip Bay. For more detailed information describing the bioregions see Kelleher *et al.* (1996) and Hewitt *et al.* (1999).

During the course of the CRIMP and the Victorian Marine and Freshwater Resources Institute (MAFRI) surveys, an additional nine introduced taxa were identified, bringing the total introduced and cryptogenic tally to 191 species from 10 phyla and five algal divisions (Figure 4). Forty-nine introduced and cryptogenic species were collected during the CRIMP surveys. Of the 108 animal target species identified by the experts (removing algae), 47.5% of the known target hard-substrate species were detected. Similarly, 48% of the target soft-substrate species were collected by the CRIMP survey.

Using the target species list of 182 taxa developed by the consultants, we evaluated the field surveys conducted by CRIMP and MAFRI. Clearly the extent to which different taxa were readily known and identifiable varied from group to group based in part on the available taxonomic knowledge; however, all specimen identifications were verified either by the taxonomic expert or by a voucher specimen provided for that purpose. The survey collected differing proportions of target species according to taxonomic group. All fish targets (Chordata – 100%) were collected while other groups such as the Ascidiacea (86%) and Crustacea (80%) were well sampled though not all target taxa were collected. Porifera (66%), Cnidaria (60%), Mollusca (57%), and Bryozoa (52%) were collected with decreasing levels of efficiency, in part suggesting that either the pattern of dispersion in these species was more widespread, or that some of these target taxa identified from historic publications and/or collections may no longer be established in PPB. The Annelida (12.5%) were poorly collected; however, this reflects a lack taxonomic knowledge of the annelids of hard substrate (where the majority of specimens were collected; Wilson 1999) and a reduced emphasis of collection in soft substrate (Campbell and Hewitt 1999b). A single target echinoderm (*Asterias amurensis*) was not collected during the survey and was subsequently found in low numbers (four in two years) prior to achieving outbreak status in 1998 (Talman *et al.* 1999). The majority of detected species (76%) were hard-substrate organisms (bryozoans, crustaceans, cnidarians, and ascidians). Introduced species were detected in all regions of the bay; however, the majority of species were found in port regions (regions 1 and 2; Figure 1).

Species origins can be determined only in retrospect based upon the date of first collection in Port Phillip Bay, the known trade activities prior to collection date, and the known international distribution of the species. Based on these criteria, species introduced to PPB have come from all regions of the world except Antarctica (Figure 5). Historically, the majority originated in the Northeast Atlantic (53) and the Mediterranean (24), while another 21 are cosmopolitan. The North Atlantic (northeast and northwest) appears to have been a consistent and significant donor region for successful invaders. This was the anticipated origin of many species given the long history of trade with Britain and the historic parliamentary limitations on traffic into PPB. A large number of invasions were from the Southern Hemisphere: New Zealand (10), Africa (East Africa, 3; West Africa, 4) and South America (South Atlantic, 6; Southeast Pacific, 3). More recently (post 1950) there has been an increase in the number of introduced

North Pacific species (Northeast Pacific, 15; Northwest Pacific, 19). Since 1990, these North Pacific species represent the single largest group of introductions, many of which have been identified as pest species.

Discussion

Port Phillip Bay represents one of Australia's largest trade regions historically and in the present. The extent to which this bay has been invaded and the patterns of invasion history provide insight into potential management activities. Invasions within PPB appear to be increasing, possibly due to an increase in modern shipping traffic and an increase in aquaculture activities (historically associated with incidental introductions). As expected, the majority of introductions are concentrated around the shipping ports of Geelong and Melbourne. However, these factors alone cannot account for the increased number of invaders. Trade with new regions, increased vessel traffic, and altered conditions (in Port Phillip Bay) may all have contributed to higher invasion rates (Carlton 1996b). Many of the modern invasions of pest species such as *A. amurensis, U. pinnatifida,* and *C. fragile* ssp. *tomentosoides* appear to have been transported as secondary inoculations from other domestic primary inoculation sites.

The numbers of invaders in Port Phillip Bay are high by world standards. Within Australia no other single port or region equals the number of recognized species. In part, this may be due to the effort expended in determining the scale of biological invasions in these locations. A National Port Survey program has been initiated by CRIMP to evaluate the scope of introductions in Australian ports using a consistent and quantitative methodology (Hewitt and Martin 1996). These surveys are designed to provide a snapshot of the current invasion diversity rather than the complete history achieved by the Port Phillip Bay Introduced Species Study due to the extensive coverage by expert taxonomists.

The number of reported introduced and cryptogenic marine and brackish water species in Port Phillip Bay exceeds similar numbers from anywhere in the world. While Cohen and Carlton's study of San Francisco Bay and Delta region (Cohen and Carlton 1995) reports a greater overall introduced and cryptogenic species richness (212 introduced and 123 cryptogenic species), it includes salt marsh and freshwater species. It is difficult to compare surveys with significantly different methodologies and approaches; however, if the total numbers are restricted to only marine and brackish water species (excluding salt marsh species), San Francisco Bay has approximately 138 introduced species (Cohen *et al.* 1998 present 95 species). This may reflect a stronger trading history of the region or may indicate a higher regional susceptibility to invasions. Regardless, the findings presented here suggest that Port Phillip Bay is one of the most invaded marine ecosystems in the Southern Hemisphere.

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Factors Limiting the Spread of the Introduced Mediterranean Mussel *Mytilus galloprovincialis* on Washington's Outer Coast

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Key words: mussel, *Mytilus galloprovincialis*; eastern Pacific, invasion, ecological resistance, survivorship, range expansion

INTRODUCTION

In the wake of fish stock overexploitation around the world (Naylor *et al.* 1998), aquaculture is increasingly being developed as a potentially more sustainable source of protein. Global aquaculture production has more than doubled in weight and volume in the last decade, and now accounts for more than one quarter of all seafood consumed by people (Naylor *et al.* 1998). One consequence of this growth in aquaculture is an increase in the transfer and spread of culture species around the world. In order to predict the ecological consequences of these introductions, we need to know where, in a given region, these introduced species will spread.

One of the most commonly used bivalves in raft culture is the Mediterranean blue mussel *Mytilus galloprovincialis*. Globally, *M. galloprovincialis* is native to the Mediterranean Sea and has spread through accidental transport and intentional introduction to Asia, North America, and the southern hemisphere (Carlton in press). In South Africa, the spread of *M. galloprovincialis* has led to a dramatic reorganization of the intertidal zone. It has replaced the native mussel *Aulacomya ater*, causing a change in algal diversity and limpet population structure, and has been correlated with an increase in oystercatcher fecundity (Griffiths *et al.* 1992). In California, *M. galloprovincialis* has replaced the native sibling species, *M. trossulus*, at a number of intertidal locations (Geller 1999).

Most recently, *M. galloprovincialis* has been introduced both intentionally and accidentally to the Pacific Northwest. It is cultured at several shellfish farms in Puget Sound, Washington, where it is favored over the native sibling species, *M. trossulus*, which succumbs to a hemolytic neoplasia before reaching market size. In addition, *M. galloprovincialis* has been identified in ballast water arriving in Oregon from Japan (Carlton and Geller 1993), and is presumably also delivered to ports of Washington and British Columbia. From these numerous sources, *M. galloprovincialis* is spreading in rocky intertidal communities of the Pacific Northwest (Suchanek *et al.* 1997). Its invasion here will be limited in part by its ability to survive wave action, desiccation stress, and predation. Here, I experimentally compare the ability of the introduced and native *Mytilus* sibling species to survive wave action, desiccation (as tidal height), and predation.

METHODS

I compared the survivorship of transplanted *M. galloprovincialis* and *M. trossulus* in the rocky intertidal of Tatoosh Island, just off the outer coast of Washington State (48°24'N, 124°41'W). At this site the native California mussel, *M. californianus*, a larger and thicker shelled species, forms conspicuous mid-intertidal beds with ephemeral gaps created by wave and storm disturbance (Paine and Levin 1981). Since *M. californianus* is not a member of the sibling species complex that includes *M. galloprovincialis* and *M. trossulus*, and plays a different ecological role (Suchanek 1978), I did not manipulate it in the current comparison of sibling species. *M. trossulus* is found in two distinct intertidal zones on Tatoosh: in the ephemeral gaps within the *M. californianus* bed, and in a more permanent band above the *M. californianus* (Suchanek 1978, as *M. edulis*).

I transplanted mussels to six sites encompassing a range of wave exposures from high to low. At each site, I planted mussels at two tidal heights: in gaps in the *M. californianus* bed, and in the *M. trossulus* zone

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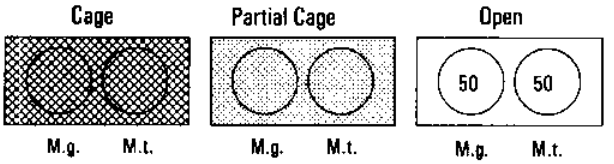

Figure 1. Mussel transplant experimental design, Tatoosh Island, Washington. Fifty individuals of the introduced *Mytilus galloprovincialis* (M.g.) and of the native *M. trossulus* (M.t.) were transplanted in three paired plots. The three plots were haphazardly assigned to cage (*i.e.*, predator exclusion), partial cage (i.e., cage control), and open treatments with no cage. This design was repeated at two intertidal heights (high and mid) at each of six sites over a range of wave exposures (high, medium, low).

above the *M. californianus*. At each height, I planted three patches of 50 mussels of each species, *M. galloprovincialis* and *M. trossulus* (Figure 1). The patches were paired by species, and pairs were haphazardly assigned to one of three treatments: fully caged to exclude predators (primarily the drilling snails, *Nucella emarginata* and *N. canaliculata*); a partial cage to allow predators in and control for unintended caging effects; and an open treatment with no cage (Figure 1). Each patch was covered with fiberglass mesh to allow the mussels to attach byssal threads to the rock. After two weeks, I removed the mesh and monitored mussel survivorship over the next 16 wk. At 2-wk intervals, I counted the number of live mussels, empty shells, and shells with drill holes in each patch. I also surveyed the density of *Nucella* spp. at each pair of transplants. Results are presented for the first six weeks.

Results and Discussion

Proportion data were arcsin (square root) transformed and count data were ln (x+1) transformed to improve normality. All analyses were conducted using JMP version 3.0 statistical software. Mussel survivorship at six weeks was analyzed by four-factor ANOVA testing for effects of species, tidal height, wave action, and treatment. The proportion of mussels that was washed away was compared for the two species using a Student's *t*-test. The proportion of empty mussel shells with drill holes was regressed linearly against the density of *Nucella* spp. in each patch.

There was no significant difference in survivorship between the two species. Both species survived significantly better in caged treatments, where they were protected from predators, than in open treatments where they were exposed to predation (Table 1; Figure 2A). Both species survived significantly

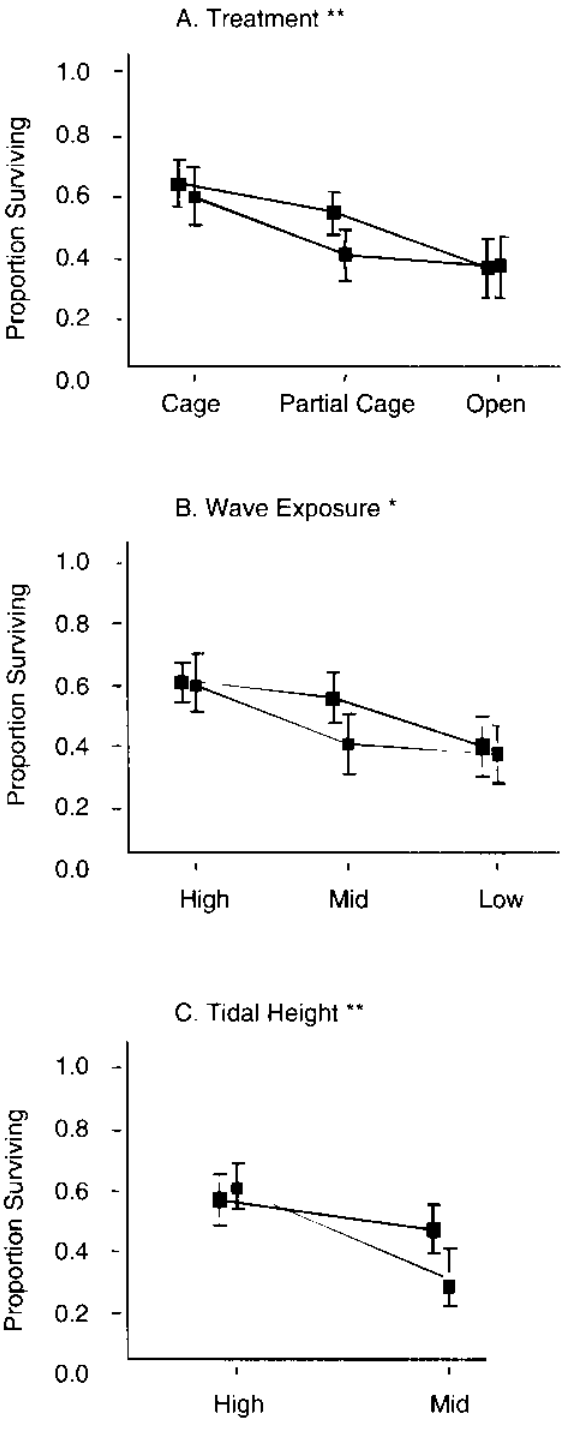

Figure 2. Transplanted *M. galloprovincialis* survived equally with *M. trossulus* in all locations and all treatments on Tatoosh Island, Washington, after six weeks. Both mussels survived significantly better (a) in the cage treatment than in the open treatment; (b) at high than low wave exposure; and (c) at high than low tidal height. Points represent the mean of n = 12 cages (a and b) and n = 18 cages (c) ±1 standard error. Significant differences indicated by * ($p < 0.05$) and ** ($p < 0.01$); horizontal lines indicate means that were not significantly different in post-hoc pairwise comparisons

Table 1. Proportion of transplanted mussels surviving after six weeks on the intertidal rocky shore of Tatoosh Island, Washington: ANOVA results. There was no significant difference between species (introduced *M. galloprovincialis* vs. native *M. trossulus*). Significant main effects were found for wave exposure (high, medium, low), tidal height (high, mid), and treatment (predator exclusion cage, partial cage, open). See also Figure 2.

Source of Variation	DF	SS	F	p
Species	1	0.092	0.765	0.385
Wave Exposure	2	0.939	3.882	0.026
Tidal Height	1	.232	10.194	0.002
Treatment	2	.300	5.375	0.007

better at high than at low wave exposure, and at high than at low tidal height (Table 1; Figure 2B, C).

Inspection of the empty shells suggested that the primary causes of mortality for these two species may not be the same, for two reasons. First, more of the empty shells of *M. trossulus* (43.7% ± 4.80) than *M. galloprovincialis* (31.8% ± 5.02) tended to remain attached to the rock after six weeks (mean ± 1 S.E., $t = 1.871$, $p = 0.066$). This suggests that *M. galloprovincialis* may have weaker byssal thread attachments than *M. trossulus*. Second, some of the empty shells had drill holes, indicating that those mussels had been eaten by the predatory snails *Nucella* spp. *Nucella* spp. density varied among sites: at sites with higher *Nucella* spp. density, the proportion of drilled *M. trossulus* shells tended to be higher (slope = 0.30, $R^2 = 0.11$, $p = 0.084$), but the proportion of drilled *M. galloprovincialis* shells did not (slope = -0.25, $R^2 = 0.05$, $p = 0.339$). This trend suggests that the native predatory snail may prefer the native mussel to the introduced mussel. These hypotheses, concerning byssal strength and predator preference, will be further tested in laboratory experiments.

Since *M. galloprovincialis* appears to survive as well as *M. trossulus* over a range of wave exposures and tidal heights, the two species are likely to overlap as *M. galloprovincialis* spreads through the Pacific Northwest. Competitive interactions between the two species may therefore be important in limiting the spread of *M. galloprovincialis*. The present results suggest that local variations in wave exposure and predator density have the potential to affect the outcome of this competitive relationship. In future field experiments I will compare the growth rates of these two species over a range of conditions. Additional factors that I have not yet addressed include the abiotic variables of water temperature and salinity; and the biotic factors of susceptibility to parasites and disease.

Acknowledgments

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The 1998 Puget Sound Expedition: A Shallow-Water Rapid Assessment Survey for Nonindigenous Species, with Comparisons to San Francisco Bay

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ABSTRACT: A rapid assessment survey for nonindigenous species at 23 primary stations and eight secondary stations was conducted September 8–16, 1998, in the inland marine waters of Washington State from Blaine, at the Canadian border, to Olympia, in south Puget Sound. The 1998 Expedition team was composed of scientists with both broad and specific taxonomic and regional expertise from universities and local and state agencies. It included core researchers from the four San Francisco Bay Expeditions of 1993–1997, where the survey techniques were developed. Using a variety of sampling methods on marina docks and adjacent shallow water benthic habitats, the 1998 team collected and identified more than 400 native species and 39 nonindigenous species (3 plants and 36 invertebrates)—taxonomic work on the samples is still underway. The number of nonindigenous species collected per site showed no obvious correlation with salinity, temperature or region. We believe that Puget Sound presently hosts more than 50 nonindigenous marine species. This is substantially fewer than the approximately 160 nonindigenous species known to be in the marine and estuarine portions of San Francisco Bay, excluding its associated Delta region. The vast difference in invasion level is noteworthy considering the close shipping links between these two estuaries for the past 150 years, although it is also partly a sampling artifact reflecting the different state of knowledge of nonindigenous species in the two estuaries.

Key words: rapid assessment, Puget Sound, Washington, Northwest Straits, San Francisco Bay, Pacific, invertebrate, alga, *Zostera*, *Spartina*, ascidian, crustacea, introduced species, nonindigenous species, estuary

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Introduction

Understanding the level of invasion and disturbance in the various ecosytems within which we operate is becoming an increasing priority as general awareness of the disruptive effects of nonindigenous species in terrestrial, freshwater, and marine ecosystems grows. San Francisco Bay is one of the best-studied marine ecosystems in terms of non-native species introduced by human activities over recent centuries (Carlton 1979; Cohen and Carlton 1995; Cohen 1996; Cohen and Carlton 1998). In addition to these well-known studies on San Francisco Bay, a series of four surveys (known affectionately as the San Francisco Bay Expeditions) was completed by a team of biologists during the 1990s. These were week-long rapid assessment surveys for introduced species in the float-fouling communities of the Bay. Following the model of the San Francisco Bay Expeditions of 1993, 1994, 1996, and 1997, we carried out a similar Puget Sound Expedition in the late summer of 1998 to inventory nonindigenous marine species in the float-fouling communities of greater Puget Sound, using the core researchers and methods pioneered in San Francisco Bay between 1993 and 1997.

The rapid assessment survey methods reported here provide estimates of species diversity. The use of rigorous quantitative methods in most modern marine ecological research precludes sampling large areas over a short time for presence or absence of species. Rapid assessment methods have been refined here for the detection of maximum numbers of marine species, allowing flexibility in sampling. Biological invasions are detectable only by looking for species, and become apparent only when accompanied by accurate taxonomic identification of the invading organisms. This rapid assessment method tests whether or not field surveys by taxonomic specialists can detect biological invasions that have been overlooked, unreported, or misidentified in standard, modern ecological studies.

Puget Sound is the southern portion of one of the three largest North American estuary systems. It is a complicated, glacier-produced inlet approximately 150 km long, with many branches providing approximately 3,350 km of shoreline. The Sound has numerous freshwater inflows and a complicated circulation pattern driven by tidal forcing. To its north, Puget Sound merges with the Strait of Juan de Fuca (which runs west to the Pacific Ocean) and connects with the Strait of Georgia (which separates Vancouver Island from the southern British Columbia mainland) via a patch of American waters sometimes called the Salish Sea or Washington Sound and recently dubbed the Northwest Straits in conjunction with a new American federal marine conservation management initiative. We sampled from south Puget Sound to the northern edge of Washington State in the Salish Sea.

Metropolitan areas are now concentrated on the eastern side of this region. The Puget Sound basin presently supports a population of nearly 4 million people. Southwestern British Columbia includes an additional 3 million. Population growth rates, primarily from immigration, are presently high on both sides of the international border, and are predicted to remain high in the foreseeable future. There is abundant cross-Sound ferry transportation, both public and private, and heavy recreational boat use. The robust present-day shipping trade derives primarily from ports up and down the west coast of North America and from other countries on the Pacific Rim. Major Puget Sound shipping destinations include the deep-water port facilities at Seattle, Tacoma, and Olympia and oil refineries in the Northwest Straits region near Anacortes and Bellingham. Each year, approximately 6000 ships enter the Strait of Juan de Fuca from the Pacific Ocean (Washington State Office of Marine Safety, now Department of Ecology 1996). About half of these enter American ports and the other half turn north into the Strait of Georgia towards Canadian port facilities around Vancouver (Niimi, this volume).

Puget Sound and San Francisco Bay have been tightly connected by shipping since the earliest days of European and Asian settlement of the Puget Sound region in the early to mid-1800s. Some of the earliest Puget Sound–Pacific Rim shipping contacts were made by the Hudson Bay Company in the 1830s, when agricultural and other natural resource products from the Puget Sound region were shipped to Hawaii, China, Australia, and Europe. By the 1850s, logs and lumber from Puget Sound were being shipped in great quantities to San Francisco. Today, both the Puget Sound and San Francisco Bay estuary systems receive enormous amounts of shipping traffic (and associated ballast water) from all over the world, but principally from other Pacific coast North American ports and Pacific Rim countries.

Comparison of the state of marine bioinvasion of San Francisco Bay and Puget Sound is therefore an important and interesting project.

Materials and Methods

The First Puget Sound Expedition took place September 8–16, 1998. The Expedition team was composed of 15–20 marine scientists with broad and specific taxonomic and regional expertise from several universities and local agencies (Cohen *et al.* 1998). Using a rapid assessment approach, we sampled 23 marinas composed of floating docks (primary sites) and eight intertidal sites (secondary sites) between Olympia and Blaine (Table 1), a distance of about 230 km. The sampling occurred over six days; we identified most of the species collected in the field or immediately afterward at the University of Washington's Friday Harbor Laboratories.

Marinas with floating docks provide important and common substrates for marine organisms in the inside waters of Washington State and British Columbia. Private and public year-round mooring facilities are located abundantly along the shoreline, sometimes accommodating many hundreds of small boats, and frequently offering covered as well as uncovered moorage space. Different organisms can be found under the covered and uncovered portions of these marinas and we sampled both whenever the situation allowed.

Our primary goal in selecting sampling sites was to achieve the widest and most uniform coverage of Puget Sound possible in the four full sampling days available to us; some additional sampling was done from Friday Harbor. The duration of the expedition was determined by time available to the majority of scientists involved. The time was then split approximately in half to allow both field and lab time. With these goals in mind, preliminary visits were made to about 60 potential sites from the Canadian border to the south Sound. Site selection was ultimately based on geographic distribution, with as many different inlets included as possible, and with sites characterized by heavy commercial use as well as by light to heavy recreational use. A few sites were eliminated on the days of sampling for lack of time, because our original schedule was too ambitious. Five sites per day seemed comfortable and realistic, although on three days we managed to sample six sites either by working late, or by sampling the sixth site more superficially. Waning daylight ultimately determined the end of each sampling day. In addition to the 50–60 minutes spent sampling each site, another half hour was required to get the gear put away and samples properly labeled and stored before moving on to the next location.

Table 1. List of stations sampled during the Puget Sound Expedition, September 8–16, 1998.

Station #	Station description	City	County
Primary sites			
1	Port of Everett Marina	Everett	Snohomish
2	Port of Edmonds Marina	Edmonds	Snohomish
3	City of Des Moines Marina	Des Moines	King
4	Harbor Island Marina, near mouth of Duwamish River	Seattle	King
5	Elliott Bay Marina	Seattle	King
6	Fishermen's Terminal	Seattle	King
7	Ole & Charlie's Marina	Tacoma	Pierce
8	Steilacoom Marina	Steilacoom	Pierce
9	Boston Harbor Marina	Olympia	Thurston
10	Port of Shelton Marina	Shelton	Mason
11	Fairharbor Marina	Grapeview	Mason
12	Kitsap Marina	Port Orchard	Kitsap
13	Brownsville Marina	Brownsville	Kitsap
14	Seabeck Marina, Hood Canal	Seabeck	Kitsap
15	Port Ludlow Marina	Port Ludlow	Jefferson
16	Port Hadlock Bay Marina	Port Hadlock	Jefferson
17	Boat Haven Marina	Port Townsend	Jefferson
18	Deception Pass Marina, Whidbey Island	north of Oak Harbor	Island
19	Blaine Marina	Blaine	Whatcom
20	Squalicum Harbor, Port of Bellingham	Bellingham	Whatcom
21	Samish Bay, small float near mouth of Samish River	Edison	Skagit
22	Cap Sante Boat Haven	Anacortes	Skagit
23	University of Washington, Friday Harbor Labs floating dock	Friday Harbor	San Juan
Secondary sites			
1	beach at Steilacoom Marina	Steilacoom	Pierce
2	beach at Boston Harbor Marina	Olympia	Thurston
3	beach at Seabeck, Hood Canal	Seabeck	Kitsap
4	beach north of the Blaine Marina	Blaine	Whatcom
5	lagoon at Port Ludlow Resort	Port Ludlow	Jefferson
6	beach at Port Ludlow Resort	Port Ludlow	Jefferson
7	Padilla Bay – *Vaucheria* flats east of the Swinomish Channel	east of Anacortes	Skagit
8	Argyle Lagoon	south of Friday Harbor	San Juan

At each primary site, we sampled a variety of habitats. Float- and dock-fouling organisms were sampled by a variety of simple manual techniques. Tools included hand scrapers, a 1.3-m long-handled scraper

with attached 3-mm steel mesh net, a 2.4-m pole with attached 1-mm nylon mesh net, and a broad array of pans, buckets and other containers. Organisms were collected individually from the substrata, or by scraping and subsequent washing through coarse sieves followed by 1.0-mm or 0.5-mm mesh sieves for final washing. We collected from concrete, styrofoam, and wooden float surfaces as well as ropes, tires, conduit, bumpers, chains, and boat bottoms within reach. At two sites we were able to examine newly removed floats because reconstruction was taking place during our visit, and at another site a heavily fouled rowboat was pulled out for us. A sample of live bay mussels (*Mytilus* sp.) was obtained from each site where they were present; these were later frozen for genetic analysis. Benthic (bottom) and plankton samples were also taken at most dock sites. (Two of the scientists were unable to participate during the first two days of the sampling, but returned to the missed sites and collected plankton and mussel samples within two weeks.) An Ekman grab was used to obtain non-quantitative bottom samples that were sieve-washed (using 1.0- or 0.5-mm meshes as deemed appropriate for the organisms being collected) and sorted on site; unsorted bottom samples were retained for later examination for foraminifera and other microfauna. A custom-made cylindrical benthic sampler fitted with 1-mm stainless steel mesh walls was thrown out on a line and dragged back along the bottom, working like a small benthic sled to collect larger infauna. Vertical plankton hauls were taken with a 0.5-m diameter, 102-µm mesh net with a 211-µm mesh cod end. Horizontal plankton tows were taken by pulling a plankton net fitted with 125-µm mesh alongside each dock. The tows were made close to the float-fouling community in an effort to obtain demersal organisms such as harpacticoid copepods. At least two team members took field notes for the group at each site. Pilings were sampled separately at several sites, and nearby intertidal sites were opportunistically sampled when the tide and shoreline geography allowed. In order to compare similar environments, docks were classified as primary sites, and other sites such as beaches were classified as secondary sites.

Surface temperature and salinity were measured at each dock site. We attempted to use two electronic (Yellow Springs Instruments) meters to obtain depth profiles of temperature and salinity, but these devices produced varying and unreliable readings (as was the experience of the San Francisco Bay Expeditions). The measurements we preferred were near-surface measurements obtained with two thermometers and two refractometers that agreed to within 0.5°C and 0.5 psu.

At each dock site, we filled a 1-L jar with a representative voucher collection, plus additional samples of material of interest. If laboratory time was scheduled soon after the field work, the samples were kept on ice; otherwise they were preserved on-site in formalin or alcohol for later analysis. Laboratory work was conducted at the King County Environmental Laboratory and the University of Washington's Friday Harbor Laboratories. The voucher collections were all re-examined in the laboratory. Most organisms were identified by team members. Some specimens were retained by individual team members for further study; some were sent to other taxonomic specialists. The voucher collections are currently held by the Washington State Department of Natural Resources, and will ultimately be deposited in an appropriate curated museum collection.

This project was conceived in March 1998, carried out in September 1998 and the results were presented at the First National Conference on Marine Bioinvasions in January 1999, all within 10 months. We had little funding by conventional standards, but the rapid timeline allowed a high level of spontaneity and enthusiasm among the participants. This was integral to the success of the 1998 Puget Sound Expedition. Not all of the data analysis was completed during the Expedition and further identifications are anticipated later at the convenience of the participants.

Results

We collected and identified more than 450 species of plants and invertebrates, providing a snapshot of the fouling fauna of Puget Sound in late summer, 1998. Most of the species were native (in the sense that they are known or assumed to be present on the Pacific coast of America naturally, that is, prior to the arrival of European colonists). Thirty-nine nonindigenous species were identified (Table 2). Eleven of these nonindigenous species are new records for Puget Sound and several more confirm unpublished reports of established nonindigenous species.

An additional 31 cryptogenic species (those whose native range cannot be determined, thus of *unknown origin*) have so far been identified in samples

Table 2. Nonindigenous species collected by the 1998 Puget Sound Expedition including geographic origins, first records and possible mechanisms of introduction; ** indicates a new record for Puget Sound. This list of species is provisional pending further taxonomic work and review by expedition members and associates and includes additions or corrections to a similar Table 2 in Cohen et al. (1998), which it supercedes (see text for sources). First records consisting of written accounts that do not state the date of planting, collection or observation are preceded by the symbol "<". Mechanisms are listed as: OA – with shipments of Atlantic oysters, OJ – with shipments of Japanese oysters, SF – in ship fouling or boring, SB – in solid ballast, BW – in shipballast water or seawater system, MR – planted for marsh restoration or erosion control (mechanisms given in parentheses are considered less likely).

Taxon	Native Range	First Record[1,2] Pacific Coast	First Record[1,2] Puget Sound	Possible Mechanism of Introduction
Phaeophyceae				
Sargassum muticum (Yendo, 1907) Fensholt, 1955	Japan	1944	1948[3]	OJ
Anthophyta				
Spartina anglica C.E. Hubbard, 1968	England	1961-1962	1961-62	MR
Zostera japonica Ascherson and Graebner, 1907	W Pacific	1957	1974[4]	OJ
Foraminifera				
Trochammina hadai Uchio 1962	Japan	1971	1971[5]	BW, SF, OJ
Cnidaria: Hydrozoa				
Cordylophora caspia (Pallas, 1771) (= *Cordylophora lacustris*)	Black Sea and Caspian Sea	ca. 1920	ca. 1920	BW, SF
Cnidaria: Anthozoa				
Diadumene lineata (Verrill, 1869) (= Haliplanella luciae)	probably Asia	1906	< 1939	OA, SF
Annelida: Polychaeta				
Hobsonia florida (Hartman, 1951)	NW Atlantic	1940[6]	1940[6]	?
Pseudopolydora paucibranchiata (Okuda, 1937)	Japan	1950	1993[7]	BW, SF
Mollusca: Gastropoda				
Batillaria attramentaria (G. B. Sowerby II, 1855) (= *B. zonalis, = B. cumingi*)	Japan	1924	1924	OJ
Crepidula fornicata Linnaeus, 1758	NW Atlantic	1905	1905	OA
Myosotella myosotis (Draparnaud, 1801) (= Ovatella myosotis)	Europe?	1871	1927	OA (SB, SF)
Mollusca: Bivalvia				
Crassostrea gigas (Thunberg, 1793)	Japan	1875	1875	OJ
Mya arenaria Linnaeus, 1758	NW Atlantic	1874	1888-1889	OApar 7560
Nuttallia obscurata (Reeve, 1857)	Japan, Korea (China?)	1991[8]	1993[8]	BW
Venerupis philippinarum (Adams and Reeve, 1850) (= *Ruditapes philippinarum* = *Tapes japonica*)	NW Pacific	1924	1924	OJ
Arthropoda: Crustacea: Copepoda				
**Choniostomatid copepod	?	?	1998	
Arthropoda: Crustacea: Cumacea				
Nippoleucon hinumensis (Gamo, 1967)	Japan	1979	mid-1990s[9]	BW
Arthropoda: Crustacea: Isopoda				
Limnoria tripunctata (Menzies, 1951)	?	1871 or 1875	1962[10]	SF

Arthropoda: Crustacea: Amphipoda				
Ampithoe valida (Smith, 1873)	NW Atlantic	1941	1966[11]	BW, OA, SF
***Caprella mutica* (Schurin, 1935) (= *C. acanthogaster*)	Sea of Japan	1973-1977	1998	BW, OJ
Corophium acherusicum (Costa, 1857)	N Atlantic	1905	1974-1975	OA, SF
Corophium insidiosum (Crawford, 1937)	N Atlantic	1915	1949[12]	OA, SF
Eochelidium sp.	Japan or Korea ?	1993?	1997	BW
Grandidierella japonica (Stephensen, 1938)	Japan	1966	1977[13]	BW, OJ, SF
Jassa marmorata (Holmes, 1903)	NW Atlantic	1941	1990?[14]	BW, SF
***Melita nitida* (Smith, 1873)	NW Atlantic	1938	1998	BW, OA, SB, SF
***Parapleustes derzhavini* (Gurjanova, 1938)	W Pacific	1904	1998	SF
Kamptozoa / Entoprocta				
***Barentsia benedeni* (Foettinger, 1887)	Europe	1929	1998	OJ, SF
Ectoprocta / Bryozoa				
Bowerbankia sp. "gracilis"	NW Atlantic?	<1923	<1953	OA, SF
Bugula sp. 1	?	?	1993	?
***Bugula sp.* 2	?	?	1998	?
***Bugula stolonifera* (Ryland, 1960)	NW Atlantic	<1978	1998	SF
***Cryptosula pallasiana* (Moll, 1803)	N Atlantic	1943-1944	1998	OA, SF
Schizoporella unicornis (Johnston, 1847)	NW Pacific	1927	1927	OJ, SF
Urochordata: Tunicata				
Botrylloides violaceus (Oka, 1927)	Japan	1973	1977	OJ, SF
Botryllus schlosseri (Pallas, 1766)	NE Atlantic	1944-1947	1970s[15]	OA, SF
***Ciona savignyi* (Herdman, 1882)	Japan	1985	1998	BW, SF
***Molgula manhattensis* (DeKay, 1843)	NW Atlantic	1949	1998	BW, OA, SF
***Styela clava* (Herdman, 1881)	China to Sea of Okhotsk	1932-1933	1998	BW, OJ, SF

[1]most pre-1979 dates are from Carlton (1979).
[2]most post-1979 dates from Cohen and Carlton (1995).
[3]first Puget Sound record from the San Juan Islands (Scagel 1956).
[4]first Puget Sound record from Padilla Bay, Washington (Phillips and Shaw 1976; Harrison and Bigley 1982).
[5]first Pacific Coast and Puget Sound record by McGann et al. (2000).
[6]first Pacific Coast and Puget Sound record from Everett, Washington (Banse 1979).
[7]first Puget Sound record from Eagle Harbor in 1993 by the Washington State Department of Ecology (K. Welch, pers. comm).
[8]first Pacific Coast record at White Rock, B. C. (Forsyth 1993), first Puget Sound record from San Juan Islands, Washington (A. Scruton, pers. comm.).
[9]first Puget Sound record from Seattle, Washington, mid-1990s by Jeff Cordell (unpublished).
[10]first Puget Sound record in Quayle (1965).
[11]first Puget Sound record from Hood Canal by Bousfield (in Bousfield and Jarrett 1981).
[12]first Puget Sound record from Totten Inlet and Whidbey Island by Shoemaker (1949).
[13]first Puget Sound record from Seattle, Washington, 1977 by Frederic H. Nichols (C. P. Staude, pers. comm.).
[14]if this species proves to be synonymous with *J. staudei*, the first Puget Sound record is from Friday Harbor, Washington by Craig Staude (C. P. Staude, pers. comm.).
[15]first Puget Sound record from San Juan Islands, Washington (Lambert and Lambert 1998).

from the 1998 Expedition; about half are organisms requiring further work to identify to the species level. When species identification is completed, some of these cryptogenic species may move to the nonindigenous species list. The (ongoing) list of cryptogenic species is available on the web at http://faculty.washington.edu/cemills/PSrecords.html.

Salinity at the sites varied between 0 and 34 psu, with all but two of the stations having salinities over 20 psu. Sea water temperatures varied from 12° to 20°C. The number of nonindigenous species collected and identified per site, not counting a number of still-unidentified annelids or peracarids, ranged from zero to eight. Plots of the number of nonindigenous species per site against salinity, temperature, or by region revealed no discernible patterns (Cohen *et al.* 1998).

Discussion

This rapid assessment survey focused primarily on non-quantitative or semi-quantitative sampling of dock fouling communities (organisms growing on the sides and undersides of floating docks and associated floats, bumpers, tires, ropes, etc.). Field identification of specimens was followed by examination in the laboratory by taxonomic experts on the team. Sampling dock-fouling communities has the following advantages:

- The habitat is easily sampled at low cost and with simple equipment.
- It can be sampled regardless of the tide level.
- There is easy and quick access to a large selection of suitable sites throughout Puget Sound.
- Most sites provide an adequate working area for a sizable team of experts to sample simultaneously, while remaining in verbal contact.
- In many coastal regions, the dock-fouling fauna includes a significant nonindigenous or cryptogenic component.
- Dock-fouling communities are normally extremely diverse and profuse, allowing rapid collection of many species.

The following disadvantages should also be ascribed to dock sampling:

- The communities are mostly restricted to hard and biogenic substrates.
- Many species of nonindigenous macrophytes (seaweeds and vascular plants) are more likely to become established in shallow benthic habitats than on docks and pilings and are therefore missed.
- It is very difficult to sample quantitatively.

We chose to sample in September mostly because that is when the participants were available. Late summer sampling turned out to be perhaps the best possible time, as annuals such as hydroids, bryozoans, and tunicates were well developed. Many of these would not have been present in such abundance at other times of the year. Tunicates were producing large numbers of larvae in September, and the morphology of the larva proved necessary for positive identification of at least one species (*Botrylloides violaceus*). Peracarid crustaceans are also at peak abundance and reproductive maturity in late summer and early fall, providing excellent specimens on which to make specific identification. Many of the hydroids were also reproductive, enabling their identification to species, although a somewhat different, and probably more diverse, set of hydroids would have been found earlier in the year (May and June). The algae would also have been more diverse if we had sampled in May and June; many early species had already died back when we sampled.

The expedition team was remarkable for the amount of local marine invertebrate and plant knowledge and specific taxonomic expertise that was represented. Recognition of newly established alien organisms requires a thorough knowledge of the local fauna and flora to allow recognition of species that are not native to the system. Identification of nonindigenous species requires familiarity with, and access to, the worldwide literature. Less experienced laboratory workers might conveniently assign names already in the Puget Sound literature to similar, new nonindigenous species. Because of their expertise, the ability of the expedition members to recognize nonindigenous species was high. There were, however, several prominent taxa of organisms that we could not identify with the specificity needed to recognize nonindigenous species. Such taxa included some hydroids, which are abundant in fouling communities throughout Puget Sound, but have never been thoroughly worked up locally to the species level; thus, we lacked the background information necessary to draw comparisons during a week-long field trip. Flatworms and sponges could not be positively identified even to genus during the expedition. The low worldwide taxonomic resolution of some of the polychaetes and peracarid crustaceans prevent distinctions between some native and nonindigenous species. Green algae *Ulva* and *Enteromorpha* were

ubiquitous at the waterline at most float sites, but their identification to species is problematic, requiring genetic or ultrastructural taxonomic expertise not available to us.

We are now compiling a master list of nonindigenous species that are presently established in Puget Sound. We anticipate that it will include a few more than 50 species. This list will be substantially shorter than the 78 species currently listed for all Washington State and adjacent marine waters by the Washington State Department of Fish and Wildlife (http://www.wa.gov/wdfw/fish/nuisance/ans4.htm). Previous lists of nonindigenous species in Puget Sound include that of Carlton (1979), who acknowledged 29 nonindigenous marine species in the region covered by the Puget Sound Expedition; Elston (1997), whose list of 31 nonindigenous species includes shared inland waters of Washington and British Columbia; and Ruiz and Hines (1997), who listed 67 nonindigenous species in the marine and estuarine waters of Washington State and British Columbia. Our expedition is the first extensive field-based study since Carlton's 1976 and 1977 fieldwork in Puget Sound and the Strait of Georgia. We collected 24 nonindigenous species not on Carlton's list, 30 species not on Elston's list, and 14 species not on the Ruiz and Hines list or the Washington State Department of Fish and Wildlife website (which is based largely on the Ruiz and Hines list).

The San Francisco Bay and estuary system now supports more than 230 nonindigenous species (Cohen and Carlton 1998). Of these, about 160 are in marine and estuarine habitats comparable to what is here considered as greater Puget Sound. The Puget Sound area has received considerably less attention than San Francisco Bay; the list of approximately 50 nonindigenous species in Puget Sound does not include fish or salt marsh organisms, as are listed for San Francisco Bay. Some of the difference between the lengths of these lists therefore continues to be a reflection of effort by scientists looking for nonindigenous species, but Puget Sound has not yet received many of the devastating new organisms now well established in San Francisco Bay. We plan to extend our studies of Puget Sound to additional habitats (see below). No list of freshwater nonindigenous species has been generated for rivers in Puget Sound that might be comparable to the 90 known nonindigenous species in the Delta region of the San Francisco Estuary (A. Cohen, unpubl. data).

While marinas provided accessible platforms for sampling subtidal communities close to the surface that are not subject to tidal exposure (thus considerably lengthening the daily sampling window), we largely missed sampling the extensive intertidal mudflat/sandflat habitat typical of much of Puget Sound. Brief stops at Samish Bay and Padilla Bay added several nonindigenous species to our list and indicate that these habitats must be sampled in the future to gain a more comprehensive picture of bioinvaders.

The northernmost site sampled in 1998 was determined by the Canadian border, which provides a politically, but not ecologically, meaningful boundary. Puget Sound and the Strait of Georgia are tightly linked ecosystems that share much of the same flora and fauna and will together share the burdens and environmental changes that come with invasions and establishment of new marine organisms from other regions.

Further complementary expeditions are planned for Puget Sound and the Strait of Georgia. Much of the original Puget Sound Expedition team plans to reassemble to sample mudflats and beaches in Puget Sound and Willapa Bay in May 2000. A team of (mostly Canadian) biologists sampled float-fouling communities on both the mainland and Vancouver Island sides of the southern Strait of Georgia in late winter 1999 and plans to repeat this exercise in late summer in the near future (C. Levings, pers. comm.).

At least one-quarter of the nonindigenous species now known to be present in Puget Sound have been previously overlooked, unreported, or misidentified. The possible impacts of these invasions thus remain unexplored by modern ecological research. Our findings demonstrate the value of rapid assessment surveys by trained systematists as a useful technique to detect marine or aquatic bioinvasions. Comparison of nonindigenous species in Puget Sound and San Francisco Bay also highlights the magnitude of the continued threat from interstate ballast water transport up and down the west coast.

Acknowledgements

This Expedition owes its success to many factors. Most important were the volunteer spirit underlying the participation of the scientists, the cooperation of marina managers in allowing us to sample, the interest and support from Tom Mumford, head of the Aquatic Resources Division of the Washington State Department of Natural Resources, John Armstrong of

the U.S. Environmental Protection Agency, and Mary Mahaffy of the U.S. Fish and Wildlife Service, and finally to the much-nicer-than-predicted weather during our long days in the field. The Washington State Department of Natural Resources provided vans, mileage, ferry fares, meal and lodging reimbursements, equipment, supplies, and employee time. The U.S. Fish and Wildlife Service, Puget Sound Water Quality Action Team, and King County Department of Natural Resources provided funds, equipment, or employee time that contributed to the success of the Expedition. Availability of space at the University of Washington Friday Harbor Laboratories and the Western Washington University Shannon Point Marine Laboratory was appreciated. Eugene Kozloff, Bruno Pernet, Scottie Henderson, Mary McGann, Doris Sloan, Craig Staude and Wim Vervoort all provided expert assistance with species identification.

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The Freshwater Expansion and Classification of the Colonial Hydroid *Cordylophora* (Phylum Cnidaria, Class Hydrozoa)

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ABSTRACT: ***Cordylophora*** **spp. is a colonial hydroid occurring in brackish and freshwater habitats. Records indicate that the distribution of this hydroid is expanding globally by increased boat travel and ballast discharge.** ***Cordylophora*** **spp. occurrences are becoming more common in freshwater habitats probably due to an increase in salts from runoff. This paper provides an updated distribution of** ***Cordylophora*** **spp. populations in several freshwater systems in the United States. DNA analyses along with interbreeding experiments using several populations of** ***Cordylophora*** **spp. are proposed to address a possible taxonomic discrepancy between two of the five documented species of** ***Cordylophora, C. caspia*** **and** ***C. lacustris.*** ***Cordylophora*** **spp. poses problems to power plants in Europe and the United States. Methods to curtail hydroid growth in pipes at a local power plant in Morris, Illinois were investigated. Laboratory experiments using cultured colonies indicate that high temperature is most effective (compared to chlorine doses) in killing or curtailing hydroid growth. Hydroids exposed to lower temperatures and chlorine regenerated due to the presence of menonts or resting stages. Elucidation of the overall distribution patterns and genetic structure of various hydroid populations will assist in confirming or modifying the taxonomy of this genus and will provide ecological information for an organism that is becoming a more prevalent invading species.**

Key words: hydroid, range, taxonomy, *Cordylophora*

INTRODUCTION

Evaluation of the distribution, classification, and biofouling potential of the hydroid *Cordylophora* spp. is necessary due to an increased occurrence of this organism in freshwater systems. The purpose of this paper is to provide an updated literature review of the biology and classification of the genus *Cordylophora*. Reasons for the range expansion of the hydroid are addressed and are related to a higher incidence of fouling problems caused by *Cordylophora* spp.

BIOLOGY OF CORDYLOPHORA

Cordylophora spp. (Class Hydrozoa, Family Clavidae) is a colonial, athecate hydroid occurring in fresh water to brackish habitats and therefore is able to live within a wide salinity range from 0 to 20 ppt (Fulton 1962; Green 1968; Roos 1979; Gaulin *et al.* 1986; Pennak 1989; Thorp and Covich 1991) (Figure 1A, 1B). Growth is optimal at 15 ppt salinity with colonies growing up to 5 cm though this varies depending on the habitat (Green 1968; Pennak 1989). Colonies are polymorphic, consisting of polyps specialized for feeding (gastrozooids) or reproduction (gonophores) and are dioecious with gonophores containing eggs or sperm (Figure 1B). A medusa stage is lacking, while a free-swimming planula larva is released from female gonophores (Pennak 1989). Colonies flourish especially during late spring and summer months with a peak in growth during July, August, and September depending on water temperature (Roos 1979; Jourmalainen *et al.* 1994; pers. obs.). Colony proliferation via asexual budding can be quite rapid if conditions are favorable. When water temperatures decrease in the fall, the colonies die back and consist simply of stalks, with portions of the coenosarc or structures referred to as menonts inside the perisarc or chitinous covering (Roos 1979) (Figure 1B, 1C). The menonts allow the hydroid to withstand periods of stress and/or cold temperatures. When water temperatures begin to increase in the spring, cells within the menonts begin dividing and new colonies are established. The presence of menonts and the ability for regeneration make this hydroid resistant to environmental changes and persistent in growth when it is responsible for biofouling.

DISTRIBUTION AND RANGE EXPANSION

Cordylophora spp. was first discovered in the Caspian and Black Seas and supposedly came to

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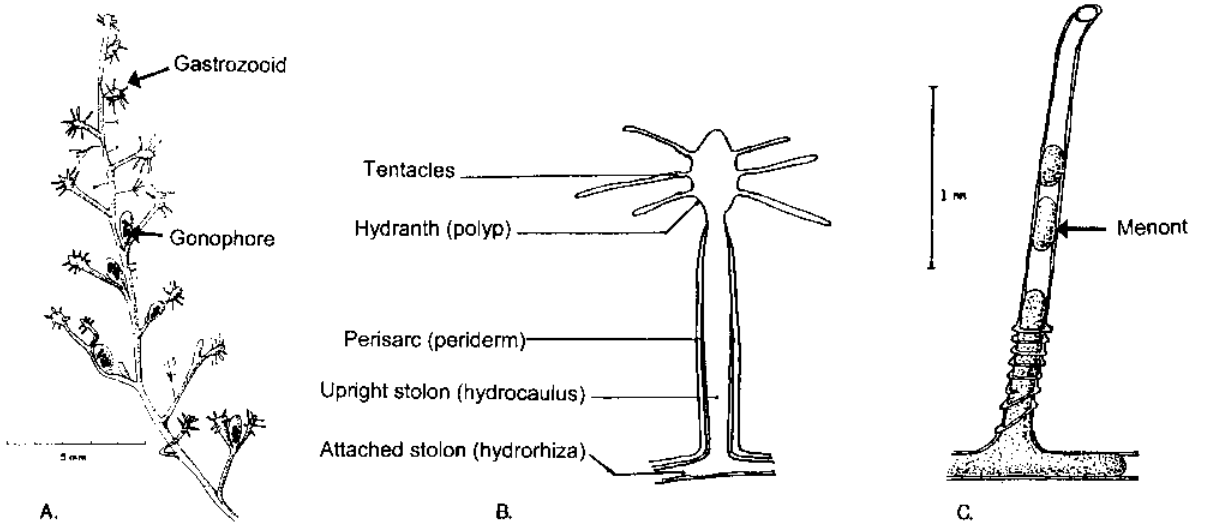

Figure 1. A. Part of a *Cordylophora* colony taken from a *Nuphar* stalk, with gastrozooids, female gonophores and epiphauna. Camera lucida drawing (Roos 1979). B. A drawing of the anatomy of an upright stalks and hydrorhiza of *Cordylophora* (re-drawn from Marcum and Diehl 1978). C. Menonts in hydrorhiza and base of hydrocaulus. Camera lucida drawing (Roos 1979).

North America in the late 1800s (Clarke 1878; Thorp and Covich 1991). There are currently five species of *Cordylophora* documented in the literature: *caspia, lacustris, annulata, pusilla,* and *inkermanica* (Roos 1979; Fulton 1962; Calder 1988; Boreo 1987; Marfenin 1983, respectively). Arndt (1984) produced a map of the global distribution of *Cordylophora caspia.* That map has been updated by adding the locations of the other four species along with additional citations for the occurrence of *C. caspia* (Figure 2). *C. annulata* occurs in Bermuda, while *C. pusilla* and *C. inkermanica* occur off the coast of Spain and in the Black Sea, respectively (Marfenin 1983; Boreo 1987; Calder 1988). The remaining locations, and thus the majority of the points on the map, refer either to *C. caspia* or *C. lacustris.* It is apparent from the updated map that the geographical distribution of *Cordylophora* spp. (especially in the United States) is expanding inland in freshwater ecosystems. The global distribution of the hydroid is in part due to its ability to tolerate a wide range of salinity along with increased boat travel and ballast discharge (Roos 1979; Thorp and Covich 1991; Carlton and Hodder 1995). It is also suggested that anthropogenic factors such as pollution or eutrophication are responsible for this expansion by altering salt concentrations in the water (Hubschman 1971). The increased prevalence of this hydroid in freshwater systems is possibly due to an increase in salts (especially chlorides) from runoff (Smith 1989).

Clarification of Classification

A need for clarification of the taxonomy relating to *C. caspia* and *C. lacustris* is evident by reviewing the literature. Pennak (1989) describes *C. lacustris* as a freshwater hydroid and states that some taxonomists think that *C. lacustris* and *C. caspia* are synonymous. Confusion exists in the literature with some biologists referring to *Cordylophora* as *C. lacustris* while others refer to it as *C. caspia* (Table 1A,B). Some authors equate the two (Roch 1924 as cited by Ramane and Schlieper 1971; McClung *et al.* 1978; Roos 1979; Smith 1989; Cohen *et al.* 1998). Smith (1989) states that *C. caspia* is used more commonly in the European literature. Jormalainen *et al.* (1994) cites

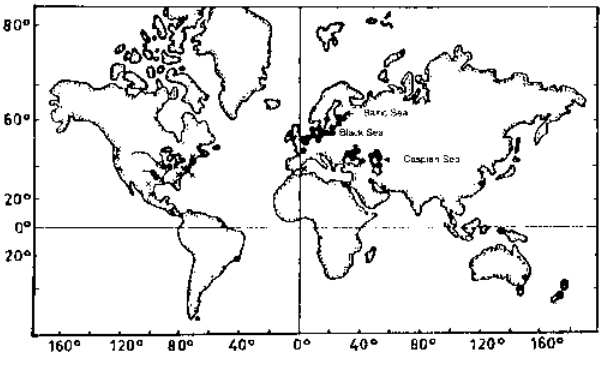

Figure 2. An updated map showing the global distribution of *Cordylophora* spp. (modified from Arndt 1984). The Xs indicate additional locations of the hydroid. There are currently five species of *Cordylophora* documented in the literature: *caspia, lacustris, annulata, pusilla* and *inkermanica.*

Table 1. A summary of the documentation of two species of *Cordylophora (C. caspia=Cc; C. lacustris=Cl)*. Some authors were more specific in providing habitat descriptions and locations. FW= freshwater habitats. Part A lists studies documenting *C. caspia* (Pallas 1771). Part B includes references to *C. lacustris* (Allman 1844).

Author	Part A. Habitat	Location
Arndt 1984	(Cc) Brackish (5-7 ppt)	Iraq
Calder 1971	(Cc) Brackish, S. Chesapeake Bay	Virginia, U.S.
Calder 1976	(Cc) Estuarine	South Carolina, U.S.
Calder 1990	(Cc) Estuarine	South Carolina, U.S.
Calmano *et al.* 1992	(Cc) Estuarine	Germany
Cohen *et al.* 1998	(Cc=Cl) Brackish San Francisco Bay	California; Washington, U.S.
Franzen 1996	(Cc) Brackish (5-6 ppt), Baltic Sea	Stockholm, Sweden
Jormalainen *et al.* 1994	(Cc) Brackish, Aurajoki River	Turku, Finland
Ringelband and Karbe 1996	(Cc) Brackish (10 ppt)	Nord-Ostseekanal, Germany
Roos 1979	(Cc=Cl) Brackish rivers	Amsterdam, Holland
Von Holle and Ruiz 1997	(Cc) Estuarine, Baltimore Bay	Maryland, U.S.
Walton 1996	(Cc) Brackish, Hudson River	New York, U.S.

Table 1 Continued: References to *Cordylophora lacustris*.

Author	Part B. Habitat	Location
Calder 1968	(Cl) FW/low salinity	Virginia, U.S.
Chain 1980	(Cl) FW, Tamar River (0-10 ppt)	Cotechele Quay, England
Clarke 1878	(Cl) Brackish, Curtisi creek	Baltimore, Maryland, U.S.
Fulton 1962	(Cl) FW	Nye Pond, North Falmouth, MA
Gaulin *et al.* 1986	(Cl) Estuarine	New Hampshire, U.S.
Hubschman 1971	(Cl) FW, Lake Erie	Pennsylvania, U.S.
Kelly and Franks 1995	(Cl) FW, Livingston Reservoir	Huntsville, Texas, U.S.
Lipsey and Chimney 1978	(Cl) FW, Baldwin Lake	Illinois, U.S.
McClung and Davis 1983	(Cl) FW, Concho River	San Angelo, Texas, U.S.
McClung *et al.* 1978	(Cl=Cc) FW, 5 rivers	Texas, U.S.
Moore 1951	(Cl) Estuarine, Tamar & Ouse River	Norfolk, England
Pennak 1989	(Cl) FW	New Jersey to Gulf of St. Lawrence, Illinois, Arkansas, Arizona, Kansas, Tennessee, Louisiana, U.S.
Siegfried *et al.* 1980	(Cl) Estuarine, Sacramento River	California, U.S.
Smith 1989	(Cl=Cc) FW	Massachussetts, U.S.
Streever 1992	(Cl) FW, Little River Spring cave system	Florida, U.S.

work by Gaulin *et al.* (1986) on *C. lacustris* by making reference to *C. caspia*. Green (1968) describes *C. caspia as* growing best at about 15 ppt while its freshwater counterpart has different "proportions" and has been described as *C. lacustris*. Differences in morphology suggest either that *C. caspia* and *C. lacustris* are two different species or that they are synonyms and that a great deal of plasticity in morphology is present. Morphological variation in *C. caspia* is caused by variation in salinity and temperature (Kinne 1956, as cited by Remane and Schlieper 1971). Kelly and Franks (1995) cite work by McClung *et al.* (1978) indicating variable colony size with increased chloride concentrations. However, the data of McClung *et al.* (1978) do not concur with Kelly and Franks' results for *C. lacustris* observed in east Texas. Kinne (1956, as cited by Remane and Schlieper 1971) reports *Cordylophora caspia* stalks being taller in brackish water (16.7 ppt) compared to colonies in freshwater. Similarly, Pennak (1989) documents that brackish water colonies of *C. lacustris* reach 20-100 mm in height while freshwater colonies rarely exceed 30 mm. Locally in Illinois, freshwater colonies obtain heights of 10-50 mm (pers. obs., Morris Illinois; Dr. Terry Marsh, pers. comm.). These colonies grow in areas of extreme flow and are at times exposed to slight increases in salinity due to evaporation caused by heating of power plant effluent water. Jormalainen *et al.* (1994) observed brackish water colonies of *C. caspia* reaching heights of 5 to > 50 mm. Remane and Schlieper (1971) also state that brackish colonies are at "their best" in the number of gonozooids and the number of tentacles on the hydranths; freshwater colonies have fewer and smaller gonophores and eggs. Roch (1924, as cited by Remane and Schlieper 1971) indicates that colonies of *C. lacustris* require more oxygen at lower salinity.

Yet another anatomical or morphological feature that can lead to confusion in classification between *C. caspia* and *C. lacustris* are rings or annulations on the perisarc of the stalk as seen in diagrams presented in the literature. For example, Pennak (1989) and Smith (1989) present diagrams of *C. lacustris* with annulations (Smith also refers to *C. caspia* and *C. lacustris* as synonymous) while Moore's (1951)

diagram of *C. lacustris* lacks rings. Green (1968), Remane and Schlieper (1971), Marcum and Diehl (1978), and Barnes (1994) present diagrams of *C. caspia* without annulations, while Roos (1979) presents a diagram of a colony (*C. caspia* = *C. lacustris*) without annulations and another drawing documenting menonts or overwintering structures with annulations on the stalk. Calder (1971) presents *C. caspia* with annulations.

All of these features, including variation in colony height, numbers of gonophores and tentacles, and the presence or absence of annulations on the perisarc indicate prominent eco-plasticity in colony morphology. This eco-plasticity may be genetically based and may lead to reclassification within the genus *Cordylophora*. The environmental habitats and locations of *Cordylophora* spp., as summarized from the literature, clearly indicate a great deal of habitat variation (Table 1B). With current taxonomic thought considering *C. lacustris* and *C. caspia* as conspecifics, Calder does encourage molecular analyses among populations for clarification (D. Calder, pers. comm.). It is unknown how much morphological variation is plastic and how much is genetically based.

Fouling by Cordylophora spp.

Cordylophora spp. causes biofouling by colonizing the inner walls of power plant pipes in Europe and the United States (Lipsey and Chimney 1978; Jenner and Janssen-Mommen 1993; Moreteau and Khalanski 1994; A. Cohen, pers. comm.; Folino and Indelicato, unpublished data; M. Khalanski, pers. comm.).

The Collins Generating Station located in Morris, Illinois, south of Chicago, is owned and operated by Midwest Generation (formerly owned by Commonwealth Edison). *Cordylophora* spp. was clogging the intake tunnel, causing blockage of filters and condenser tube sheets at the plant. Funding was granted to the author and student assistants to develop a treatment protocol for curtailing growth of the hydroids in unwanted areas. Colonies obtained from the Des Planes River in Joliet, Illionois were cultured and exposed to thermal treatments of 35°C, 36.5°C, 37.7°C, and 40.5°C for varying lengths of time ranging from 1 to 8 hr. Several experimental sets of colonies were also exposed to chlorine levels ranging from 0-5 ppm for 20 min, 105 min, and for three 20-min doses in a 24-hr period. Colonies did not survive

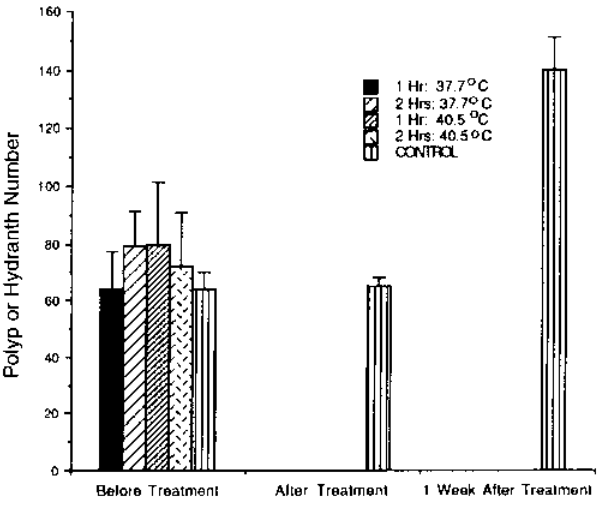

Figure 3. Results of the 37.7 and the 40.5°C treatments. Colonies were exposed for 1 and 2 hrs. Polyp or hydranth number was assessed before, immediately after and 1 week after each treatment. None of the colonies survived.

at the two highest temperatures (Figure 3), while colonies at the lower temperatures and at all of the chlorine concentrations exhibited varying degrees of survival and regeneration relative to exposure time (Folino and Indelicato, unpubl. data). It was clear that the presence of the menonts in the chitinous perisarc allowed for colony regeneration and growth. Thermal treatments appear to be the more effective and ecologically sound approach to addressing this biofouling problem at this particular plant. In other related studies dealing with pests such as *Corbicula fluminea* (asiatic clams) (Cameron *et al.* 1989) or *Drissena polymorpha* (Van Benschoten *et al.* 1995; Harrington *et al.* 1997), chlorine and/or exposure to high temperatures were used to control or curtail growth.

In addition to fouling problems, some have suggested that this hydroid impacts freshwater/estuarine communities. Von Holle and Ruiz (1997) document the negative effects of *Cordylophora caspia* on ciliates and bryozoa, while the hydroid attracted barnacles, amphipods and polychaetes. They suggest that this hydroid is a major restructuring force in the fouling community of Baltimore Harbor, Maryland.

Cordylophora spp. also occurs with the zebra mussel, *D. polymorpha*, at locations in Europe (Jenner and Janssen-Mommen 1993; Moreteau and Khalanki 1994) and in Illinois (J. Stoeckel, pers. comm.; pers. obs.). Competition for space may occur between the hydroid and *D. polymorpha* (Smit *et al.* 1993; Walton 1996). The presence of *Cordylophora* spp.

may enhance or facilitate recruitment of *D. polymorpha* (Dean and Hurd 1980; Moreteau and Khalanski 1994; M. Khalanski, pers. comm.). The hydroid may also provide a physical barrier to *D. polymorpha* settlement and/or may act as a larval filter (Standing 1976). Lastly, filtering by clumps of *D. polymorpha* may provide a more favorable substrate for *Cordylophora* spp. by enhancing flow. This type of symbiotic relationship is similar to associations between sponges and brittle stars in the Caribbean (Hendler 1984). Perhaps some ecological association exists between these organisms that enhances their roles in biofouling (Walton 1996). This is yet to be investigated.

Future Research

With the range of *Cordylophora* spp. expanding, it is of great interest to identify the factors responsible for this expansion. Future research with populations of *Cordylophora* spp. should address the following: (1) global population locations and physiochemical water conditions at each population location, (2) hydroid morphometric features such as colony height and polyp morphology, (3) genetic similarities and differences among populations collected, and (4) interbreeding capabilities among populations sampled.

As mentioned previously, *C. caspia* and *C. lacustris* are considered conspecifics though the existence of different species is possible. The species names *caspia* and *lacustris* have been used interchangeably without clear scientific documentation of anatomical or genetic differences for various *Cordylophora* spp. populations. To ascertain whether different species exist, morphological and genetic data need to be carefully analyzed.

Distinguishing morphological and genetic differences in populations of *Cordylophora* spp. will provide information to trace invasion routes and help explain the range expansion of *Cordylophora*. It is of great interest to aquatic biologists to understand reasons for a given organismís range expansion, since it may become a larger fouling problem for power companies and may have a major impact on community structure in freshwater and estuarine ecosystems.

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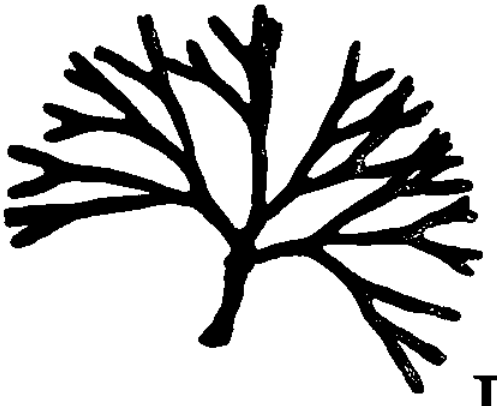
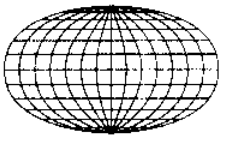


Ecological and Evolutionary Consequences

'There is a great need to merge data collection with models in order to extrapolate the results of experiments and sampling procedures on small spatial and temporal scales to predictions at larger scales.'



Ecological and Evolutionary Consequences Overview

Ecological and Evolutionary Consequences of Invasions: Addenda to the Agenda

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Key words: ecological process, evolutionary consequence, molecular technique, Bayesian statistics, frequentist statistics, population viability analysis

Introduction

The invasion of coastal marine and estuarine systems by nonindigenous species is simultaneously a widely heralded phenomenon and one that is poorly understood. This paradoxical situation has involved immense media attention and subsequent increase in public awareness, which, in part, has created problems for resource managers and regulatory agencies burdened with the job of "doing something about nonindigenous species" with little or no data upon which to act. Only recently have national funding solicitations targeted exotic species beyond oyster diseases and the zebra mussel, *Dreissena polymorpha,* woes of the Great Lakes. However, this new funding is still supporting a comparatively narrow range of projects focused on estuarine and marine nonindigenous species. A much broader research agenda is needed both to develop a more general understanding of the invasion process in the habitats as well as to generate the data needed by managers and decision makers.

My goal is not simply to review studies of nonindigenous species that have already contributed to what we know about impacts of introduced species. Rather, I attempt to distinguish the areas being addressed from those that are largely being ignored, in order to emphasize the need for directing resources to these holes in the agenda and to identify them as productive areas for future research. To some degree, I will highlight areas that are generally ignored by ecologists and evolutionary biologists working in marine and estuarine environments, whether they are concerned with nonindigenous species or not. However, the particular areas of emphasis upon which invasion biologists have to focus are particularly idiosyncratic and represent an even narrower subset of ecological and evolutionary endeavors. My desire is to make a plea for broadening the invasion biology agenda in marine and estuarine systems so that we develop a more general understanding of the ecological and evolutionary impacts of invaders. This should not delay providing answers to decision makers, but rather will broaden the sphere of answers available and permit refining the most needed approaches.

The Problem

Most experimental approaches available to benthic ecologists are useful only at small spatial scales. Unfortunately, the kinds of perturbations produced by introduced species are not unlike regional climatic events in that they may occur over broad spatial scales, certainly at scales beyond which experimental manipulation is feasible. In many cases, finding appropriate control sites for study may be difficult if the invading species has been undergoing rapid range expansion. Baseline data collected before an invasion, whether ecological, morphological, or genetic, are often few and scattered, as those trying to interpret impacts due to oil spills or other natural or man-made disasters have found. Unfortunately, research often begins after the fact and after the invading species has become a problem, typically several years after it has become established and pre-invasion data are no longer available. And unlike broad-scale envi-

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ronmental fluctuations (El Niño events, hurricanes), the introduced species does not go away and come back again permitting baseline data to be collected in the future.

Ecological Consequences

Nonindigenous species may have a range of negative (and some positive) impacts on the systems they invade. These include ecological impacts and economic impacts on fisheries, recreation, commerce, and other human activities in estuarine and marine environments. I will focus on the ecological impacts without regard to the economic consequences, although none of my conclusions would change in any case. The numbers of studies that have quantitatively documented the impacts of nonindigenous species are few. These studies have been reviewed recently by Ruiz *et al.* (1997) as part of a broader review of mechanisms, rates, and extent of invasions. Although there are many studies with anecdotal information about impacts, fewer than two dozen studies actually measure impacts quantitatively and test hypotheses about the causes. A few additional newer studies have also occurred since this review (Crooks 1998).

Single Species to Communities

Most of the studies in Ruiz *et al.* (1997) focused on impacts of a single nonindigenous species or a few species. Much of this work has focused on impacts to prey and competitors of the nonindigenous species of interest. This is not surprising, since prey and competing species are most likely to be affected by the introduction.

Unlike the direct impacts of predation or competition due to nonindigenous species, only a handful of studies to date have considered indirect effects of introduced species (Alpine and Cloern 1992; Kimmerer *et al.* 1994; Cloern 1996; Grosholz *et al.* in press), although these are likely to be as important and pervasive as direct effects (Menge 1995; Menge 1997). Much of ecology now focuses on indirect effects: for example, positive indirect effects on nonprey species; however, these are rarely considered in studies of invasive species.

Finally, few, if any, studies have investigated the impacts of exotic pathogens or parasites on host populations, although many studies have examined impacts on individual hosts or the abundance of the parasite (*e.g.*, Grosholz and Ruiz 1995; Hines *et al.* 1997). This is particularly disturbing given the potential for parasites and disease to have far-reaching impacts on native communities (Lessios 1995).

Food Webs and Ecosystems

Missing almost entirely from these studies are any implications for food web or ecosystem-level processes, except for studies of the Asian clam, *Potamocorbula amurensis* (Alpine and Cloern 1992; Kimmerer *et al.* 1994; Cloern 1996). These studies have shown changes in phytoplankton production caused by this bivalve that have profound implications for the entire food web in San Francisco Bay, CA. A few other studies have documented the impacts of habitat modification due to nonindigenous species, arguably a type of ecosystem-level change, either by providing new habitat or stabilizing and changing sediments (Posey *et al.* 1993; Crooks 1998). But given the potential importance of these kinds of changes and the consequences seen in terrestrial systems (Vitousek *et al.* 1996), it is surprising that so few studies have been conducted.

Physical Transport Mechanisms

The process of range expansion of nonindigenous species is much easier to observe than tracking a rare allele in a native population, and, thus, monitoring dispersal along a coastline early in an invasion can provide rare insights into the physical transport mechanisms that are so important for understanding recruitment processes in marine and estuarine systems. Monitoring the range expansion of nonindigenous species in the context of models of surface advection may provide unique information that may aid in further model development. However, this goes beyond the realm of basic science, because understanding the mechanism and rate of range expansion for unwanted nonindigenous species is critical for predicting the arrival of a given species at a particular location. Understanding the rate of spread of one marine species may be the best guide we have for predicting the subsequent rate of spread for another exotic species with a similar life history.

Multiple Approaches

The nature of invasions may only infrequently lend itself to the use of controlled manipulative experimentation on scales with which ecologists working in benthic marine systems are familiar. If experimental manipulations of the invader or its prey

are possible, these should receive the highest priority. The numbers of studies in which one or the other is manipulated are growing, but they still represent only a small portion of the study of marine exotics. In most cases, a broader range of approaches that are now reasonably common in other systems is going to be needed to interpret the often imperfect and after-the-fact data that invasion studies often generate.

One method is the use of path analysis as a way of inferring direct and indirect effects of the exotic species on the community of native species. This method has been used successfully in marine intertidal systems for addressing the impacts of predators both native and introduced (Wootton 1994; Grosholz *et al.* in press). In short, this method is a structured multiple-regression approach that allows the specification of causal pathways based on an understanding of the natural history of the system. It allows the construction of a model with causal pathways involving several dependent and independent variables, and permits the decomposition of the correlation between two variables (due to direct and indirect effects) into causal and noncausal components. Preferably, this should be accompanied by manipulative experiments to test the strength of the causal pathways postulated by the model, but the path analysis framework is a good beginning for investigating the multiple impacts of an invader in a complex system.

Another method that can and should be used frequently on broader spatial and temporal scales that are not amenable to experimental manipulation is to compare the site(s) impacted by the invader with appropriate control sites, ideally both before and after the impact. The use of these before-after control-impact (BACI) designs (Osenberg *et al.* 1994; Underwood 1996) will greatly strengthen inferences about the impact of the invader through comparisons with typically several control sites that have been sampled both before and after the invasion. A weaker but still useful comparison is a simple comparison of the impacted site with multiple control sites after the invasion has taken place. One way of strengthening the inferences based on this method involves gathering measurements of impact (on prey or competing species, habitat variables, etc.) as sites become sequentially invaded. The difference in the timing of the impacts witnessed at these sequentially invaded sites would be predicted to be equivalent to the difference in timing of invasion. For example, if two sites were invaded in different years (site one invaded in year one, and site two invaded two years later in year three), the changes in species composition or habitat variables witnessed at site one would be predicted to occur after a two-year delay at site two. If this can be accomplished with several sites, a match between the temporal offset in time of invasion and the delay in the corresponding changes due to the invader can provide a means of testing for invasion impacts.

Additional inference about the impact of invaders may be constructed through the use of Bayesian statistics (Ellison 1996). These methods are frequently used in ecology, including situations involving introductions (albeit native species) of predators and competitors (Stow *et al.* 1995; Pascual and Kareiva 1996; Stow *et al.* 1997). Although the validity of this statistical approach is continually debated (Dennis 1996), Bayesian methods may be particularly important in situations where significant information from prior invasion exists, or for which only a single time series exists with no control sites. Assuming that sufficient knowledge of the system exists with which alternative causal models can be constructed to explain the changes that may be ascribed to the invader, Bayesian methods permit the selection of the model most likely to produce the data in hand. By contrast, the more universally used frequentist statistics assume that the data in hand are a sample from the theoretical true population of values, and then estimate the probability that the observed data are caused by the model hypothesis (the invader) or its null alternative. In addition, Bayesian methods are especially useful if data from previous studies of the invader are available. This information can be included in the estimation of a prior probability which is an integral part of model testing. Thus, information from previous invasions can be used to inform interpretations of a current invasion.

Evolutionary Consequences

Consequences for the Invading Species vs. the Native Species

Unlike ecological studies, in which the focus is typically the resident native species (occasionally other exotics), evolutionary studies of invasions in marine and estuarine systems have focused almost entirely on how the invasion process has molded the

morphology or genetics of populations of the invading species. There are a few notable exceptions where the impact of the invader on the genetics or morphology of the native species has been investigated (*e.g.,* Vermeij 1982; Trussell 1996). This is perhaps not surprising, given that hard evidence for large impacts on native species is limited, since pursuing the genetic or morphological consequences of invasion impacts on populations might be unproductive. However, the invading species may be acting as a significant new selective force even though its ecological impacts on the native population may be minimal. Even if the invading species is rare, with little or no numerical impact at all on the native species, there is the potential for hybridization with closely related indigenous species, a point that remains almost entirely untested for any marine or estuarine species. Hybridization would of course represent a potentially enormous threat to the integrity of native species.

Allometry and Functional Morphology

Characters that might influence the invading species' performance (*e.g.,* trophic morphology) are perhaps the most obvious starting point for studies of functional morphology. Past studies mentioned above have examined the consequences of predation by the European green crab, *Carcinus maenas,* in eastern North America, with regard to changes in the shape of the shells of their snail prey (Vermeij 1982; Trussell 1996). Similar changes may occur in the timing or size of reproduction as well as growth rates in the invading species and perhaps the native species as well. Extensive predation on prey taxa may provide novel selection pressure for altered behavior, distributional changes, reduced mean size, early reproduction, altered growth rates, etc. Much work needs to be done in any or all of these areas.

Hybridization

One of the most significant consequences of an invading species is in regard to its potential for reproductive compatibility and possible hybridization with native species. The potential for loss of the native genotypes may be great, depending on the reproductive life history of the species; the impacts may range from the introgression of a few exotic genes into the native gene pool to complete loss of native genotypes.

The consequences of the invasion for the invader may be significant as well and can include the loss of rare alleles and even a reduction in overall heterozygosity if the founding population remains small for an extended period of time. The loss of genetic variation may have important, but as yet unmeasured for marine organisms, implications for the performance of the invader in the new range. Patterns of spatial and temporal genetic variation resulting from subsequent founder events in the range-extension process may further influence the potential of the invader to adapt to the novel local conditions.

Cryptic Species

The invasion process, either through ballast transport or other vectors, may create the possibility that close relatives of the initial invader may also become established. The possibility that what is viewed as a single invasive species may, upon closer examination, be two or more morphologically similar species has already been realized for at least one invasion (Geller *et al.* 1997) and may be the case for several others (Cohen, unpub. data).

Population Structure

Investigations of population structure of the invading species provide several opportunities for understanding pathways of invasion and the potential for regional dispersal, and for providing insights into physical processes that may influence dispersal (Kordos and Burton 1993; Hare and Avise 1996; Burton 1998). Estimating gene flow between populations of an invading species will provide critical data for assessing the potential for any local control measures. If reproduction and recruitment for a species are local and not regional, then some kind of population reduction measure may prove successful. Gene flow estimates could suggest that recruitment at a particular site is not limited to the local pool, thus making control or reduction at that site much less likely.

Multiple Approaches

A number of molecular techniques are available to ask questions about pathways of invasion, species identity, extent of hybridization, population structure, etc. (Avise 1994; Burton 1996; Ferraris and Palumbi 1996). Questions about the population structure of invader and native can be investigated with allozyme techniques as well as with DNA and RNA methods. However, the potential for limited genetic variation in the invading species resulting from the colonization bottleneck may limit the use

of some techniques such as allozymes or more slowly evolving genes due to lack of appropriate variation. Rapidly evolving microsatellite loci may be particularly useful for distinguishing population structure in these cases. To infer phylogeographic patterns en route to determining pathways of invasion, distinguishing hybrids, or separating single from multiple invasions, several different kinds of sequence data may be required, (depending on the level of variation needed), including mitochondrial DNA, internal transcribed spacer (ITS), or microsatellites (Burton 1996; Farraris and Palumbi 1996).

Finally, crossing or breeding design experiments can be employed to determine the contribution of heritable genetic variation to morphological or even behavioral characters that appear to be variable between invasions or between native and invaded populations. Crosses between species may also be informative for determining hybrid viability between closely related native and invading species.

Future Opportunities

The invasion of coastal habitats by nonindigenous species is a cause of great concern, and only significant investments of effort and resources will halt the spread of existing invaders and prevent future invasions. At the same time, much can be learned and should be learned about the natural or seminatural systems that we have left by studying the ecological and evolutionary consequences of these invasions. Invasions bring novel predators, competitors, prey, and habitat changes to our coastal environments. Moreover, the invaders are likely undergoing rapid evolution in their new habitat, with changes in prey choice, habitat usage, reproductive life history, and many other characters. Current evidence suggests that the founding populations of marine invaders may be very small (Bagley and Geller, unpub. data), which in a novel environment often hundreds or thousands of miles from their native range creates an exceptional opportunity for rapid evolution. The opportunities for parallel studies of ecological and evolutionary processes here are unprecedented.

What To Do Next?

I have already outlined many ways in which the studies of invasions in marine systems can not only advance our understanding of ecological and evolutionary processes in these habitats, but can also coalesce into a coherent body of information about a very general process. However, a few important areas need to be developed immediately if the science of invasion is to move forward.

First, like most other areas, baseline data are critical to any future interpretations of invasions on any scale, particularly larger ones. However, this includes not only numerical abundance data for native populations, but also morphological and genetic data from these same populations. The difficulty of distinguishing cryptogenic natives from true natives and obviously introduced exotics means that we need to collect at least modest numbers of voucher specimens from a range of native species, hopefully the same species and populations from which we are gathering baseline ecological data. Furthermore, the possibility of hybridization with an invading species that may or may not leave a morphological signature means that we need to develop a systematic collection of voucher specimens as soon as possible. In any case, the uncertainty of the identity of any invading species should dictate that each and every researcher should collect voucher samples for morphology and genetics from all invading populations with which they work. It would also be desirable if all of those samples were carefully georeferenced using the best equipment available to the investigator (a handheld Geographical Positioning System at a minimum) to avoid clumsy site descriptions and approximate locations.

Demographic data such as size- or age-specific birth, death, growth, survival, or reproduction are tragically limited for invaders and even most native species. These data are essential if we are to understand the impacts of these invaders, or if we are to successfully design any type of control program for the invading species in the future. For instance, to design an intelligent control program for an invader, we need to have enough demographic data to know which life stages contribute most to the population growth of the invader (how sensitive lambda is to changes in growth, survival, and reproduction of different demographic stages). With these data, we can target specific life stages and implement control measures that would result in the greatest reduction of population growth of the invader, or provide the most "bang for the buck." This analogue of a population viability analysis (PVA) could be called a population inviability analysis. Without these data, we risk enormous and costly investments that may result in embarrassing and tragic failures.

Analysis of recruitment patterns for any invasive species would provide desperately needed information about potential rates and extent of range expansion. Coupled with genetic analyses, this could answer questions about the extent to which recruitment is local or regional. As mentioned above, this is critical to determining the extent to which control measures at one site may be swamped by recruitment from outside the control area. There is reason to believe that this is not a hopeless situation, as illustrated by the many species now established in San Francisco Bay that have failed to colonize similar habitats only tens of kilometers away (Grosholz, unpub. data).

Finally, given the need for a predictive understanding of invasion outcomes, just as in other areas of ecology, there is a great need to merge data collection with models in order to extrapolate the results of experiments and sampling procedures on small spatial and temporal scales to predictions at larger scales. There are many classic examples in the ecological literature in which models have been successfully used with data to develop a predictive mechanistic framework (Roughgarden *et al.* 1989; Kareiva 1990). Hopefully, as invasion biology develops a broader empirical base, more attempts will be made in which the development of data and theory are joint endeavors.

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Scale-Dependent Effects of an Introduced, Habitat-Modifying Mussel in an Urbanized Wetland

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Key words: benthos, *Chione* spp., competition, habitat alteration, *Musculista senhousia*, mytilid

INTRODUCTION

The potential consequences of biological invasions by nonindigenous species (NIS) vary widely, and a complete list of the effects of invasive aliens would resemble a list of the roles of any species in an ecosystem. Nonetheless, it is useful to categorize known effects of exotics because doing so can offer insight into the dynamics of biological invasions and aid management efforts. A survey of the burgeoning literature on NIS (*e.g.*, Vitousek 1990; Williamson 1996; Parker *et al.* 1999; Ruiz *et al.* 1999) reveals several often-considered mechanisms by which exotics can affect native species and the ecosystem (Table 1).

Exotics may alter the genetic make-up of native populations through hybridization or affecting gene flow. They also may transmit or be pathogens or parasites. Interspecific interactions between exotics and natives are typically considered in negative terms, and may involve predation, herbivory, and competition. Less frequently considered are positive interactions such as mutualism or facilitation (or commensalism). Introduced species also may benefit resident species by serving as trophic resources. These interspecific effects may translate into alterations of community structure, such as local increases or decreases in species diversity. Although exotics may affect ecosystems in a variety of manners, effects of exotics on ecosystem-level properties are typically grouped into three main classes: alterations of food webs (or productivity), nutrient cycling, or disturbance regimes (Vitousek 1990; Cushman 1995; Williamson 1996). By breaking down biogeographic barriers and affecting evolutionary processes within invaded systems, non-native species also can have effects at large spatio-temporal scales.

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Table 1. Common effects of exotic species. Note that ecosystem engineering is recognized as a major class of ecosystem-level alteration, encompassing a variety of known effects.

Genetics	Hybridization of natives and exotics	
Disease/Parasitism	Exotics as parasites/pathogens	
	Exotics as carriers of parasites/pathogens	
Interspecific Interactions	Competition	
	Exotics as predators or consumers	
	Exotics as prey or primary producers	
	Commensalism/facilitation	
Community Structure	Altered diversity/dominance patterns	
Ecosystem-level Alterations	Food webs/productivity	
	Nutrient cycling	
	Ecosystem engineering	
		Disturbance regime
		Soil/vegetation structure
		Microhabitats
		Geomorphology
		Habitat destruction
		Habitat construction
Biogeographical and Evolutionary Patterns		

THE MUSSEL MUSCULISTA SENHOUSIA

Musculista senhousia (Benson in Cantor) is an Asian mussel that has invaded three continents, and is capable of having a range of effects within invaded ecosystems (Table 2). This small mytilid typically lives in soft sediments, where it uses its byssus to create a cocoon on the surface of the sediment (Crooks 1992). When the mussel occurs in high densities

Table 2. Examples of *Musculista senhousia* effects.

Effect	Description	Reference
Carrier of parasite	Intermediate host to unidentified species of flatworm parasite (final host is probably a fish or shorebird).	Crooks 1992; P.S. Perkins, UCSD, pers. comm.
Exotics as predators	Consumes plankton through filtration of water column.	Cloern 1982; Reusch and Williams 1999
Exotics as prey	Consumed by fish (*e.g.*, yellowfin and spotfin croakers, sargo), snails (*e.g.*, *Pteropurpura festiva*), and shorebirds (*e.g.*, willets, godwits and ducks).	Crooks 1992, pers. obs.; Reusch 1998; Carlton et al. 1990
Competition	Inhibits suspension-feeding bivalves such as *Chione undatella* and *C. fluctifraga*, and has negative effects on vegetative spread of eelgrass.	Creese *et al.* 1997; Crooks 1998b; Reusch and Williams 1998
Ammensalism	May inhibit subsurface deposit feeders and some tube-builders.	Crooks 1998a, b; Crooks and Khim 1999
Facilitation	Facilitates small infauna within mats (mediated by habitat creation). Benefits eelgrass blade growth by biodeposition (mediated by changes in nutrient cycling)	Crooks 1998a; Crooks and Khim 1999; Reusch and Williams 1998
Community structure	Changes in local species richness, density of individuals, and spatial heterogeneity on tidal flat.	Creese *et al.* 1997; Crooks 1998a; Crooks and Khim 1999
Nutrient cycling	Increased nutrient supply (combustible organic matter and ammonium) through biodeposition.	Crooks 1998a; Reusch and Williams 1998
Ecosystem engineering	Creation of byssal mats on soft sediments, which increases structural complexity, shear strength, and amounts of fine sediments and organics.	Crooks 1998a; Crooks and Khim 1999

(5,000-10,000 m^{-2}), a dense byssal mat is formed. Thus, *M. senhousia* fundamentally alters the nature of the benthic habitat through the construction of these byssal carpets and by causing changes in sediment grain size, organic content, and shear strength of sediments (Crooks 1998a; Crooks and Khim 1999). Studies on interrelationships between *M. senhousia* and resident biota in Mission Bay, San Diego, California, have revealed that the effects of the mussel appear scale-dependent. The mats created by *M. senhousia* increase the structural complexity of the benthos, and a suite of small macrofauna (*e.g.*, the tanaid *Leptochelia dubia*, the gastropod *Barleeia subtenuis*, amphipods, and midge larvae) able to live within this biogenic habitat are facilitated (Crooks 1998a). Most macrofaunal organisms found in higher abundances within mussel beds respond to the physical structure of the mat, although the biological activities of the mussel do affect some taxa (Crooks 1998a; Crooks and Khim 1999).

At somewhat larger scales, however, organisms not able to live within the mussel mats can be outcompeted. For example, large clams are often reported to be in lower abundances in the presence of *M. senhousia* (*e.g.*, Sugawara *et al.* 1961; Uchida 1965; Creese *et al.* 1997), and experimental work in Mission Bay has demonstrated that two species of cockles (*Chione* spp.) can be severely inhibited by the presence of the mussel and its mats (Crooks 1998b). Decreases in survivorship and growth of these clams probably occur by competition for both space and food. Over the last three decades, such interactions may have led to observed declines in surface-dwelling, suspension-feeding clams within a Mission Bay salt marsh tidal creek. Over the same time frame, a deeper-living deposit-feeding clam, *Macoma nasuta*, showed an increase in abundance in the tidal creek, and experimental results demonstrate that *M. senhousia* does not negatively affect this organism. Negative effects of *M. senhousia* are not limited to

animals, as the mussel can inhibit the vegetative propagation of the native eelgrass, *Zostera marina* (Reusch and Williams 1998, 1999). Interestingly, however, the mussel can also benefit eelgrass by regeneration of nutrients that stimulate blade elongation (Reusch and Williams 1998).

Exotic Ecosystem Engineers

Like *M. senhousia*, a number of other exotic species are reported to modify the physical nature of ecosystems, including snails that bulldoze sediments (Bertness 1984), aquatic plants that create meadows (Posey 1988; Posey *et al.* 1993), and goats that overgraze islands (VanVuren and Coblentz 1988). However, the general idea that exotics can fundamentally alter ecosystems and affect resident biota by constructing, destroying, or otherwise modifying the physical nature of habitats, termed ecosystem engineering (Jones *et al.* 1994, 1997), has yet to be incorporated into invasion biology. The recognition of this concept provides a valuable framework with which to view the effects of exotics. It appears to encompass alteration of disturbance regimes as well as other assorted ecosystem-level affects (Table 1). In the case of *M. senhousia* and other exotics that create physical structure, this habitat creation may benefit some resident biota, which again tends to receive little attention in invasion biology.

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Potential Impact of the Introduced Bryozoan, *Membranipora membranacea*, on the Subtidal Snail, *Lacuna vincta*, in the Gulf of Maine

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ABSTRACT: To examine the potential impact of the introduced bryozoan, *Membranipora membranacea*, on the snail, *Lacuna vincta*, an experiment to determine the growth rate of *L. vincta* fed on kelp (*Laminaria saccharina*) with and without *M. membranacea* was undertaken. In addition, field samplings were conducted at a series of water depths in order to compare density, size, and the number of egg masses of *L. vincta* with percent cover of *M. membranacea* on kelp blades. The results showed that *M. membranacea* had a potential negative impact on the *L. vincta* population associated with kelps. The growth rates of *L. vincta* fed on kelp with *M. membranacea* were lower than *L. vincta* fed on kelp without *M. membranacea*. The results of the field study showed a positive correlation between the percent cover of *M. membranacea* and water depth and positive correlation between the percent cover of *M. membranacea* and the density of *L. vincta* on kelp blades. The results suggest that the overgrowth of *M. membranacea* on kelp blades decreases the available grazing spaces for *L. vincta*, and affects the growth rate of *L. vincta*. Competition between *M. membranacea* and *L. vincta* for space on kelp blades may occur.

Key words: *Membranipora membranacea*, *Lacuna vincta*, *Laminaria*, competition, bryozoan

INTRODUCTION

Recently, several introduced species have become established in the subtidal ecosystems in the Gulf of Maine. The bryozoan *Membranipora membranacea*, the tunicates *Styela clava*, *Diplosoma* sp., and *Botrylloides diegensis*, the green alga *Codium fragile* ssp. *tomentosoides*, and the red alga *Bonnemaisonia hamifera* are all examples of recent introductions (Carlton and Scanlon 1985; Prince 1988; Berman *et al.* 1992; Lambert *et al.* 1992; Harris and Tyrrell, in review). The impact of the introduced bryozoan, *M. membranacea*, on a native kelp herbivore, *Lacuna vincta*, is the focus of this study. *M. membranacea* was first observed in the Gulf of Maine in 1987 (Lambert 1990; Berman *et al.* 1992; Lambert *et al.* 1992), and was thought to be introduced from Europe or the Pacific coast (Ryland 1970; Yoshioka 1982; Berman *et al.* 1992). The life stages of this bryozoan include a planktonic larva and a benthic adult (Ryland 1970; Yoshioka 1982). The adult *M. membranacea* is found primarily on laminarian and fucoid algae (Ebling *et al.* 1948; Sloane *et al.* 1957; Ryland 1970). In the Gulf of Maine, *M. membranacea* is mostly found on *Laminaria* spp. during the fall and winter (Berman *et al.* 1992). The overgrowth on kelp blades by the bryozoan is reported to have a negative impact on the health of kelp because it inhibits photosynthesis (Lambert *et al.* 1992). Overgrown kelps appear to be susceptible to a decrease in structural integrity of the blade (Lambert *et al.* 1992; Harris and Tyrrell, in review) which may also affect other marine organisms such as *L. vincta* that utilize kelp as a food source and habitat (Lambert *et al.* 1992).

L. vincta, a herbivorous snail, is one of the most abundant native organisms in the subtidal zone in the Gulf of Maine. *L. vincta* normally feeds and lives on kelp blades (*Laminaria* spp.), which are high in nutrition (Martel and Chai 1991). *L. vincta* can also be found on other algae such as *Fucus edentatus*, *Chondrus crispus*, *F. serratus*, *Gigartina stellata*, *Laurencia pinnatifida*, *Codium fragile* ssp. *tomentosoides* *Ulva lactuca*, *Desmarestia viridis*, *Chordaria flagelliformis*, and *Antithamnionella floccosa* (Shacklock 1981; Southgate 1982; Thomas and Page 1983; pers. obs.); however, they are not preferred food and habitat (Martel and Chai 1991; pers. obs.).

The purpose of this study was to increase the understanding of the potential impact of the introduced bryozoan, *M. membranacea*, on *L. vincta* popu-

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lations in the Gulf of Maine. Both *M. membranacea* and *L. vincta* use laminarian algae as their habitats but little is known about the interaction between these two competitors. The coexistence of both species on the same resource may have a negative impact on either or both of these species by creating intraspecific and interspecific competition for space. A combination of laboratory feeding experiments and field sample analyses was conducted to compare interrelationships between the percent cover of *M. membranacea* and density, growth, and reproduction of *L. vincta*.

Methods

Both field sampling and laboratory experiments were conducted to determine the potential impact of *M. membranacea* on *L. vincta* populations.

Field Sampling

Study Site and Sampling Location

Samples of the snails, *L. vincta,* were collected from Cape Neddick, York, Maine (43° 10' N, 70° 36' W) (Figure 1) using SCUBA techniques. This site is moderately exposed, particularly to swells and storms out of the northeast.

Sampling Methods and Schedules

The collections were made during October and November 1998. Field samples were collected at 3, 6, and 9 m (10, 20, and 30 ft) along transects set on depth contours. Five replicates of kelp sporophytes (*Laminaria* spp.) were collected at each depth. The replicates were taken 5 m apart. To collect the samples, plastic bags were used to cover the kelps, and then the kelps were removed from the substrates within the plastic bags. All the samples were brought back to the University of New Hampshire. In the laboratory, the snails and snails' egg masses were counted and measured. A grid was used to determine the percent cover of *M. membranacea* on each kelp blade. Both sides were measured and the results of each side were averaged to arrive at the mean percent cover per blade. In addition, kelp blades were dried and weighed. Weight of *M. membranacea* was determined and subtracted to give algal biomass. The percent of *Laminaria* weight covered by *M. membranacea* was also subtracted to give area and biomass of *Laminaria* available to *L. vincta*. Then, density, length, and the number of egg masses of *L. vincta*

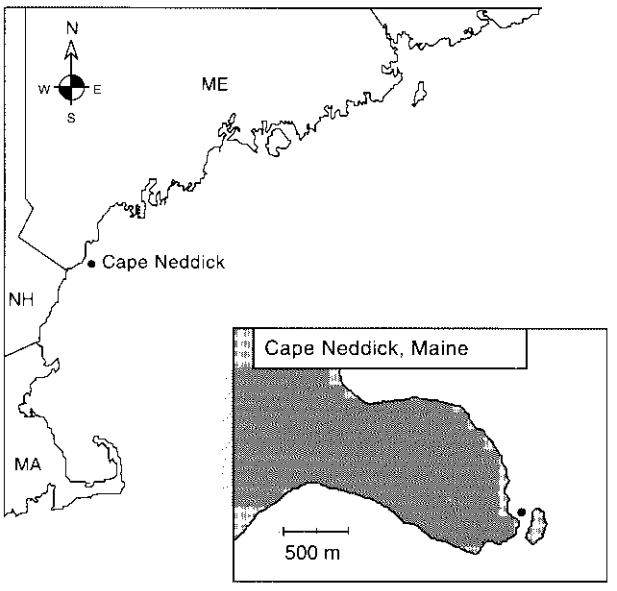

Figure 1. Map of the study location: Cape Neddick, York, Maine.

were related to the percent of available gm of algal dry weight.

In addition, monthly field samplings of *L. vincta* were conducted to examine the abundance of *L. vincta* on seven algal species. In this report, only the results from April 1998 are discussed. Five replicate samples of each algal species–*Laminaria saccharina, U. lactuca, Desmarestia aculeata, D. viridis, C. flagelliformis, C. crispus,* and *A. floccosa*–were collected at approximately 7.5-m water depth. The snails were sorted, counted, and measured in the laboratory. Algal biomass was also measured. Snail population parameters were then analyzed and compared on a gram-per-algal-species basis.

Laboratory Experiment

Impact of M. membranacea Cover on L. vincta Growth

To examine the potential impact of *M. membranacea* on the growth rate of *L. vincta*, an experiment was conducted to compare the growth rate of snails fed on kelp (*L. saccharina*) with *M. membranacea* and kelp without *M. membranacea.* All snails used in the experiment were 2 mm in length. There were three treatments with ten replicates each. Each 7-cm-diameter container received either one snail and one piece of kelp (approximately 3 cm x 3 cm) with 100% cover by *M. membranacea*, one snail and one piece of kelp (approximately 3 cm x 3 cm) with-

out *M. membranacea*, or a control with one snail and no kelp. Shell length of the snails was measured every week for six weeks.

The Structural Integrity of Kelp Fronds

The results of the field sampling showed that *M. membranacea* and *L. vincta* coexist on kelp blades. An experiment was conducted to determine whether *M. membranacea* or *L. vincta* had more effect on the structural integrity of kelp fronds. Thirty kelp sporophytes were collected randomly from Cape Neddick, Maine using SCUBA techniques, and all samples were brought back to the laboratory for analysis. Each sample of wet kelp was hung blade down from its holdfast, and lead sinkers were attached at the tip of each blade with a C-clamp. Weights were gradually added until the blade tore apart or a total of 2,940 g was reached. The location of any break was recorded and the presence of *L. vincta* grazing holes and/or *M. membranacea* cover was noted.

Prior to testing, the length and width of each kelp blade was measured, and the number of holes and percent cover of *M. membranacea* was recorded. The maximum weight of lead sinkers used in this experiment was 2,940 g. If a kelp blade did not break after the addition of 2,940 g, we recorded that kelp blade as intact.

Statistical Analysis

The mean plus 1 standard error (SE) was calculated for percent cover of *M. membranacea* and the length, density, and number of egg masses of *L. vincta*. Pearson correlation tests were used to examine the relatonship between water depth, percent cover of *M. membranacea*, density, size, and number of egg masses of *L. vincta*. In addition, a two-way ANOVA was used to test differences between times and the growth rates of *L. vincta* in difference treatments. Tests were done using Systat 7.0 (Systat 1997).

Results

Field Sampling

L. vincta are associated with a wide diversity of algal species. Figure 2 shows the density of *L. vincta* per gram of algal dry weight on seven algal species commonly found at Cape Neddick. The density of *L. vincta* was highest on *U. lactuca* and *C. flagelliformis*. *L. vincta* was found at medium density on *L. saccharina*. On the other hand, the largest individuals

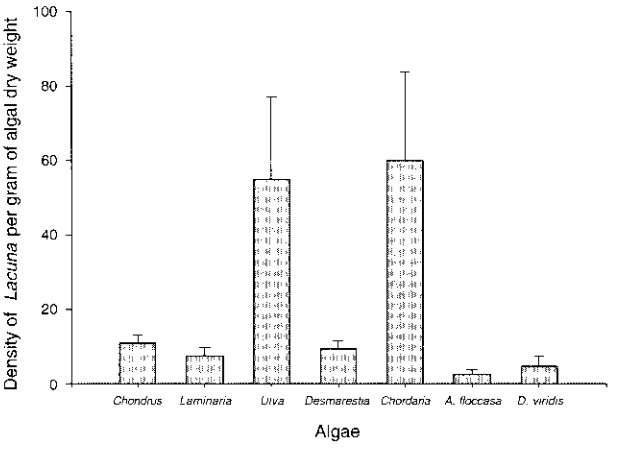

Figure 2. Mean density plus 1 SE of *Lacuna vincta* per gram of algal dry weight on seven algal species.

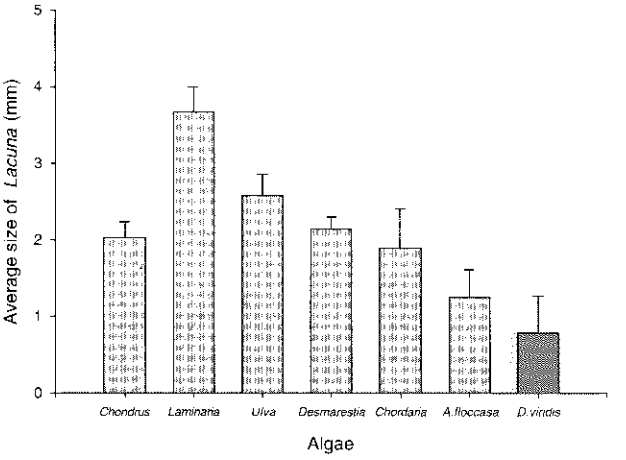

Figure 3. The mean length plus 1 SE of *L. vincta* on seven algal species

of *L. vincta* were found associated with *L. saccharina* (Figure 3).

The patterns of percent cover of *M. membranacea* and the density, the size, and the number of egg masses of *L. vincta* on *Laminaria* spp. at different depths are summarized in Figure 4. There was a significant positive correlation between the water depth and percent cover of *M. membranacea* on kelp blades ($P < 0.01$) (Table 1) with percent cover increasing from about 5% at 3 m to 35% at 9 m (Figure 4a).

There was also a pattern of increasing density of *L. vincta* per available gram of kelp with depth (Figure 4b), although the correlation of density and depth was not statistically significant (P=0.07). The highest density of *L. vincta* was found at 9 m (mean density of 137 per available g of algal dry weight), but the variation between blades was too great for statistical significance (range 17 to 478 per g).

Table 1. Results of Pearson correlation on the relationship between the depths of water, percent cover by *Membranipora membranacea*, and density, size, and number of egg masses of *Lacuna vincta*. Values given are P values.

	Depth	Percent cover *Membranipora*	*Lacuna* Density	*Lacuna* Size	*Lacuna* Egg Masses
Depth	-	-	-	-	-
Percent cover *Membranipora*	0.006	-	-	-	
Lacuna Density	0.070	0.004	-	-	-
Lacuna Size	0.1654	0.235	0.418	-	-
Lacuna Egg Masses	0.096	0.178	0.394	0.463	-

Table 2. Results of two-way ANOVA test for differences between times and the growth rate of *Lacuna vincta* in different treatments.

Sources	df	*F*	*P*
Treatments	2	13.576	0.000828
Times	6	10.309	0.00
Treatments x Times	12	2.646	0.003

There was a positive correlation between percent cover of *M. membranacea* and *L. vincta* density on kelp ($P<0.005$) (Table 1). There was no correlation between water depth and size of *L. vincta* ($P>0.1$) (Table 1). The highest mean size of *L. vincta* was found at 6 m depth (2.16 mm), and the average size of *L. vincta* decreased at 9 m depth (2.06 mm) (Fig 4c). Egg masses were found only at 6- and 9-m depths, and the number of egg masses per blade was similar for both depths (Figure 4d).

Laboratory Experiments

Impact of M. membranacea Cover on L. vincta Growth

There were significant differences in the growth rate of *L. vincta* fed on *L. saccharina* with and without *M. membranacea* ($P<0.001$) (Table 2). The growth rate of *L. vincta* fed on *Laminaria* without *M. membranacea* was higher than *L. vincta* fed on *Laminaria* with *M. membranacea* in every week ($P<0.005$) (Table 2; Figure 5).

The Structural Integrity of Kelp Fronds

There were clear differences in the structural integrity of kelp blades with and without *L. vincta* feeding holes and *M. membranacea* (Figure 6). None

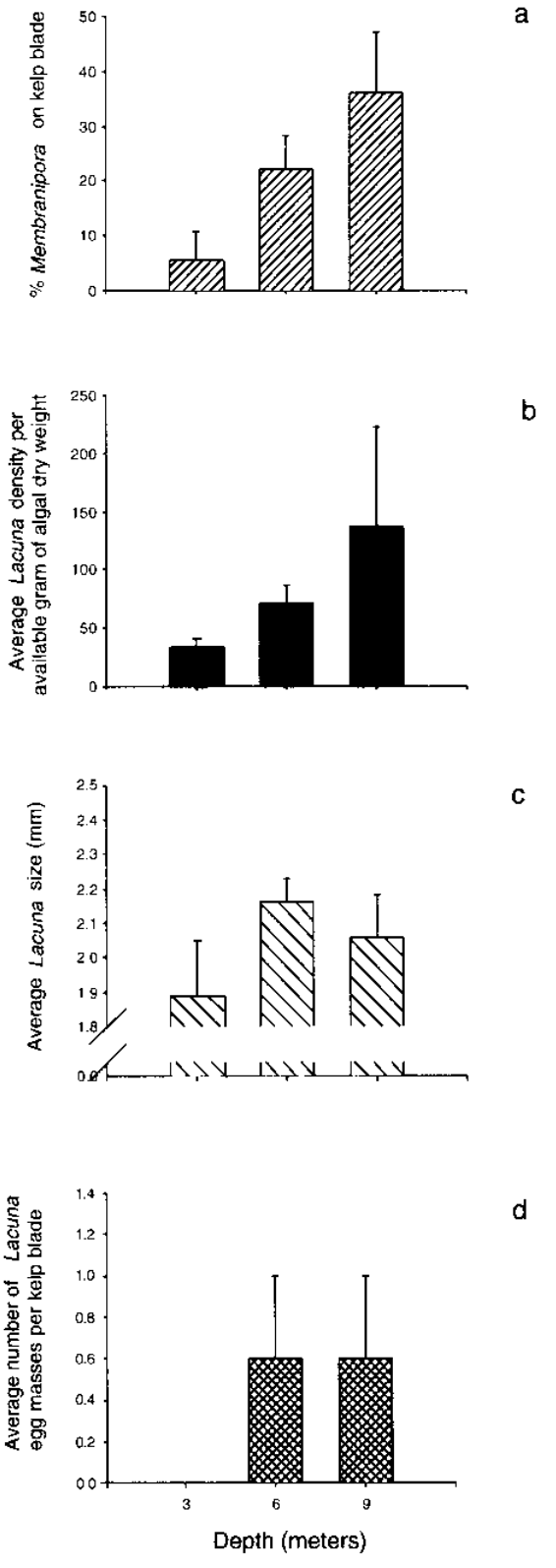

Figure 4. The percent cover of *Membranipora membranacea* (a), and average density per available gram of algal dry weight (b), average size (c), and average number of egg masses (d) of *Lacuna vincta* on *Laminaria* spp. at different depths of water.

of the kelp blades without holes broke during the tests. In contrast, 82% of kelp blades with only *L. vincta* feeding holes broke and 88% of those blades with holes and *M. membranacea* cover separated. The breaks were always in areas containing feeding holes. In the case of the blades with both holes and *M. membranacea*, 62.5% were in areas with only holes while 25.5% were in areas of holes overgrown by *M. membranacea*.

Discussion

L. vincta is a common herbivorous gastropod in subtidal habitats in the Gulf of Maine (Maney and Ebersole 1990). It occurs on a variety of algal species (Figure 2), including kelps of the genus *Laminaria*. *Laminaria*. spp. is a preferred food of *L. vincta*, and the largest individuals tend to be found associated with this kelp (Figure 3), at least in part due to its high nutritional content (Martel and Chai 1991). Grazing by *L. vincta* has been implicated as having a negative impact on *Laminaria*. populations (Fralick *et al.* 1974). *M. membranacea*, which first appeared in 1987, also occupies *Laminaria*. blades and has been described as having a negative impact on kelp population (Lambert *et al.* 1992).

In the field study, both the percent cover of *M. membranacea* and the density of *L. vincta* on kelp blades increased with increasing water depth. At greater water depths, there is less wave action and this may be beneficial for *M. membranacea* and *L. vincta* stability on the kelp blades (Ryland 1970). In this study, the statistical analysis showed that there was a positive correlation between the percent cover of *M. membranacea* and the density of *L. vincta* on kelp blades (Table 1). The results suggest that these two species coexist on kelp blades (Figure 4). *M. membranacea* uses laminarian algae for its substrate while *L. vincta* uses kelp for both habitat and a food source (Ryland 1970; Lambert *et al.* 1992; Martel and Chai 1991; Berman *et al.* 1992). The coexistence on the same habitat may have a negative impact on organisms either directly or indirectly, and may create intraspecific and interspecific competition for the resource (Jensen 1985; Jensen and Kristensen 1990). When *M. membranacea* covers the surface of a kelp blade, the space available to *L. vincta* for food decreases, resulting in an increased density of snails in the open area (Figure 4b).

The effect of coexistence of *M. membranacea* and *L. vincta* may also have an impact on the size of *L.*

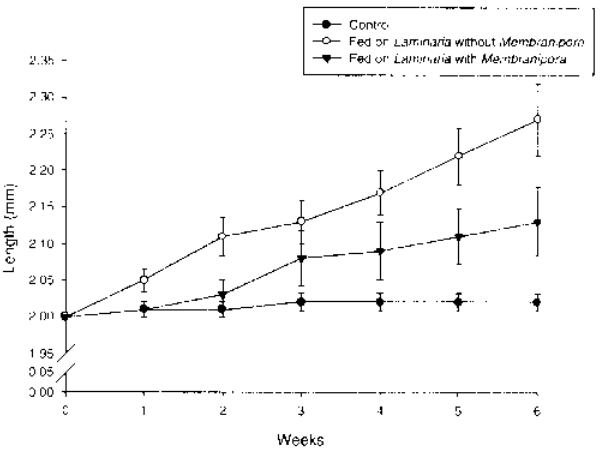

Figure 5. The growth rate of *Lacuna vincta* fed on *Laminaria saccharina* with and without *Membranipora membranacea*.

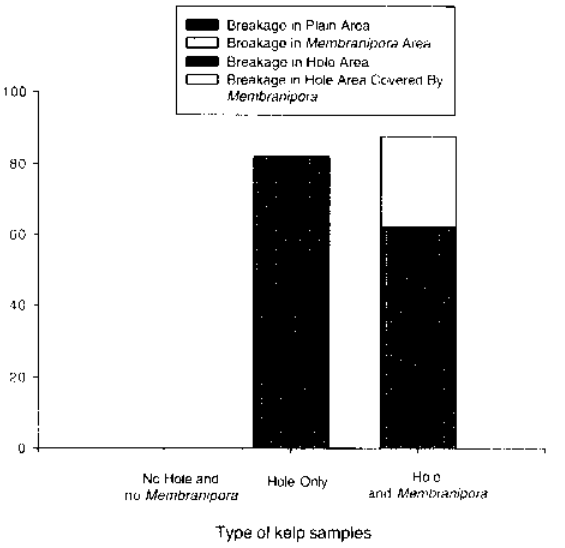

Figure 6. The results of the experiment on breakage areas of kelp fronds.

vincta as shown in Figure 4. The largest *L. vincta* were found at 6 meter depth, where densities were intermediate (Figure 4c). The density and mean size of *L. vincta* on *Laminaria* at 6 m were comparable to the densities and size values seen on *U. lactuca* in (Figures 2 and 3). The densities were lower, and mean size was larger on *Laminaria* (figures 2 and 3), which suggest that crowding may inhibit larger *L. vincta* from occupying *Laminaria*. under crowded conditions. Mean size of *L. vincta* declined but densities increased at 9 m (Figure 4c), which would seem to support this pattern.

The laboratory results showed that the growth rate of *L. vincta* fed on kelp with *M. membranacea* was

lower than the growth rate of *L. vincta* fed on kelp without *M. membranacea*. When *M. membranacea* overgrows kelp blades, it reduces the grazing area for *L. vincta*, which cannot penetrate into the surface of blades covered by encrusting bryozoans like *M. membranacea*. The laboratory studies provide evidence for reduced growth rates due to limited food availability, which may explain the smaller mean size at 9 m depth. Further studies are needed to determine whether the higher densities result in smaller sized individuals due to slower growth rates or avoidance by larger individual *L. vincta*.

Berman *et al.* (1992) reported that *M. membranacea* is a seasonal bryozoan, and is found abundantly on kelp blades during the fall and winter at Cape Neddick, Maine. *L. vincta* can be found year round in the Gulf of Maine, and has year-round reproduction (Maney and Ebersole 1990). Even though *M. membranacea* and *L. vincta* coexist on the same kelp resource, the overlap in spatial distribution is seasonal and the ability of *L. vincta* to utilize alternative algae may reduce the actual competition between the two species.

It is possible that *L. vincta* may have a negative impact on *M. membranacea* occupying *Laminaria* blades. The results from the laboratory stress experiment showed that the structural integrity of kelp fronds was decreased by holes made by *L. vincta* and the overgrowth of *M. membranacea*. Kelp blades are more easily broken in areas with holes caused by *L. vincta* grazing than with *M. membranacea* because the holes reduce the cross-sectional area of the kelp blade.

Fralick *et al.* (1974) suggested that grazing by *L. vincta* and the resulting holes were an important factor leading to the severe breakage of kelp fronds observed at Cape Neddick in the fall of 1973. Lambert *et al.* (1992) reported that the occurrence of *M. membranacea* on kelp fronds played an important role in the defoliation of kelp blades at Cape Neddick in the fall of 1991. This study shows that both *L. vincta* and *M. membranacea* can be the important factors leading to the breakage of kelp (Figure 6). When both *L. vincta* and *M. membranacea* occur on *Laminaria* blades, they may make the blades more susceptible to breakage by wave action. If *M. membranacea* is absent, *L. vincta* normally grazes on the tip and the middle areas of kelp blades (Johnson and Mann 1986; pers. obs.). Similar to *L. vincta*, *M. membranacea* recruits most heavily near the tip of the kelp blades and grows toward the base of the kelp fronds (Ryland 1970). When *M. membranacea* settles and overgrows portions of the kelp blades, it may impact the *L. vincta* population by obstructing its preferred grazing area.. Therefore, *L. vincta* may migrate toward the base of the kelp fronds and increase in density on a smaller portion of clear blade. The heavier grazing in a limited area may increase the likelihood of water-motion-induced breakage closer to the base of the blade. The coexistence of these two species may create more defoliation of kelp blades, and may change the area of kelp breakage.

Membranipora membranacea overgrowth may actually reduce its own survival through the winter. The areas that have *M. membranacea* are heavy and less flexible. In addition, *M. membranacea* overgrowth concentrates *L. vincta* grazing in the remaining free area close to the base of the blade. When the breakage does happen, the distal part of kelp blade covered by *M. membranacea* is most likely lost (see Figure 6). Studies by Harris and Tyrrell (in review) suggest that *M. membranacea* may be altering its habitat preference to other algal species. An indirect effect of reducing *L. vincta* grazing space on *Laminaria* blades may be decreased winter survival of *M. membranacea* colonies through blade loss, which would facilitate the shift to alternative algal substrates.

In summary, *M. membranacea* appears to have a negative impact on the *L. vincta* populations occupying *Laminaria* spp. The overgrowth of *M. membranacea* on kelps reduces the grazing spaces for *L. vincta* and creates intraspecific and interspecific competition for space on kelp blades. The competition between these two species may also have a negative impact on *M. membranacea* populations occupying *L. saccharina*, which may be facilitating the shift of this bryozoan to new algal substrates. Further studies of the interactions between the introduced bryozoan and this common herbivorous snail should provide interesting insights into the mechanisms governing their competition for a common resource, which is also an important component of benthic communities in the Gulf of Maine.

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Harris, L. G. and M. C. Tyrrell. Changing community states in the Gulf of Maine: Synergism between invaders, overfishing, and climate change. *Biological Invasions*. (submitted manuscript)

Ecological Interactions of Invading Ascidians Within Epifaunal Communities of Southern New England

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ABSTRACT: Within the last 25 years, four species of sessile marine ascidians, *Botrylloides diegensis*, *Styela clava*, *Diplosoma macdonaldi*, and *Ascidiella aspersa*, have invaded marine rocky shallow subtidal habitats of New England. Although all four species produce short-lived, poorly dispersing larvae, they have spread over a broad geographic area from Connecticut to Maine. Within this range their local distributions are fairly patchy, as they frequently occur in high abundance at some sites while being rare or absent at similar sites nearby. Since 1987 we have conducted experimental field studies in Vineyard Sound and eastern Long Island Sound, examining the ecological interactions between these ascidians and the native community. We have found that (1) adult ascidians transplanted to sites where they are rare or absent survive and grow at rates similar to those found at sites where they are abundant, (2) the ascidians are not strongly inhibited by any single resident epifaunal species from recruiting onto occupied substrates but recruitment success does decline as the diversity of resident species increases, (3) at field sites where these ascidians are rare, 1- to 3- day-old recruits are commonly preyed on by at least three species of very abundant small snails, *Anachis lafresnayi*, *Anachis avara*, and *Mitrella lunata*, (4) juvenile solitary ascidians are also preyed on by fish, mostly the cunner *Tautogolabrus adspersus*, and (5) *Botrylloides* and possibly *Styela* seem to escape predation at an earlier age and smaller size than similar native species. From our studies we feel that these species are able to invade local communities because (1) they have short range larval dispersal that allows them to build up abundant, self-sustaining local populations, (2) they can rapidly invade disturbed areas where the diversity of resident sessile species is low, and (3) some are less vulnerable to predation than similar resident species. However, they do appear to be excluded from some habitats such as more exposed open coastlines and even when present they seem to become well integrated into the local communities.

Key words: *Botrylloides*, *Styela*, *Ascidiella*, *Diplosoma*, predation, ascidian, sessile community, closed population

INTRODUCTION

Four species of sessile marine ascidians, the colonial species *Botrylloides diegensis* and *Diplosoma macdonaldi* and the solitary species *Styela clava* and *Ascidiella aspersa*, have recently invaded the marine rocky subtidal habitats of New England (Carlton 1989, pers. comm.). *Botrylloides* and *Styela* were first observed in the early 1970s (Carlton 1989; Berman *et al.* 1992), while we first encountered *Ascidiella* and *Diplosoma* in eastern Long Island Sound in 1989 and 1991, respectively. As with most ascidians, all four species produce non-feeding, short-lived planktonic larvae. Other than possible rafting by adult colonies attached to boat bottoms or drift debris such as eel grass (Worcester 1994), larvae are the principal dispersal stage of these ascidians. Previous studies of colonial ascidian larvae and settlement patterns (*e.g.*, Davis and Butler 1989; Stoner 1990; Grosberg 1987) have indicated that few travel more than 10 m before settling and most disperse much shorter distances. Given this limited mobility, we would expect the speed and range of their geographic spread to be limited. However, both *Styela* and *Botrylloides* have spread over a broad geographic area from Connecticut to Maine in fewer than 10 yr (Berman *et al.* 1992). Contrary to their rapid spread throughout this range, their local distributions along the

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New England coastline appear to be fairly patchy. Both species often occur in high abundance at some sites and are rare or absent at virtually identical sites nearby (Osman *et al.* 1990, 1992; Osman and Whitlatch 1995a). These patterns are intriguing and present a dual paradox. First, it is unclear how species with short-lived larvae can so rapidly spread along the coastline. Secondly, the rapid regional spread of the species is coupled with persistent discrete local populations which appear to be quite stable. Our research has been directed at uncovering the ecological processes that contribute to these patterns as well as understanding the short- and long-term effects of the invaders on resident epifaunal communities.

Our research on introduced ascidians is part of a series of ongoing studies that began in 1987 (Osman *et al.* 1989, 1990, 1992; Osman and Whitlatch 1995a, b, c, d, 1996, 1998; Whitlatch *et al.* 1995; Zajac *et al.* 1989; Stachowicz *et al.* 1999). We have focused on the ecological interactions between introduced ascidians and the resident epifaunal community in southern New England. We performed a variety of experimental field studies in Vineyard Sound near Woods Hole, MA and eastern Long Island Sound near Groton, CT. (Figure 1). In addition we have conducted surveys in a variety of habitats along the New England coast from Connecticut to Maine. Several general patterns have emerged from these studies:

1. Throughout the region we have found ascidian-dominated epifaunal communities at some sites and epifaunal communities dominated by bryozoans and other sessile invertebrates at similar sites, often less than 1 km away. In general, the ascidians are more likely to dominate subtidal hard substrates in protected areas such as embayments and harbors, whereas bryozoans, sponges, and cnidarians are the usual dominant subtidal epifauna in more exposed open-coast areas. Both of these patterns seem to have existed within the New England region prior to the invasion of *Botrylloides*, *Diplosoma*, *Styela*, or *Ascidiella* and have persisted over many decades. For example, Grave (1933) reported dominant ascidians in Eel Pond, Woods Hole, MA prior to any invasion, and after the invasion of *Botrylloides* and *Styela*, this dominance continued to be found at this site by Grosberg (1981) and later by Osman *et al.* (1990, 1992). Likewise, we observed the almost complete dominance by the colonial bryozoans *Schizoporella errata* and *Bugula turrita* on the pilings of the Marine

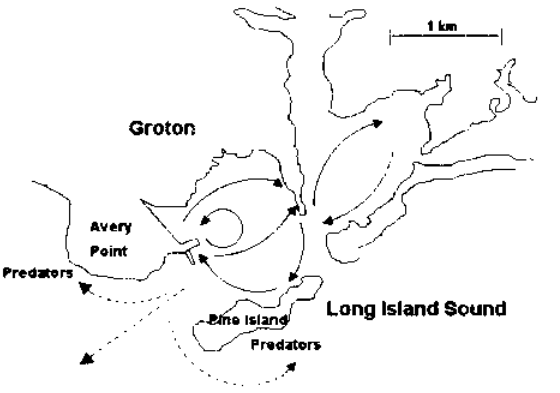

Figure 1: Conceptual model of closed population larval transport of invading ascidian species in the Poquonnock River estuary. Solid arrows indicate larval transport among sites within the embayment population. Dashed arrows indicate the occasional transport of larvae out of the embayment on ebb tide. Predators at the more exposed sites consume most ascidians that settle in these locations.

Biological Laboratory (MBL) water intake pier in 1987-1988 (Osman *et al.* 1990, 1992) that matched similar patterns observed in the early 1970s (Osman, pers. obs.). This site was less than 1 km from the Eel Pond site by water (< 100 m by land). Also, adult *Botrylloides* and *Styela* transplanted from Eel Pond to the bryozoan-dominated MBL pier suffered little mortality and showed no significant difference between treatments open to and caged from large predators (Osman *et al.* 1990). Juvenile *Botrylloides* colonies transplanted from Eel Pond to the MBL pier grew significantly faster than control colonies in Eel Pond (Osman *et al.* 1992).

2. Similarly, since 1989 we have observed complete ascidian dominance on substrates at a protected site in eastern Long Island Sound (the Avery Point, CT breakwater) and bryozoan dominance at a more exposed site (Pine Island) less than 1 km away (Osman *et al.* 1992; Osman and Whitlatch 1995a, 1996, 1998). Although epifaunal species dominance differed between sites, larvae of all species were found to settle at both sites. Surprisingly, Rogers (1998) found consistently greater numbers of solitary ascidian larvae in larval traps deployed at the exposed Pine Island site than at the protected breakwater site.

3. Even though larvae of all species settled at all sites, ascidian recruitment was almost completely eliminated at the bryozoan-dominated sites. Observations (Osman *et al.* 1990) and subsequent

experiments (Osman *et al.* 1992; Osman and Whitlatch 1995a, 1996, 1998) indicated that this resulted primarily from predation on newly-settled ascidians by the tiny snails *Mitrella lunata*, *Anachis avara*, and *Anachis lafresnayi*, and possibly other small predators. All three species of gastropods were found at our bryozoan-dominated sites in Woods Hole and Long Island Sound but not at the ascidian-dominated sites.

These results and observations suggest that there are at least four potential contributors to the success, distribution, and spread of the invading ascidians, including (1) environmental constraints or limits on where populations can successfully establish themselves, (2) life-history constraints that influence larval transport and distribution, (3) how the native sessile community interacts with the invading ascidians, and (4) whether there are differences between resident epifauna and introduced ascidians in their vulnerability to predators that could influence their eventual ability to occupy certain sites as well as to expand within a region. Our goal is to examine the role of each of these on the four introduced ascidians by summarizing, reviewing, and contrasting the results of over 10 years of research examining these species. Much of our research has concentrated on the latter two processes and only recently have we begun to examine the influences of environmental factors and larval distribution on ascidian populations. Our conclusions regarding these, therefore, are more speculative.

In reviewing our results, we first present an overview of our methods, which generally involved field experiments directed at testing specific hypotheses. We then summarize our results for each of the four invading species in order to elucidate the similarities and differences among them. Finally, we will use these results to examine the more general questions concerning what has made these species successful invaders and their paradoxical distribution.

Methods

Our investigations can be categorized into four general areas (1) discerning patterns of distribution of the invading ascidians relative to the resident fauna, including temporal and spatial patterns of recruitment, (2) experimentally examining the interactions between the epifaunal community and the invaders, (3) determining the role of predators in limiting the recruitment of the invaders, and (4) investigating any environmental constraints on the recruitment of the ascidians. Below we summarize the methods used in the studies we have conducted. More detailed descriptions are in the publications cited.

Temporal–Spatial Patterns

Although we have recently conducted surveys of the habitat distributions of the four species along the New England coast between Connecticut and Maine (Whitlatch and Osman, this volume), most of our studies have focused on spatial and temporal patterns of recruitment and community development. In these studies 100-cm^2 substrates were exposed for periods of 2 d to measure settlement (Osman *et al.* 1992), 1 wk to measure recruitment (Whitlatch *et al.* 1995; Osman and Whitlatch 1998), or several months to measure community development (Osman and Whitlatch 1998). Factors affecting the distribution of ascidian predators have also been measured recently (Rogers 1998; Berger 1998).

Interactions with Resident Species

The examination of interactions between the invaders and the attached epifaunal community focused mostly on the effects of attached adults on recruitment. Substrates with different densities of a single species were exposed to competent larvae in the laboratory (Osman *et al.* 1989) or to naturally occurring larvae in the field (Osman and Whitlatch 1995b, c; Whitlatch *et al.* 1995). Settlement was measured after 1-2 d (Osman *et al.* 1989; Osman and Whitlatch 1995b), recruitment after 1-wk (Osman and Whitlatch 1995c), and survival and growth of recruits after 1 mo (Osman *et al.* 1989). In addition, food competition between adult and juvenile oysters was investigated in the laboratory (*Crassostrea virginica*) and *Botrylloides* or *Styela* (Zajac *et al.* 1989; Osman *et al.* 1990).

Most recently, we have also begun to examine whether variations in the resident community can influence the ability of invaders to successfully recruit. In these studies communities were constructed with 1-4 species of residents. The invader (*Botrylloides*) was allowed to recruit into these communities and the invasion was judged to be successful if the *Botrylloides* survived to maturity and reproduced (Stachowicz *et al.* 1999).

Effects of Predators on Recruitment and Colonization

Experiments that were conducted in 1989 through 1992 (Osman *et al.* 1990, 1992; Osman and Whitlatch 1995a) examined the effects of single individuals of the predators *Mitrella lunata* or *Anachis* spp. on recruitment of sessile species. These experiments were conducted at the Avery Point breakwater using paired surfaces of 18-cm^2 panels with one surface exposed to a predatory snail and the other surface acting as a control. Companion experiments were also conducted in which the recruitment densities of *Styela clava* were manipulated to examine the effects of prey density on *Mitrella* and *Anachis* predation (Osman and Whitlatch 1995a).

Because these experiments did not test whether predator control of recruitment operated in the field under natural densities of predators, we conducted a series of experiments between 1992 and 1994 at a field site near Pine Island less than 1 km from the Avery Point breakwater (Osman and Whitlatch 1995d, 1996, 1998). These experiments were conducted using artificial pilings that were 75-cm tall, 28-cm diameter PVC pipe secured upright to weighted frames to mimic pilings and natural boulders. In each experiment 100-cm^2 substrates attached to replicate pilings were used as sampling units. In most experiments, 4 piling treatments were used with 5 pilings/treatment and 1-4 substrates/piling. Treatments were: (1) open pilings, exposed to all predator guilds, (2) caged (1-cm^2 mesh) pilings which excluded all but the snails *Mitrella* and *Anachis* and other micro-predators, (3) screened (1-mm^2 mesh) pilings which excluded all predators, and (4) partially screened pilings to control for artifactual environmental changes associated with screening the pilings. Each experiment was conducted with 1 of 3 life-stages of an epifaunal species: 1- to 3-d-old recruits, 2- to 3-wk-old juveniles, or adults. In each experiment panels were exposed for 3-6 d and then retrieved and mortality and/or growth were estimated.

Predator effects on colonization and community development were examined by transplanting ascidian-dominated communities from the Avery Point site to the Pine Island site (Osman and Whitlatch 1998). Transplanted substrates contained natural communities that were 1-, 2-, 3-, and 4-wk-old. Substrates were placed on (1) open experimental pilings exposed to all predators, (2) caged pilings exposed to only micro-predators, or (3) suspended in the water column on racks away from all predators. Community development was measured weekly for 3 mo.

Effects of the Physical Environment

The effects of environmental variables, particularly photoperiod and tidal currents, on recruitment were measured using either an automated device (Whitlatch and Osman 1998) or a piling array deployed with gravid adults on a central piling and pilings 1-3 m distant to measure the local spatial distribution of recruiting offspring (Osman and Whitlatch 1998).

Results

The effects of predators on the four invading ascidians, the types of interactions we have found between the invaders and the resident epifaunal community, larval life-history patterns and distributions of the invaders, and environmental effects on their settlement are compared in Table 1. In this table we have tried to summarize and contrast the outcome and consequences of each of these processes. Below we briefly summarize the results for each of the four species.

Botrylloides diegensis

Of the four invading species, *Botrylloides* has been in New England epifaunal communities the longest, since its release in 1972 (Carlton 1989; Berman *et al.* 1992). In southern New England, *Botrylloides* appears to be a semelparous, sub-annual species with two distinct cohorts per year (Malatesta 1991). In this sense its life-history is very similar to that of *Botryllus schlosseri* (Grosberg 1988) with which it often co-occurs. Recruitment in Long Island Sound revealed significant seasonal and year-to-year variations (Whitlatch *et al.* 1995). There are typically two recruitment peaks, a smaller one in early summer presumably produced by over-wintering colonies and a larger one in late August and early September produced by the early summer cohort.

Botrylloides colonies can grow rapidly and fill available substrate space. Few if any species are able to attach and recruit onto the surface of adult *Botrylloides* colonies and we have observed little overgrowth of *Botrylloides* by other species. Because of

Table 1. Summary of the effects of predators on the four species, interactions with the resident epifaunal community, measured patterns of larval distributions and settlement season, and effects of the physical environment. Some processes were not measured for all species and only those measured in experiments are included. Most experiments were conducted 3 7 days. Long-term patterns refer to colonization experiments that were conducted over several months. Numbers refer to the following studies: 1. Osman *et al.* 1989, 2. Zajac *et al.* 1989, 3. Osman *et al.* 1990, 4. Osman *et al.* 1992, 5. Osman and Whitlatch 1995a, 6. Osman and Whitlatch 1995b, 7. Osman and Whitlatch 1995c, 8. Osman and Whitlatch 1995d, 9. Whitlatch *et al.* 1995, 10. Osman and Whitlatch 1996, 11. Osman and Whitlatch 1998, 12. Whitlatch and Osman 1998, 13. Rogers 1998, 14. Berger 1998, 15. Holt 1994, 16. Malatesta 1991, 17. Sedgwick-Springer 1992, 18. Stachowicz *et al.* 1999

	Botrylloides	***Diplosoma***	***Styela***	***Ascidiella***
1. Predators				
Anachis	Minor Reduction of New Recruits [4,5]	Large Reduction of New Recruits [4,5] Long-term Abundance Reduction [15]	No Effect on New Recruits [8, 11]	
Mitrella	No Effect on New Recruits [4,5]	No Effect on New Recruits [4] Elimination of New Recruits [5, 10, 11] Long-term Abundance Reduction [15]	Elimination of New Recruits [5, 11]	
Micro-Predators	Some Long-term Abundance Reduction[11] No Effect on Recruits [10] No Effect on Juveniles [3,10,11] No Effect on Adults[10,11]	Reduction of New Recruits [10, 11] Long-term Abundance Reduction [11]	Reduction of New Recruits [8] Long-term Abundance Reduction [11]	Reduction of New Recruits [8, 11] Natural Colonization Reduction [11,12]
Fish or Macro-invertebrates	Reduction of New Recruits [8, 10, 11] Minor Reduction of Juveniles [3] No Effect on Juveniles [8] Some Long-term Abundance Reduction[11]	Elimination of New Recruits [10]	Elimination of New Recruits [8] Reduction of Juveniles [3] Elimination of Juveniles [8, 10, 11] No Effect on Adults [3] Long-term Abundance Reduction [11]	Elimination of New Recruits [8, 10,11] Long-term Abundance Reduction [11]
Carcinus	Reduces Micro-Predators [14]	Reduces Micro-Predators [14]	Reduces Micro-Predators [14]	Reduces Micro-Predators [14]
2. Competitors				
Effects on Resident Species by Invader	Reduced Space for Settlement [1, 6] Inc. Settler Density in Open Areas [1, 6] Caused Mortality by Overgrowth[1] Reduced Growth by Food Competition [2] Reduced Space for Recruitment [7] Reduced Recruitment [5, 9] Changed Relative Species Abundances [9]	Reduced Space for Settlement [6] Inc. Settler Density in Open Areas [6] Reduce Space for Recruitment [7] Overgrew Recruits [7]	Predation on Oyster Larvae [1] Reduced Growth by Food Competition [2,3] Reduced Settlement [9,17] Reduced Recruitment of *Botryllus, Bugula* but not *Spirorbis, Balanus, Botrylloides* [5, 7]	
Effects on Invader by Resident Species	Reduced Space for Settlement [6] Increased Settler Density in Open Areas [6] Reduced Space for Recruitment [7] Inc. Resident Diversity Reduced Recruit Survival[18]	Reduced Space for Settlement [6] Inc. Settler Density in Open Areas [6] Reduced Space for Recruitment [7]		
3. Larval Processes				
Distribution	Local Settlement Near Parents [11]		Larvae Found at All Sites [13] Most Larvae at Site where Adults Absent and Predators Abundant [13]	Larvae Found at All Sites [13] Most Larvae at Site where Adults Absent and Predators Abundant [13]
Season of Settlement	June-Nov, Small July and Large Sept Peaks (2 Cohorts) [9,16]	July-Nov, High Annual Variability	June-Nov, Annually Variable Peaks (Jul-Oct)	Mar-Oct, Annually Variable Peaks (Jun-Sept)
4. Environment				
Current	Most Settlement at Slack Water	Most Settlement at Slack Water	Most Settlement at Slack Water	Most Settlement at Slack Water
Light	Most Settlement Dawn to Noon [12]	Most Settlement Dawn to Noon [12]		Most Settlement Dawn to Noon [12]

this, the space occupied by *Botrylloides* is unavailable to other species and *Botrylloides*' presence either significantly decreases settlement and recruitment by other species or causes the density of recruits to increase in unoccupied open spaces (Osman *et al.* 1989; Osman and Whitlatch 1995b, c). However, we also found that *Botrylloides* could not recruit onto many other species and its settlement and recruitment were also reduced in the presence of some native species. In recent experiments, we have found that the survival of new *Botrylloides* recruits is significantly reduced as the diversity of the native community increases (Stachowicz *et al.* 1999). This appears to result from the reduction in the amount and variability of open space as the number of species increases. Finally, a series of laboratory studies Zajac *et al.* (1989) demonstrated that its presence reduced the growth rate and survival of juvenile oysters, presumably as a consequence of interspecific competition for limited food.

Compared to all other ascidians we have investigated, *Botrylloides* suffered the least mortality from predators. In experiments with individual predators (Osman *et al.* 1990, 1992; Osman and Whitlatch 1995a), the recruitment of *Botrylloides* was, at best, weakly reduced by *Anachis* and almost never by *Mitrella* predation. Although recruitment was usually reduced in the presence of the predators, the difference between control and predator treatments was seldom significant. This pattern was also seen in the field. *Botrylloides* recruitment was reduced significantly on open pilings exposed to fish and macro-invertebrates, and we concluded that these taxa were the major source of recruit mortality. These results differed sharply from those for the other colonial ascidians investigated, *Botryllus* and *Diplosoma*, which suffered high rates of recruit mortality in the presence of micro-predators. In piling experiments with juvenile and adult *Botrylloides* (Osman and Whitlatch 1996), no differences in mortality were seen among the treatments. From these results it would appear that *Botrylloides* escapes predation by the time it is approximately a week old.

Finally, these patterns were repeated in the 1994 colonization experiments (Osman and Whitlatch 1998). When 1-wk-old substrates from the ascidian-dominated breakwater site were transplanted to the site with abundant predators (Pine Island), only those on suspended racks isolated from predators developed a community dominated by *Botrylloides*. Substrates on open and caged pilings had no *Botrylloides* or any other ascidian. However, when substrates with 2-, 3-, and 4-wk-old communities were transplanted, *Botrylloides* remained a dominant species on all three treatments throughout the experiment.

Botrylloides recruitment is also strongly influenced by the physical environment. As with many ascidians and other epifaunal species, larval release is stimulated by light. Because larvae probably settle quickly, most settlement occurs between dawn and midday (Whitlatch and Osman 1998). *Botrylloides* also releases its larvae during periods of slack water (Worcester 1994) and most settlement occurs at this time (Whitlatch and Osman, unpublished data). This apparent ability to settle quickly after release resulted in very localized recruitment in experiments conducted at a site with strong tidal currents (Osman and Whitlatch 1998). Most recruitment occurred in the immediate vicinity (1-3 m) of the experimental adult colonies, suggesting the ability of this species to maintain self-sustaining local populations.

Diplosoma macdonaldi

We did not encounter *Diplosoma* in any of our studies until 1989, but by the late summer of 1990 it was one of the dominant species recruiting in eastern Long Island Sound (Osman *et al.* 1992). Although *Diplosoma* recruited throughout much of the summer, recruitment was generally highest in late August and September. Unlike the other three species, *Diplosoma* also exhibited large differences in abundance and recruitment rate at our study sites. Often, years when *Diplosoma* was a dominant species at the breakwater site (*e.g.*, 1993) were followed by years when it was exceedingly rare at this site (*e.g.*, 1994 and 1995). However, its absence was not regional since it remained abundant at neighboring sites in the Mystic River and at Millstone Point (Whitlatch, pers. obs.) located 5-8 km away.

In most of our studies *Diplosoma* was very similar to *Botrylloides* in its interaction with other co-occurring epifauna. However, it was much more vulnerable to predators and in this sense was very similar to the resident colonial ascidian *Botryllus schlosseri*. As we found in *Botrylloides*, few species could attach to the tunic of *Diplosoma* and it had a negative impact on the settlement and recruitment of other species by occupying available space (Osman and Whitlatch 1995b, c). In addition, we found that it also rapidly

overgrew newly settled individuals of other species, causing large reductions in recruitment density (Osman and Whitlatch 1995c). As with *Botrylloides*, its settlement and recruitment was restricted to substrate not occupied by other species.

Both *Mitrella* and *Anachis* preyed on *Diplosoma* recruits, resulting in large reductions in recruitment in both our experiments with individual predators (Osman *et al.* 1990, 1992; Osman and Whitlatch 1995a) and the piling experiments (Osman and Whitlatch 1996). Similar to *Botrylloides*, in the colonization experiments (Osman and Whitlatch 1998) 1-wk-old *Diplosoma* survived only on panels on the suspended racks isolated from predators. *Diplosoma* did not occur on any of the transplant series and the colonization success of-older colonies could not be ascertained.

Finally, diurnal variations in *Diplosoma* recruitment tend to mimic *Botrylloides* with highest levels found between dawn and mid-day (Whitlatch and Osman 1998) .

Styela clava

The solitary ascidian, *Styela clava* first appeared in Long Island Sound in 1973 (Carlton 1989). The 5- to 15-cm long ascidians can occur in densities of 500- to 1500 individuals m^{-2} (Whitlatch *et al.* 1995). They are most common in areas generally not exposed to strong wave action or currents (Abbott and Johnson 1972). At present they are a common species on floating docks, pilings, and piers in marinas from Connecticut to Maine (Whitlatch and Osman, this volume).

In our earliest experiments we found that *Styela* can act as a larval predator (Osman *et al.* 1989). We also found that its presence can reduce the growth (Zajac *et al.* 1989; Osman *et al.* 1990) and survival (Zajac *et al.* 1989) of co-occurring species, presumably through competition for food. Unlike *Botrylloides* and *Diplosoma* its effect on recruitment by other co-occurring species was mixed. In general, the presence of *Styela* reduced the settlement of most other species (Sedgwick-Springer 1992 Whitlatch *et al.* 1995). However, recruitment, integrated over a longer time period, was only reduced significantly for some species (the ascidian *Botryllus schlosseri* and the bryozoan *Bugula turrita*). No significant effect of *Styela* was seen on the recruitment of barnacles or the polychaete *Spirorbis* (Whitlatch *et al.* 1995). Some species can also settle on the tunic of *Styela* (Sedgwick-Springer 1992). Given *Styela*'s small area of attachment to the substrate and the ability of many species to recruit onto its tunic, its mixed effect on recruiting competitors is as expected.

In experiments with individual predators, *Styela* recruits suffered high mortality from *Mitrella* but not from *Anachis* (Osman and Whitlatch 1995a). This was a pattern seen in the same study with two other solitary ascidians, *Molgula manhattensis* and *Ciona intestinalis*. New recruits of all of these species are much smaller than those of the colonial ascidians. In studies with larger, 4-7-da-old *Molgula*, we found significant predation by *Anachis* (unpublished data). Given that we have observed *Anachis* preying on all the solitary ascidians, we suggest that *Styela* is initially too small to be preyed on by *Anachis*, but not *Mitrella*. However, within a few days to a week it will be sufficiently large to be eaten by *Anachis*.

In the piling experiments, 1- to 5-da-old *Styela* recruits suffered 100% mortality on open pilings and fairly high mortalities in all treatments except the screened pilings (Osman and Whitlatch 1995d, 1996, 1998). These data suggest that mortality from predation by small micro-predators was very high. In an experiment conducted with 2- to 4-wk-old juvenile *Styela*, the juveniles suffered 100% mortality when exposed on open pilings. Survival in all other treatments was much higher, suggesting that fish and possibly larger benthic predators excluded by any form of caging, were a major source of mortality to juvenile *Styela*. In earlier studies done in Woods Hole (Osman *et al.* 1990), adult *Styela* transplanted to a site with known fish predators suffered no significant mortality. Finally, in the 1994 colonization experiment (Osman and Whitlatch 1998), *Styela* and all other solitary ascidians were found only on the suspended rack treatment isolated from predators for panels transplanted with 1- and 2-wk-old communities. The solitary ascidians were also found on the caged piling treatment for those panels transplanted with 3- and 4-wk-old communities, confirming earlier studies showing that even at 4 wk of age *Styela* and the other solitary ascidians were still vulnerable to fish predation, but that after 2 wk they escaped predation by micro-predators. All these data indicate that *Styela* is extremely vulnerable to predation by micro-predators as a new recruit and to fish predation as an older juvenile, but that it ultimately escapes predation as an adult.

Ascidiella adspersa

Ascidiella is the most recent invader to the New England region and we did not observe it at any of our study sites until 1991. Recruits were not seen in any of our studies until 1993 and our data are limited. Competition studies with *Ascidiella* produced results similar to those for the other species we have examined. *Ascidiella*'s primary effect was the reduction of available substrate on which other species could recruit successfully. A few species can recruit on the tunic of *Ascidiella*; however, not with the same intensity as was found with *Styela* (Whitlatch and Berger, unpublished data).

In a piling experiment conducted with new recruits of *Ascidiella* (Osman and Whitlatch 1996, 1998), survival was significantly lower on the open treatment than on the screened treatment, indicating clear predation by fish or macro-invertebrates. Survival was intermediate on the caged and partial-screen treatments suggesting that micro-predators also preyed on newly recruited *Ascidiella*. As already discussed with *Styela*, the results of the colonization experiment (Osman and Whitlatch 1998) suggest that *Ascidiella* is vulnerable as a new recruit and young juvenile to predation by micro-predators and to fish predation as an-older juvenile. It possibly escapes predation as an adult.

Finally, diurnal variations in *Ascidiella* recruitment were similar to the other ascidians with highest levels found between dawn and mid-day (Whitlatch and Osman 1998) .

Discussion

In many ways *Botrylloides, Diplosoma, Styela,* and *Ascidiella* differ little from other ascidians such as *Botryllus, Molgula,* or *Ciona,* which are common to the southern New England epifaunal community. Although the four invaders can be dominant space occupiers, we see little evidence of them competitively excluding resident epifaunal species. Their influence on the recruitment of other species seems to be principally the occupancy and removal of available substrate space and their recruitment is affected in a similar manner by other species. These ascidians are generally restricted to embayments and harbor areas and in these they are often the spatial dominants. Our research indicates that their restriction to these habitats results largely from small micro-predators, such as the gastropods *Mitrella lunata, Anachis avara,* and *A. lafresnayi,* which can clearly eliminate ascidian recruitment in many open coastal habitats. Furthermore, if the solitary ascidians *Styela* and *Ascidiella* escape this micro-predation filter, they also can suffer severe mortality as juveniles from larger fish predators such as cunners. Thus, in this sense the four species of introduced ascidians do not seem to differ from resident species in that their local distributions are likely to be controlled by predators. If the predators are present, recruitment can be greatly limited and, for some species, juvenile mortality can be equally as high.

However, the experiments also indicate that the severity of predation on at least *Botrylloides* is much less than that on the resident colonial ascidian *Botryllus*. Although we observed occasional mortality of *Botrylloides* recruits from predation by *Mitrella* and *Anachis* (Osman and Whitlatch 1995a), in the piling experiments the mortality of *Botrylloides* recruits was significantly increased only when exposed to larger predators (Osman and Whitlatch 1996, 1998). In addition, *Botrylloides* juveniles-older than 1 wk exhibited little or no predation-related mortality, which contrasted with still high mortalities for *Botryllus* at this age (Osman and Whitlatch 1996). Finally, only when *Botryllus* colonies reached an age of 3- to 4-wk did they experience the same reduction in mortality as observed for *Botrylloides*.

There is some suggestion that *Styela* and *Ascidiella* mortalities at the field site may be lower than those of the resident ascidians *Ciona* and *Molgula*. Recruits of both introduced species exhibited higher survival than *Ciona* recruits in all treatments (Osman and Whitlatch 1995d) and juvenile *Styela* suffered lower mortalities in caged and partially screened treatments than did *Molgula*. These differences could result from reduced mortality from micro-predators but the differences are not as striking as those between *Botrylloides* and *Botryllus*. Also, newly settled *Ciona* are often weakly attached to the substrate, which may contribute to their high losses in all treatments at the field site. *Molgula* also is less firmly attached than either of the introduced species, making it easier to be removed by predators or water currents.

Thus, overall, the local distributions of the four species of introduced ascidians appear to be controlled by the same environmental parameters as resident species. Their abundance is strongly influenced by the presence of both large and small predators.

However, they may escape the high mortalities resulting from predation more quickly than resident species. This was indicated by a series of colonization experiments conducted in 1994 (Osman and Whitlatch 1998). In these experiments substrates were exposed at the ascidian-dominated breakwater site for 1, 2, 3, and 4 wk and then transplanted to the exposed site. For the 1-wk series, mortalities of all ascidians were extremely high and a native bryozoan community developed. Substrates transplanted after 2 wk at the breakwater site (older and larger individuals) became dominated by bryozoans and *Botrylloides*. *Botryllus* only became abundant on substrates transplanted after 3 and 4 wk and solitary ascidians (*Molgula*, *Ciona*, *Styela* or *Ascidiella*) were only found on caged pilings.

If local distributions of the four introduced species are controlled by interactions with the resident fauna, then what are the features common to all four species that have enabled them to both successfully invade and rapidly spread throughout the New England coastline? We hypothesize the following reasons:

1. All four species have limited larval dispersal capabilities. Most larvae released from the two colonial species likely settle within a short distance of the parent (Grosberg 1987; Davis and Butler 1989; Stoner 1990). In fact, we found that even in areas of high current flow a sufficient number of *Botrylloides* larvae settled within meters of parental colonies to allow local populations to self-replicate (Osman and Whitlatch 1998). Even though the larvae of the solitary species are longer-lived (usually settling within a day or two), the embayments and harbors where these species are commonly abundant often have longer water residence times. Thus, such areas offer habitats where local populations of these ascidians can rapidly increase in size, forming a stable foothold in an area. Since many of these areas are in or near harbors and marinas, boats may contribute to the long-range transport of these species with new localities quickly colonized by the local recruitment of offspring of founder individuals.

2. Recruitment of these species is not strongly inhibited by any particular resident sessile species (Osman *et al.* 1989; Osman and Whitlatch 1995a, b; Whitlatch *et al.* 1995). We have found little indication that predation on larvae or competition with any single resident species can significantly reduce the recruitment of these species. However, at the community level, we have also found that as the local diversity of resident species increases, recruits of at least one species (*Botrylloides*) suffer increasing mortality (Stachowicz *et al.* 1999). This increased mortality seems a consequence of the more consistent occupancy of space by communities with more species. Many of the epifaunal species (particularly the ascidians) are fairly short-lived and their mortality opens new space in which new species can recruit and grow. However, in more diverse communities this new space can be quickly occupied by other species already present, resulting in less space in which new species can successfully recruit. Thus, it is possible that disturbed harbor, marinas, etc. with a supply of new manmade structures as well as an epifaunal community dominated by ascidians and other short-lived species may offer a sufficient supply of open space for the invaders to be successful.

3. All four invaders are equally or less vulnerable to predation than similar resident species. Native predators, both large and small, do not limit the invaders' distributions any more than they limit the distributions of resident epifauna. In fact, given the reduced effect of predators on *Botrylloides* and possibly *Styela* and *Ascidiella*, it is somewhat surprising that these species have not invaded habitats not currently utilized by resident ascidians. We feel that even for *Botrylloides* the predation pressure on recruits is sufficient to prevent expansion into areas with predators, especially when coupled with highly localized larval distributions that would limit the actual numbers of larvae arriving at such locations.

4. Embayments and harbors provide ideal habitats for maintaining both resident and introduced ascidian populations which can then be sources of recruits for colonizing new areas. The long-term and apparently continuous presence of ascidian populations in Eel Pond, MA (Grave 1933; Grosberg 1981; Osman *et al.* 1990, 1992), for example, is strong evidence that ascidian populations can be maintained in such areas for long periods. Given the vulnerability of these species to predation, they would seem restricted to these less-exposed, predator-free sites.

Harbors seem to provide an ideal habitat because of (1) the abundance of habitable pilings, docks, and other structures, (2) the potential retention of larvae, and (3) the general absence of predators, particularly those that prey on ascidian recruits. Although we have been able to maintain *Mitrella* and *Anachis* in cages at the breakwater site, they are clearly absent

from the breakwater and docks there. While Rogers (1998) demonstrated that their larvae can colonize this site, Berger (1998) suggests that predation by the green crab, *Carcinus maenas* may severely limit the ability of these micro-predators to maintain sufficient population densities to have an effect on ascidian recruit abundances. Berger (1998) and others (*e.g.* Edwards *et al.* 1982; Menge 1983) have found that green crabs, which are generally found in more protected areas, can be important and voracious predators on small gastropods. Others have shown that *Mitrella* is consumed by other crabs found in protected areas, such as the blue crab, *Callinectes sapidus* (Martin *et al.* 1989) or *Neopanope texana* (Schneider and Mann 1991). In addition, floating docks, which often contain large ascidian populations, can provide a refuge from predators.

As is illustrated in Figure 1, we suggest that the short larval life of the four invading ascidians results in continued localized recruitment within embayments and harbors such as at our breakwater site in the lower Poquonnock River. This results in persistent, self-replicating, local populations. These populations can export larvae to areas outside, but in many of these areas predators will eliminate most, if not all, recruits. It is also possible that the diversity of the native epifauna in these areas also limits the ability of the ascidians to invade (Stachowicz *et al.* 1999). In addition, the short larval life of the ascidians will also limit their dispersal distance and thus the number of larvae reaching new areas.

Given their short-lived nature, the rapid spread of these species throughout New England most likely occurred by the rafting of adult colonies on debris, seagrass blades, and macrophytes or their transport on fouled ships and other structures. Thus we feel the success of these species results from their ability to main self-replicating local populations that can produce generation after generation of potential new invaders and their paradoxical absence from nearby habitats is a result of their interactions at some life-stage with native predators as well as the epifaunal community.

We therefore expect the range of these species to expand geographically but their habitat distribution should remain restricted, unless predators are absent in any new region. However, it should also be noted that we have not observed any decline in other epifaunal species as a consequence of the invasion of these particular species. As we argued more than 20 years ago (Osman and Whitlatch 1978), because of their patchy distributions marine benthic communities often have a remarkable capacity to absorb new species. This does not mean that introductions or their potential for causing devastating changes to ecosystems should be ignored, but rather that we must recognize that many invasions, whether anthropogenic or natural, occur with little impact on the native community (Williamson 1996). Therefore, it is vital to go beyond documenting introductions and examine the interactions of invading species with native communities if we are to understand the long-term consequences of these invasions.

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Source of Unpublished Material

Carlton, J. T., Williams College-Mystic Seaport, Mystic, CT.

Grazing Pressure on Invasive and Endemic Subspecies of the Green Alga *Codium fragile*

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Key words: invasive species, *Codium fragile*, sea urchin, grazing, plant-herbivore interaction

INTRODUCTION

The green alga *Codium fragile* ssp. *tomentosoides* is a successful invader in areas of the northwest Atlantic where it is common subtidally. In other regions, the alga has a more limited subtidal distribution: in England the introduced alga is completely confined to the intertidal (Chapman 1999); in New Zealand the introduced alga appears to have an intertidal refuge from herbivory (Trowbridge 1995); and in the eastern Pacific, near Friday Harbor, Washington, an endemic subspecies of *C. fragile* is confined to the intertidal. These regional trends in the subtidal vs. intertidal distribution of *C. fragile* may reflect regional differences in the intensity of herbivory and correlate with *C. fragile*'s ability to invade communities. This study compared grazing pressure on the invasive, subtidal *C. fragile* ssp. *tomentosoides* in the northwest Atlantic Ocean to grazing pressure on the endemic, intertidal *C. fragile* in the eastern Pacific Ocean to determine whether lower levels of herbivory in the Atlantic may have facilitated the spread of the invasive subspecies.

RESULTS AND DISCUSSION

In laboratory grazing assays involving a number of invertebrate herbivores found on or near *C. fragile* in the field, the urchins *Strongylocentrotus droebachiensis* and *S. franciscanus* and the sacoglossan sea slug *Placida dendritica* consumed significant amounts of algae relative to controls; the Pacific urchin *S. franciscanus* having the greatest effect (Figure 1). It is noteworthy that *S. droebachiensis* from the northwest Atlantic and the eastern Pacific consumed the alga

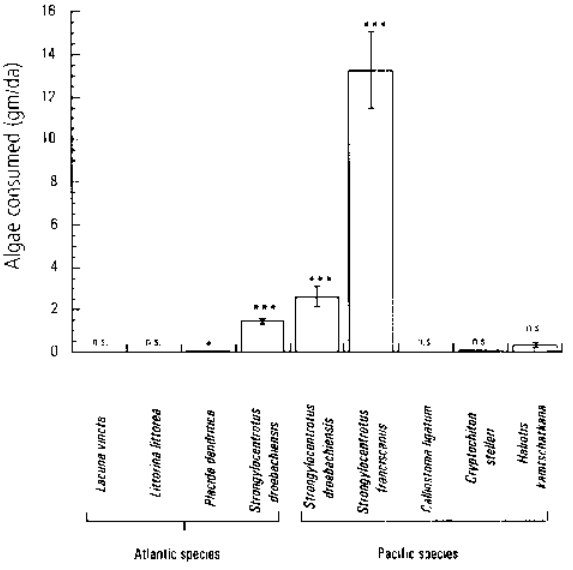

Figure 1. Amounts of *C. fragile* (ssp. *tomentosoides* in the Atlantic) consumed by various herbivores (± s.e.). Amounts consumed are weight loss of algal controls subtracted from weight loss of algae exposed to grazing. Mann-Whitney U test comparing samples exposed to grazing to simultaneous controls ("n.s" = not significant, * = $0.05 > p > 0.01$, and *** = $p < 0.001$)

at similar, but relatively low, rates (Mann-Whitney, $U = 46$, $p = 0.3252$). This suggests that the shorter period of contact between Atlantic *S. droebachiensis* and the invasive subspecies does not explain differences in grazing pressure between ocean basins. Instead, behavioral or morphological constraints are likely limiting both *S. droebachiensis* populations in their ability to consume *C. fragile* (see below). Of the large herbivores assayed, *S. franciscanus* is the most abundant in the Friday Harbor area (normally more abundant than *S. droebachiensis*), while *S. droebachiensis* is the only abundant large herbivore in the northwest Atlantic.

In field experiments near Friday Harbor, trans plants of *C. fragile* from intertidal beds to 6-7 m

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below MLW were grazed significantly by locally abundant *S. franciscanus*. However, in field experiments conducted in the northwest Atlantic, *C. fragile* ssp. *tomentosoides* transplanted into *S. droebachiensis* barrens and also into cages containing *S. droebachiensis* were not grazed significantly. Together, these results suggest that grazing pressure from *S. franciscanus* is responsible for confining *C. fragile* primarily to the low intertidal in areas in the eastern Pacific. In contrast, the absence of grazing pressure from such a large urchin species in the Atlantic has allowed *C. fragile* ssp. *tomentosoides* to spread to subtidal areas. Intense subtidal grazing by local herbivores may explain the lower invasion success of *C. fragile* ssp. *tomentosoides* in New Zealand (Trowbridge 1995) and the restriction of the alga to the intertidal in England (Chapman 1999).

Laboratory, single-diet grazing experiments on urchins from the Pacific and Atlantic compared the amounts of *C. fragile* and *Laminaria* spp. consumed by large and small *S. droebachiensis* (test diameters approx. 68 and 31 mm, respectively) and large and small *S. franciscanus* (test diameters approx. 125 and 58 mm, respectively). These experiments indicated that (1) large *S. franciscanus* consumed significantly more *C. fragile* than *Laminaria* spp., (2) similar-sized *S. franciscanus* and *S. droebachiensis* consumed *C. fragile* and *Laminaria* spp. at similar rates, and (3) there was a size-related shift in the ability of *S. droebachiensis* to consume *C. fragile* ssp. *tomentosoides* relative to *Laminaria* spp., in which small *S. droebachiensis* consumed significantly less *C. fragile* than large *S. droebachiensis* but consumed *Laminaria* spp. at rates similar to large conspecifics. These grazing experiments are summarized in Figure 2.

In a separate assay for chemical deterrents to herbivory, crude extracts of *C. fragile* ssp. *tomentosoides* and/or *Laminaria saccharina* were imbedded in carrageenan molds and exposed to grazing by *S. droebachiensis*. Results from this assay showed no evidence of a chemical deterrent in *C. fragile* ssp. *tomentosodies* to herbivory. The absence of a chemical deterrent to grazing and the apparent trend for larger urchins to consume disproportionately more *C. fragile* than *Laminaria* spp. (Figure 2) suggest a morphological or behavioral shift in grazing ability relative to urchin size.

The size-related shift in an urchin's ability to consume *C. fragile* may explain regional differences

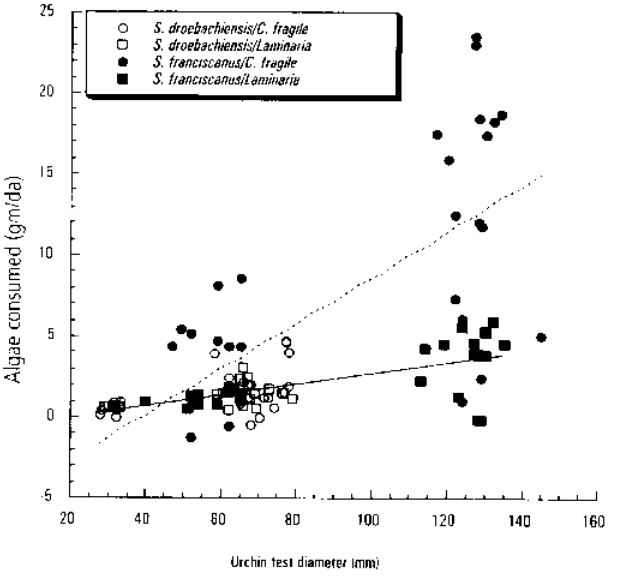

Figure 2. Summary of single-diet grazing experiments in which *Strongylocentrotus franciscanus* and *S. droebachiensis* of various sizes were fed either *Laminaria* spp. or the local *C. fragile* (in New England the invasive subspecies *tomentosoides*). Amounts consumed are weight loss of algal controls subtracted from weight loss of algae exposed to grazing. Regression lines indicate *C. fragile* (dashed) and *Laminaria* spp. (solid) consumed.

in grazing pressure on the alga. In areas with large endemic urchin grazers (*e.g.*, *S. franciscanus* in the Pacific), the alga is largely confined to the intertidal. In contrast, smaller endemic urchin grazers (*e.g.*, *S. droebachiensis* in the Atlantic and Pacific) may have little impact on the distribution of the alga. In the northwest Atlantic, where smaller urchins are the dominant grazers, the alga is not subjected to heavy subtidal grazing pressure, and has successfully invaded this habitat. These results have implications for the future success of the invasive *C. fragile* ssp. *tomentosoides* in regions with differing endemic urchin grazers (*e.g.*, Atlantic, eastern Pacific, New Zealand, Australia) and may be influenced by general decreases in urchin sizes in the Atlantic and Pacific due to local urchin fisheries.

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Predation on Native and Nonindigenous Amphipod Crustaceans by a Native Estuarine-Dependent Fish

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ABSTRACT: **The importance of nonindigenous species within guilds supporting native species in higher trophic levels is a critical concern in the biology of invasions. We find that predator-prey co-evolution relations may not allow predictions about the order of consumption and selection of similar prey types. We conducted experiments to test for native and nonindigenous prey selection by juvenile English sole (*Pleuronectes vetulus* - native to the west coast of North America), using native amphipods (*Corophium salmonis* and *C. spinicorne*) and northwest Atlantic amphipods (*C. acherusicum* and *C. insidiosum*). Single-species prey consumption in sand substratum was greater on *C. spinicorne* and *C. acherusicum* than on *C. insidiosum* and *C. salmonis*. Prey selection on both nonindigenous species was significantly greater than on native species over mud substratum but not over sand substratum. Predation was greater over sand substratum than over mud substratum. No sex-selective predation occurred on any species in either substratum type and prey size-selection was only suggested for *C. acherusicum* in both substrata types. Interspecific prey selection may vary with visibility, substratum type and prey behavior. Nonindigenous amphipods are potentially capable of supporting higher trophic levels of native species.**

Key words: prey selection, consumption, experiment, indigenous species, nonindigenous species, amphipod, *Corophium*, juvenile English sole, *Pleuronectes vetulus*

INTRODUCTION

Estuaries in the northeast Pacific are among the most invaded aquatic habitats in the world (Carlton 1979; Chapman 1988; Cohen and Carlton 1995) but almost nothing is known of the ecological effects of these invasions. Consumption of nonindigenous prey by native fish in estuaries can be substantial (Castillo *et al.* 1996). This study is the first comparison of selection for native and nonindigenous prey species in estuaries by native fish (*i.e.*, the proportion of a given prey in the diet relative to its proportion in the environment). The study by Meng and Orsi (1991) suggests that the larvae of the introduced striped bass (*Morone saxatilis*) select one coevolved copepod species over two non-coevolved introduced copepod species. In the Yaquina Bay and Alsea Bay estuaries in Oregon, predator-prey coevolution may not affect the order of prey selection by two species of juvenile flatfish (G.C. Castillo, pers. obs.).

Prey selection by fish may depend on: visibility and exposure (McCall 1992; Schlacher and Wooldridge 1996); activity (Ware 1973; Magnhagen 1986); evasion (Fulton 1982); absolute and relative density of prey (Magnhagen 1985); size (Ringler 1979); social facilitation (Brawn 1969; Olla and Samet 1974) and water temperature (Moore and Moore 1976). We measured differences in consumption and selection of two native and two nonindigenous amphipods (*Corophium* spp.) by juvenile English sole (*Pleuronectes vetulus*). If juvenile *P. vetulus* tends to prey opportunistically at equal prey densities, we would expect to find higher consumption and selection toward the most vulnerable prey species, regardless of a potential coevolved predator-prey relation. We tested whether the number of prey

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consumed by *P. vetulus* varies with species, size, or sex of amphipods, and whether prey selection varies in the presence of alternative prey or substratum type. We also tested whether the visibility and activity of prey differ among the four amphipod species.

Juvenile *P. vetulus* use Northeast Pacific estuaries as rearing areas where amphipods are an important part of their diet (Haertel and Osterberg 1967; Toole 1980). *Corophium salmonis* and *C. spinicorne* are both temperate species native to the west coast of North America. *Corophium insidiosum* and *C. acherusicum* are both semitropical species inadvertently introduced into U.S. west coast estuaries from the east coast of North America (Carlton 1979). All four species are abundant in the estuaries of Oregon and the northeastern Pacific.

The increased invasion rate in San Francisco Bay (Cohen and Carlton 1998) suggests that the abundances of introduced species have also increased in other northeast Pacific estuaries. Whether native amphipods are being replaced by nonindigenous amphipods in Northeast Pacific estuaries, as reported for freshwater amphipods in Ireland (Costello 1993), is uncertain but the native amphipod, *Corophium brevis*, may be extinct in San Francisco Bay and populations may have declined in Humboldt Bay, California, following the introduction of at least one amphipod (J.W. Chapman and T.W. Miller, unpubl. data).

Methods

Juvenile *P. vetulus* were collected during summer 1996 from intertidal flats of Yaquina Bay by seining and were then transported to the Hatfield Marine Science Center (HMSC) on the same day. The fish were treated with a 1:4,000 formalin solution for 1 h to kill parasites and reduce fish mortality (Kamiso and Olson 1986). The fish were sorted by size and maintained in continuous water flow and natural photoperiod. Fish used in experiments ranged from 5.1 cm to 6.6 cm total length (mean = 5.7 cm, sd = 0.35, n = 90). Juvenile fish were fed live *Tubifex*, defrosted *Artemia salina*, and 1-mm food pellets (Bioproducts). The varied diet conditioned the fish to multiple food types.

Native amphipods were collected from marina floats and mudflats of Yaquina Bay and used directly in experiments. Populations of the nonindigenous *C. acherusicum* and *C. insidiousum* were collected from floats and boats in Humboldt Bay and Yaquina Bay and cultured in the laboratory. *Corophium acherusicum* were also provided by John Sewall from U.S. EPA laboratory cultures (HMSC, Newport, OR). Cultured amphipods were held in aerated rectangular 8.7-L dish pans at 30 ppt and 25°C. Cultured diatoms (*Chaetoceros calcitrans*) were provided twice weekly and a mixture of powdered dry food (parts per ingredient: 1.3 Neonovum, 10 alfalfa, 20 Tetramin, and 10 wheat grass leaves) was provided every other day. The four amphipod species were treated with antibiotics (after Pelletier and Chapman 1996) for 3 d prior to the experiments to increase survival. Older juveniles and adults retained on a 351-mm sieve (Tyler Standard) were used in the experiments.

All amphipod prey populations were maintained at ca. 20°C for a minimum of 4 d prior to the experiments in the fall of 1996. Twenty-four acclimated amphipods were introduced into 5.8L glass aquaria (23cm L × 15cm W × 17cm D) containing aerated brackish water (30 ppt salinity, 14°C) and a 0.5-cm layer of benthic sediment. Amphipods were left undisturbed for 24 h to allow tube building in the sediment. Then one juvenile *P. vetulus* was introduced into each tank. The fish were left undisturbed in the tanks for 48 h (from 6 p.m. to 6 p.m.) and then removed. The water and sediment of each tank were sieved to recover amphipods. All amphipods remaining on the sieve were then counted. The number of prey consumed was estimated from the initial number of prey minus the number found on the sieve.

Single fish were placed in tanks containing 24 defrosted *Artemia salina* during each of the four species treatments to control for variations among predators. A given treatment was considered invalid and repeated when *Artemia* were not consumed in the control. Twenty-four amphipods were added to each of three tanks and maintained without predators to control for losses other than to fish predation. Both controls were used in single- and mixed-species experiments. The χ^2 statistic was used to test the significance of the difference between treatment and control means. The Tukey multiple range test (hereafter, HSD, Sokal and Rohlf 1995) was used to test the significance of pairwise differences between treatment means.

Single-Species Experiments

Prey Behavior

Prey visibility: Predator-free experiments were used to test whether amphipod visibility in tanks

with sandy sediment varies among species at 14°C. Amphipod visibility was assessed from 2-min observations for individuals swimming, walking, or partially visible above the sediment of 10 tanks. Twelve males and 12 females per species were introduced in each tank 1 h before the first observation. These observations were repeated after 1 h, after 3 h, and then every 3 h up to 24 h.

Prey activity: Interspecific differences in amphipod activity was tested in predator-free experiments. Activity was estimated from the average distance traveled per 5 sec in plastic containers (8 cm L × 8 cm W × 10 cm D) under the following conditions: no sediment, water at 14°C and at 24°C and 30 ppt salinity. One amphipod per species and sex was introduced in each of 10 replicated containers and left undisturbed for 24 h before the tests. Amphipod activity was measured with and without disturbance caused by suctioning from a small pipette as a proxy predation attempt (after Meng and Orsi 1991).

Prey consumption

All single-species predation experiments were performed on sandy substrates (98% sand and 2% mud) since *P. vetulus* occurs predominantly in sandy substrates in Yaquina and Alsea Bay estuaries. Single-species treatments consisted of 24 amphipods (12 randomly individuals of each sex) in a tank with sandy substratum. Treatments were replicated 10 times for each amphipod species. Fish were deprived of food for 72 h prior to the feeding trials.

The Strauss'(1979) index of food selection (L) is adapted to estimate size-dependent selection by fish:

$$L_i = r_i - p_i$$

where L_i is the selection for prey of size $_i$; r_i and p_i are the proportions of prey of size $_i$ consumed and available prior to the feeding trials, respectively. The value of L ranges from -1 to +1. Thus, the prey sizes selected most often will correspond to the highest L_i values. Alternatively, size selection was estimated from the cumulative difference in size distributions between eaten and uneaten individuals (Kolmogorov-Smirnov test, Tate and Clelland 1957).

Precise determination of prey size and sex were obtained from the length and morphology of the fourth article of the second antenna. Measurements were made using a stereomicroscope equipped with an ocular micrometer. The size distribution of consumed amphipods in each tank was estimated from the size distribution of prey measured before the experiments minus the size distribution after the experiments.

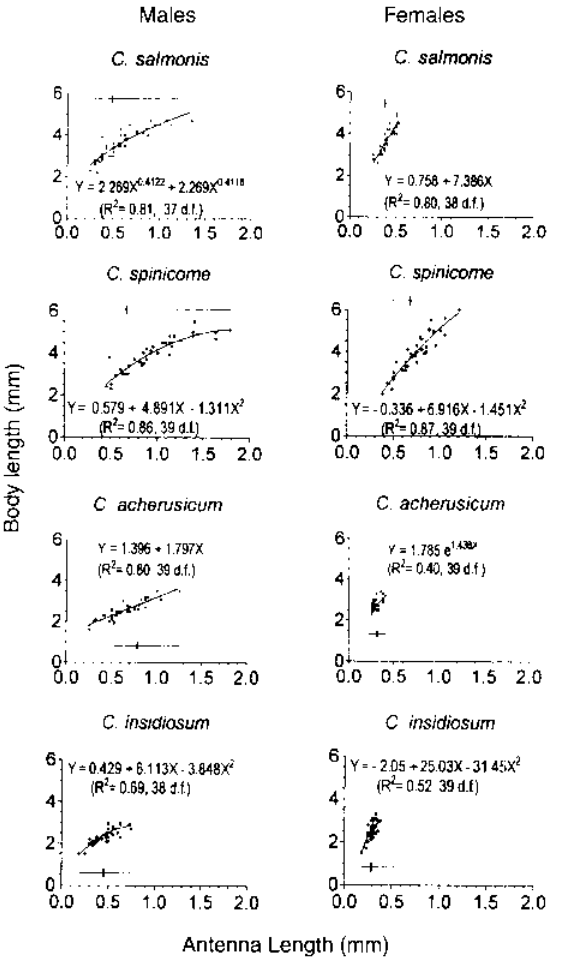

Figure 1. Amphipod body length (from telson to eye, Y) with length of 4th article 2nd antenna (X) by species and sex. Species origin: native (*C. salmonis* and *C. spinicorne*); nonindigenous (*C. acherusicum* and *C. insidiosum*). All correlations are significant ($P < 0.05$). Initial mean prey size and range (+–) used in single-species experiments is shown in each case.

Mixed-Species Experiments

The mixed-species treatments consisted of 24 amphipods (3 females and 3 males per species) in sandy substrate (98% sand and 2% mud) and muddy substrate (2% sand and 98% mud). Each treatment was replicated 25 times in the same aquaria used in single-species experiments. Variation in amphipod visibility with substratum type was assessed for all species combined in the 25 tanks prior to the feeding experiments. Fish were starved for the 48 h preceding these experiments. The proportion of the tank bottom free of sand upon completion of the experiments was used as a proxy measure of fish distur-

Table 1. Walking (WA), swimming (SW) and partially visible (PV) *Corophium* spp. in sand substrate from three observations per time interval in 10 tanks (sd = ± sample standard deviation). Rank 1 = Highest visibility.

Species	Individuals (No./tank/2 minute observation) (7:00-12:00) PM				(3:00-9:00) AM				(12:00-6:00) PM			
	WA	SW	PV	Rank	WA	SW	PV	Rank	WA	SW	PV	Rank
C. salmonis												
mean	0.53	0.03	0.30	4	0.17	0.00	0.17	4	0.37	0.00	0.40	2
sd (±)	0.21	0.06	0.10		0.15	0.00	0.15		0.06	0.00	0.10	
C. spinicorne												
mean	2.70	0.37	1.63	2	0.57	0.07	0.53	2	0.23	0.00	0.37	3
sd (±)	2.25	0.21	0.35		0.31	0.11	0.42		0.11	0.00	0.15	
C. acherusicum												
mean	2.40	0.40	3.37	1	1.43	0.17	1.17	1	1.00	0.03	1.23	1
sd (±)	0.95	0.26	2.06		0.40	0.12	0.38		0.35	0.06	0.55	
C. insidiosum												
mean	0.57	0.87	0.10	3	0.23	0.23	0.00	3	0.13	0.03	0.00	4
sd (±)	0.06	0.31	0.10		0.15	0.15	0.00		0.15	0.06	0.00	

bance. Fish disturbance in mud treatments was evaluated from the proportion of tanks in which the bottom was visible.

Results

We compared prey size relations, single-species prey behavior (visibility and activity), single-species consumption, and mixed species visibility and selection.

Prey sizes

The body lengths of the two native species are greater than those of nonindigenous species (Figure 1) and antenna lengths of males are greater for *C. acherusicum* than for native species (Figure 1). Sexual dimorphism based on antenna length is apparent in all species except *C. spinicorne* (Figure 1).

Prey behavior

Visibility: Prey exposure was the main factor accounting for differences in consumption among species. The visibility of all amphipod species in tanks with sand (*i.e.*, walking, swimming or partially buried individuals) differed among species over the 24-h period ($P < 0.05$, ANOVA) and over the last 12-h period ($P < 0.01$, ANOVA). *Corophium salmonis* and *C. insidiosum* largely settled into the sediment during the first 6 h of the test (Table 1). The decreasing order of prey visibility over the entire daily period was: *C. acherusicum*; > *C. spinicorne*, > *C. insidiosum* > *C. salmonis*. Most visible individuals were walking (Table 1).

Activity: Undisturbed amphipod activity did not vary by sex or species at 14°C or 24°C ($P > 0.10$, ANOVA; Figures 2a and 2b). At 14°C, activity following simulated predation attempts did not vary among females ($P > 0.30$, ANOVA; Figure 2C) and

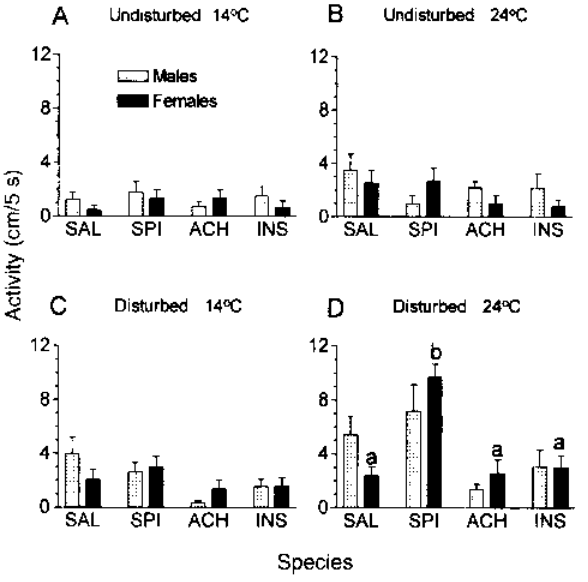

Figure 2. Mean activity (distance traveled in 5 s) by 10 males and 10 females of each *Corophium* species held at 14°C and at 24°C. SAL = *C. salmonis*, SPI = *C. spinicorne*, ACH = *C. acherusicum*, INS = *C. insidiosum*. Standard error scale over each bar and significant differences denoted by letters a and b.

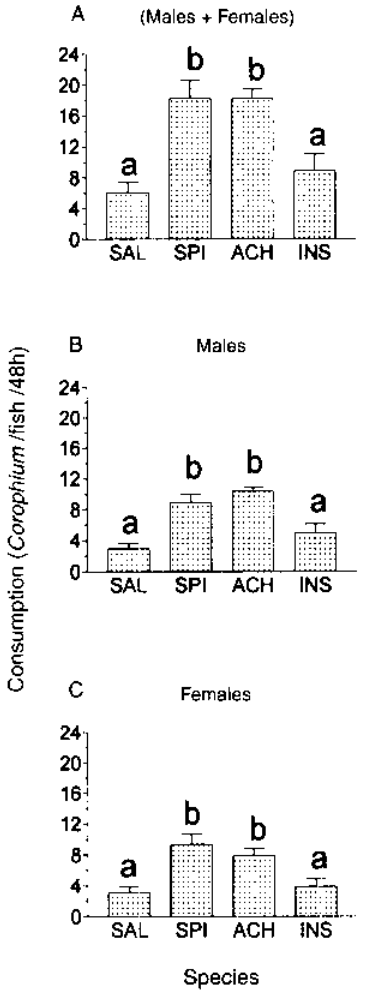

Figure 3. Mean number of *Corophium* consumed by *Pleuronectes vetulus*, in single-species experiments. SAL = *C. salmonis*, SPI = *C. spinicorne*, ACH = *C. acherusicum*, INS = *C. insidiosum*. Standard error scale indicated over each bar and different letters above bars showing significant differences among species.

varied only marginally among males (P = 0.05, ANOVA; Figure 2C) with *C. salmonis* most active and *C. acherusicum* least active (94% HSD test).

At 24°C, disturbance increased only the activity of female *C. spinicorne* (P <0.01, ANOVA, 95% HSD test). Activity of disturbed amphipods for all four species was greater at 24°C than at 14°C (males: P <0.05, females: P = 0.01, ANOVA; Figures 2C and 2D).

Single-Species consumption

Pleuronectes vetulus feeding varied among species (Figure 3A) and between sexes (P <0.05, ANOVA; Figures 3B and 3C), with predation on *C. spinicorne*

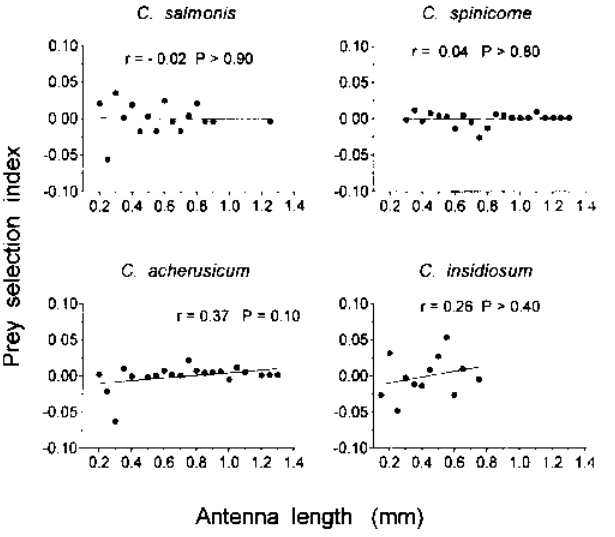

Figure 4. Strauss' selection index by prey size (4th article 2nd antenna) of *Corophium* species consumed by *Pleuronectes vetulus*.

and *C. acherusicum* nearly twice as high as on *C. salmonis* and *C. insidiosum* (95% HSD test). Significant differences in prey survival between treatments and controls occurred in all species except *C. salmonis* (1-tailed X^2 test, P <0.05). Size of *P. vetulus* did not affect prey consumption (r = 0.03, P > 0.05, n = 40).

Sex-selective predation was not apparent (P > 0.10, 2-tailed X^2 test; Figures 3b and 3c). Although size-selective predation was not suggested from the Strauss' index (Figure 4), size selection based on size distribution of prey may occur for *C. acherusicum* (P < 0.01, Kolmogorov-Smirnov test; Figure 5).

Mixed-Species Experiments

Corophium visibility was greater in sand than in mud both 1 h and 24 h following amphipod introduction (P <0.05, ANOVA; Table 2). Except for *C. insidiosum*, predation was higher in sand than in mud (P <0.001, ANOVA; Figures 6A and 6B). Species-selective predation occurred in both substrata (P <0.05; ANOVA; Figures 6A and 6B). In sand, *C. acherusicum* was selected more frequently than both native species (95% HSD test). In mud, selection was higher on both nonindigenous species (95% HSD test; Figure 6B). Differences in prey survival between treatments and controls were suggested for all species except *C. salmonis* in sand (P <0.05, 1-tailed X^2 test) and for no species in mud (P >0.05, 1-tailed X^2 test).

Sex-dependent predation was not apparent on either substratum for any species (P >0.05, 2-tailed

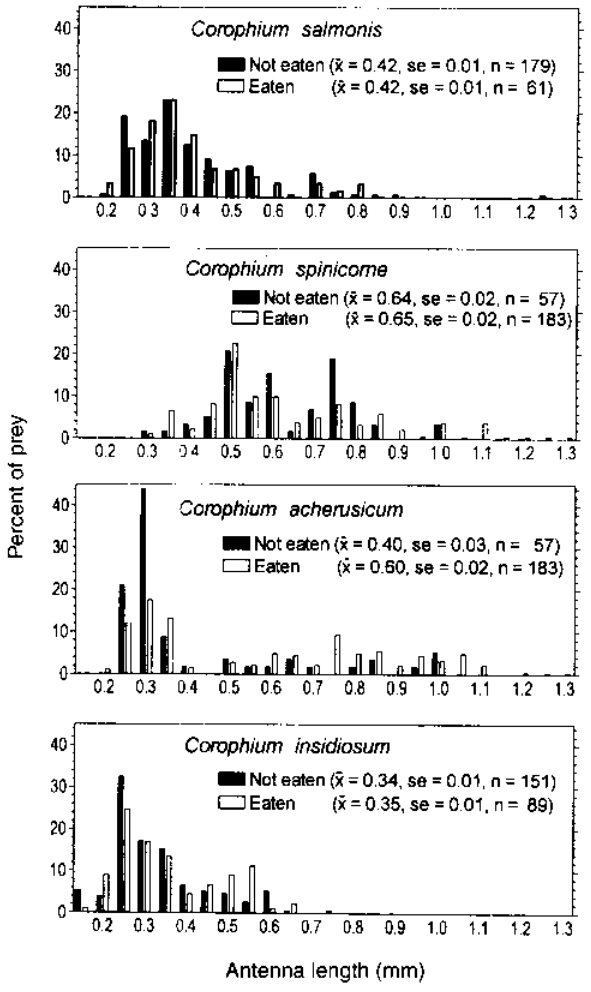

Figure 5. Percent of eaten and uneaten *Corophium* by size (4th article 2nd antenna) in 10 tanks with sand substratum. Mean antenna length (mm), standard error and total number of prey shown in parentheses

X^2 test; sand: Figures 6C and 6E; mud: Figures 6D and 6F).

Substratum type had no significant influence on the size distribution of uneaten prey ($P > 0.05$, Kolmogorov-Smirnov test; Figure 7). Except for male *C. insidiosum* in sand and *C. acherusicum* in mud ($P < 0.05$, ANOVA), size-selective predation was not suggested (data available upon request). Fish size seemed independent of overall prey consumption in sand ($r = 0.10$, $P > 0.05$, $n = 25$) and mud ($r = -0.20$, $P > 0.05$, $n = 25$).

Discussion

Juvenile *P. vetulus* preyed on all *Corophium* species used in our study. The rank of predation in single-species experiments was not consistently related to species origin as seen in mixed-species experiments.

Table 2. Walking (WA), swimming (SW) and partially visible (PV) *Corophium* in sand and mud substrata from three observations per time interval in 25 tanks (sd = ± sample standard deviation).

Substrate	Individuals (No./tank/2 min observation) (7:00-12:00) PM			(3:00-9:00) AM)			(12:00-6:00) PM		
	WA	SW	PV	WA	SW	PV	WA	SW	PV
Sand									
mean	1.24	0.57	0.60	0.61	0.17	0.24	0.68	0.27	0.33
sd (±)	0.62	0.09	0.18	0.16	0.17	0.07	0.21	0.12	0.17
Mud									
mean	0.55	0.44	0.31	0.49	0.11	0.36	0.44	0.01	0.35
sd (±)	0.49	0.11	0.36	0.14	0.10	0.08	0.07	0.02	0.06

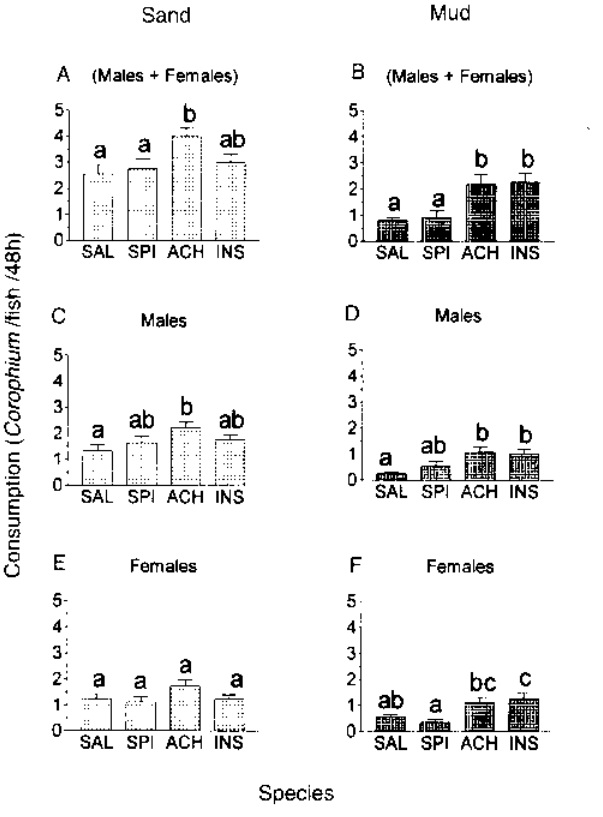

Figure 6. Mean number of *Corophium* consumed by *Pleuronectes vetulus* in mixed-species experiments. SAL = *C. salmonis*, SPI = *C. spinicorne*, ACH = *C. acherusicum*, INS = *C. insidiosum*. Standard error scale over the bars and different letters above bars indicating significant differences among species.

In the latter case, predation was more intense on sand than on mud and the ranking of prey selection was higher for nonindigenous species in both substrata. Thus, consumption of each species depends on the substrata and the species composition of the prey populations.

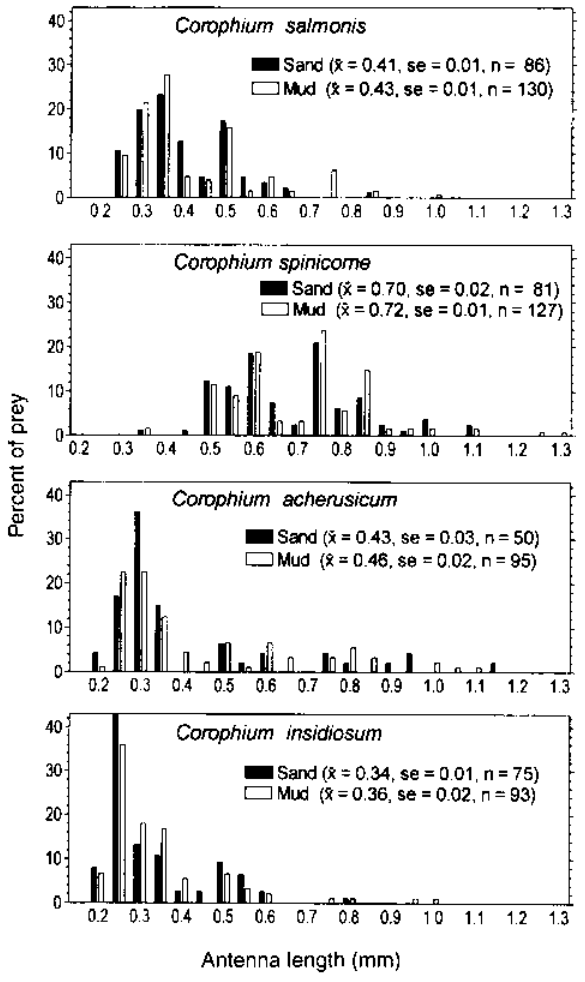

Figure 7. Percent of uneaten *Corophium* by size (4th article 2nd antenna) in sand and mud substrata. Mean antenna length (mm), standard error and total number of uneaten prey in 25 tanks per substratum are shown in parentheses.

The two most consumed species in single-species experiments (*C. spinicorne* and *C. acherusicum*) were the most visible over the 24-h observation period. Therefore, *P. vetulus* seems to be an opportunistic predator and predation risk varies with prey behavior and sediment type. Every other factor (*e.g.*, prey origin, size, sex, activity, water temperature) seemed less consequential in determining potential vulnerability to predators. Our finding of significantly higher selection for both nonindigenous species in mud is tentative due to the reduced predation in this substrate. However, fine-medium sand is the major sediment type in Yaquina Bay (Kulm and Byrne 1967). Thus, our selection experiments in sand are more representative of natural conditions than are the experiments in mud.

Increase of activity with temperature were evident for both male and female *Corophium* and escape responses may be faster at higher temperatures for *C. spinicorne* than for the other species (Figure 2D). Yet, growth is reduced at temperatures over 25°C in *C. salmonis* and *C. spinicorne* but enhanced in *C. acherusicum* and *C. insidiosum* by even higher temperatures (J.W. Chapman, pers. obs. 1997). For *C. spinicorne* reduced growth may thus be a trade-off for decreased predation at higher temperatures.

Morphological and/or behavioral differences between male and female *Corophium* did not result in sex-elective predation by juvenile *P. vetulus* in our laboratory experiments. Moreover, field sex selection for *C. salmonis* by *P. vetulus* was suggested at only one of the three intertidal sites examined in Yaquina Bay and Alsea Bay (Castillo 1998). In that study, *C. salmonis* in benthic core samples from three sites showed an increase in the average female/male ratio with water depth from about 1.0 (0-40 cm depth) to about 1.4 (80 cm depth). Thus, a spatially heterogeneous sex ratio of prey in the environment may confound the actual prey availability.

Reimers *et al.* (1978) found that juvenile chinook salmon (*Oncorhynchus tshawytscha*) in the Sixes River estuary, Oregon, consumed more male than female *Corophium*. They also reported that male *Corophium* occurs more often on the surface of the substrate than females, but females outnumber males. Higher selection of male over female amphipods (*Grandidierella lignorum*) was also reported for juveniles of another fish (the sparid *Lithognathus lithognathus*), and selection resulted from increased exposure of males to predators (Schlacher and Wooldridge 1996). The difference in sex-selective predation between our experiments and the previous two studies can be explained in part by differences in fish foraging. Unlike *O. tshawytscha* and *L. lithognathus*, a significant proportion of the diet of juvenile *P. vetulus* comprises infauna (Castillo *et al.* 1996). Thus, sex-related differences in prey accessibility may be minimized by juvenile *P. vetulus*.

Observation of *P. vetulus* feeding behavior in the present study was limited by the reduced fish activity following their introduction into the tanks. Most fish remained in a fixed area on the bottom or partially buried in the sediment for several hours.

Preliminary observations in which prey were added from the top of the tank showed eye movements in the settled fish. Once the prey reached the bottom of the tank, the fish used its dorsal and anal

fins to support its body and "shuffled" forward toward the prey, after which it quickly consumed the prey. Several fish attacks followed by regurgitation were needed to successfully ingest large *C. spinicorne* of either sex. In one case a large *C. spinicorne* male oriented its second antennae sideways when confronted by a fish, a defense mechanism also reported by Reimers *et al.* (1979).

Foraging juvenile and adult *P. vetulus* use their pointed snouts as a shovel to extract infauna (Ambrose 1976; Hulberg and Oliver 1978). Despite such behavior, the percent of the tank bottom area free of sand at the end of the experiment was not correlated to total prey consumption ($r = 0.17$, $P > 0.05$, $n = 25$) suggesting that overall sediment disturbance by fish does not enhance prey consumption.

Juvenile *P. vetulus* may be visually oriented predators. Stomach fullness of juvenile *P. vetulus* increases throughout daylight hours in nearshore areas (Hogue and Carey 1982) and estuaries (G.C. Castillo, pers. obs.). Fish-induced turbidity in the mud treatment reduced visibility and could explain the relatively low predation on *Corophium* in mud (Figure 6).

Due to turbidity, the tank bottoms of most mud replicates were not visible from the water surface at the end of the experiments. However, predation on amphipods was not significantly higher in mud replicates where the bottom was visible on completion of the experiment (mean consumption = 7.1 prey, $n = 7$ fish) than in tanks in which the bottom was not visible (mean consumption = 5.8 prey, $n = 18$ fish, $P > 0.50$, ANOVA). Thus, differences in amphipod behavior between sand and mud as well as reduced prey visibility in mud could account for the greater consumption by fish in the sand replicates.

Although the Strauss' prey size selection index was not significantly correlated to prey size for any species, *C. acherusicum* showed the highest correlation (Figure 4). Thus, this index is partially consistent with the significantly greater mean size of consumed prey for the latter species (Figure 5).

Our experiments support field observations in the sense that some nonindigenous invertebrate prey can be highly selected by juvenile *P. vetulus* (G.C. Castillo, pers. obs.). With the exception of *C. insidiosum*, field data for Yaquina Bay and Alsea Bay estuaries have shown that *P. vetulus* and starry flounder (*Platichthys stellatus*) prey on all *Corophium* species considered here (Castillo *et al.* 1996). The low abundance of nonindigenous *Corophium* in the fish diet in that study may be due to the high intertidal distribution of introduced *Corophium*. Although direct negative trophic effects of nonindigenous *Corophium* spp. on *P. vetulus* are not implied from our study, we can not predict the effect of an increasing number of nonindigenous species on the abundance of *P. vetulus*. Yet, additional guilds in the food-base of native predators could lead to a decline in community stability (Castillo *et al.*, in press).

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Ecological Interactions and Impacts of Invasive *Kappaphycus striatum* in Kane'ohe Bay, a Tropical Reef

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ABSTRACT: **The introduction and consequent spread of the red alga, *Kappaphycus striatum*, has recently become a management concern and has led to studies that examine the ecological impacts and interactions of the alga. To determine if herbivory plays a role in the abundance of *K. striatum*, predator exclosures were placed in areas of high and low algal abundance to estimate differences in grazing intensity. It was observed that, in areas of high *K. striatum* abundance, there was no significant difference in growth between the caged and uncaged treatment, suggesting that grazing intensity is low and unable to affect algal biomass. In an area of low macroalgal abundance, however, differences in growth rates between uncaged and caged treatments were significant. Growth rates were negative in the uncaged treatments, suggesting that grazing intensity was high. This higher grazing intensity may be due to a larger grazing population or to lack of alternative food sources. Comparison of growth rates in the caged treatments allowed assessment of growth rate potential among sites. Additional studies to assess the ability of the alga to reproduce vegetatively, as well as a qualitative assessment of the possible impact of algal overgrowth on live coral, will allow estimation of further spread, habitat alteration, and ecological impact.**

Keywords: introduction, macroalga, *Kappaphycus*, herbivory, fragmentation, habitat alteration, tropical reef

INTRODUCTION

The importance of understanding the mechanisms and impacts of biological invasions remains crucial in our efforts to preserve biodiversity and to minimize environmental degradation. The invasion of marine and estuarine systems by non-native species has become a widely recognized phenomenon that continues to take place around the world (Grosholz and Ruiz 1996). Studies of biological invasions have documented substantial alteration of pre-existing communities by displacing native species through predation, hybridization or competitive interactions (Lodge 1993; Ricciardi *et al.* 1995). In comparison to the extensive literature that documents invasions of terrestrial and freshwater habitats, relatively little is known of the history or impact of marine invasions (Lodge 1993). Quantitative studies and long-term experiments to distinguish the effects of an introduction from natural variation in the marine environment remain rare (Grosholz and Ruiz 1996).

Three marine red algal species, *Eucheuma denticulatum, Kappaphycus alvarezii*, and *K. striatum*, were introduced to the fringing reef area at the Hawaii Institute of Marine Biology (HIMB) on Coconut Island in the early 1970s. The introductions took place as part of aquaculture studies motivated by the increasing commercial value of the carrageenan found in the cell walls of these species. There has been relatively little documentation of the locations of the original plantings or the specific species of *Kappaphycus* and *Eucheuma* that were planted. Russell (1981) documented the early history of the *Kappaphycus* and *Eucheuma* spp. establishment and dispersal in Kane'ohe Bay from 1974 to 1976. A more recent study by Rodgers and Cox (1999) documented the current distribution of *Kappaphycus* spp. in the bay (Figure 1).

The apparent overgrowth of the *K. striatum* on live coral, as well as its spread, has become a management concern. Characteristic of weedy species, its phenotypic plasticity as well as its ability to repro-

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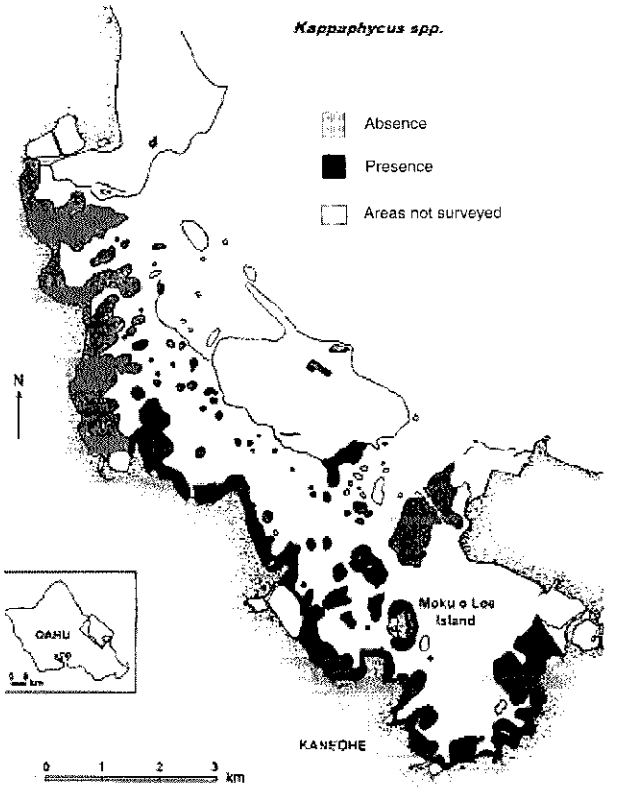

Figure 1. Distribution of *Kappaphycus* species within Kane'ohe Bay as surveyed in 1996. Reproduced with permission from Rodgers and Cox (1999).

duce vegetatively has most likely contributed to the spread of this species throughout Kane'ohe Bay. Since its introduction nearly 30 years ago, *K. striatum* has not spread outside the bay, nor reached the northernmost regions. However, the alga has a patchy distribution, with areas of very dense coverage found in close proximity to areas of low abundance. The possibility of the continued spread and dominance of *K. striatum* led to the following on-going study.

One goal of this study is to determine the factors that may play a role in determining the distribution of *K. striatum*. Herbivorous fishes in the families Acanthuridae (surgeonfishes) and Scaridae (parrotfishes) are an important component, both ecologically and evolutionarily, of the herbivore guild on many tropical reefs (Randall 1965; Wanders 1977; Steneck 1983; Hay 1984; Lewis and Wainwright 1985; Lewis 1986). Several studies have documented the influence of herbivory in coral reef community structure (Stephenson and Searles 1960; Randall 1961; Wanders 1977; Ogden and Lobel 1978; Sammarco 1982; Lewis and Wainwright 1985; Lewis 1986). An investigation conducted by Lewis (1985) demonstrated that when grazing intensity of adult acanthurids and scarids was experimentally reduced, rapid and dramatic shifts in benthic community structure followed. As portions of coral colonies were overgrown and subsequently killed by macroalgae, total macroalgal abundance significantly increased with a decrease in algal turf, crustose coralline algae, and coral abundance. The study suggests that grazing by herbivorous fish helps to maintain a tropical benthic assemblage dominated by algal turfs and crustose coralline algae by reducing abundances of macroalgal species with superior overgrowth abilities.

To determine if herbivory plays a role in the success of *K. striatum*, predator exclosures of paired caged and uncaged treatments were placed in areas of high and low algal cover. Differences in grazing pressure were elevated by comparison of the growth rates in the paired caged and uncaged treatments. We hypothesized that low grazing intensity may lead to high *K. striatum* abundance. In addition, comparison among sites of growth rates in caged treatments will allow determination of growth rate potential. The conditions at the densely covered sites may allow higher growth rates and may lead to the subsequent dominance *K. striatum*.

K. striatum rarely reproduces sexually and its ability to reproduce vegetatively is an important component of its invasive success. Small branch tips may break off due to physical disturbance and be carried by currents and waves to new locations where they may settle and establish. Assessment of the minimum fragment size capable of maintaining positive growth becomes an important issue when considering remediation efforts. Attempts at manual eradication could possibly result in spread rather than removal. In addition, the ability of fragments to survive in a variety of environments is an important determination. The fragments of *K. striatum* may have a relatively high sinking rate relative to other invasive, weedy species, but if these fragments are capable of surviving long periods of reduced light at depth, the probability of dispersal, settlement, and establishment becomes much higher.

Apparent overgrowth by algae on live coral is critical as Kane'ohe Bay historically has had remarkable disturbances associated with urbanization of the coastal zone. Quantification of significant alterations of the habitat would require long-term studies in order to differentiate changes brought about by the

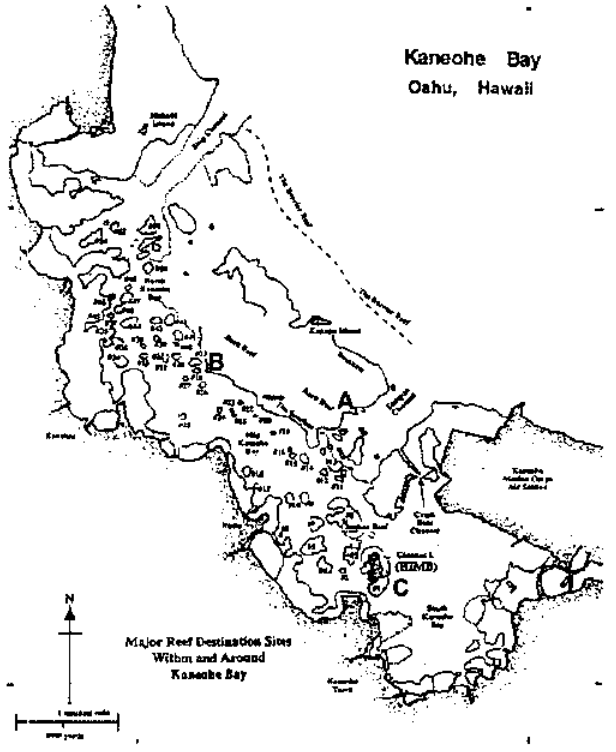

Figure 2. Map of Kane'ohe Bay with locations of predator exclosure study sites: Site A: High *K. striatum* abundance, back reef area; Site B: High *K. striatum* abundance, patch reef #29 Site C: Low *K. striatum* abundance, fringing reef of Coconut Island

algae from natural variation. For the purposes of management decisions, a more rapid qualitative assessment may be made by monitoring specific areas over a one-year period through monthly photographs taken in areas of possible algal encroachment on live coral.

Materials and Methods

Growth Potential and Herbivory

The growth rates and grazing effects of *K. striatum* were examined using paired predator exclosures at three sites (Figure 2). Site A was located in an extensive barrier reef complex with high *K. striatum* abundance and high wave energy. Site B was a patch reef with *K. striatum* dominating the reef flat and reef crest. Site C included a section of a fringing reef with very low macroalgal abundance and was located on the windward side of the Hawaii Institute of Marine Biology, a marine protected area where only limited collecting is allowed.

Large thalli were collected in the back reef area and returned to the laboratory in seawater. The thalli were broken into smaller thalli weighing 20-25 g (wet weight), and approximately 6 cm in length. Weighed thalli were randomly assigned to two levels of herbivory: caged and uncaged. Caged and uncaged thalli were attached to vinyl-coated 2.5-cm wire mesh platforms (30 cm x 15 cm). Rather than clamping or tying the algae to the platform, coiled, plastic-coated, single-strand electrical wire was threaded through the thalli. The coils were 2 cm in diameter and contained 6-7 rings. Coils were tied to the platform where fish could graze on algal tips and branches that extended out of the coil. Cages had walls, roofs, and floors of 0.5-cm vinyl-coated mesh. All cages were 8 cm L x 8 cm W x 8 cm H. The platforms holding caged and uncaged thalli were placed on rubble terraces on the reef crests in areas B and C. At site A, platforms were attached to non-living reef substrate using two metal stakes. Individual thalli were weighed at the start of each experimental period and reweighed after approximately 5 da in the field. Two separate experiments were conducted, one each in November and December 1998.

Growth rates were determined by subtracting initial weight from final weight and dividing by the number of days in the field. Growth potential was determined by examining caged growth rates among sites. Grazing intensity was calculated as the difference between paired caged and uncaged growth rates. Very low negative values occasionally resulted due to breakage during handling in the field and were not included in the analysis. Results were analyzed using an unbalanced two-way analysis of variance (ANOVA) and Tukey HAS multiple comparisons (Systat 8.0).

Fragmentation Success

Thalli were collected from the field and placed in running seawater tables. Small fragments were removed from larger thalli, weighed, and placed in one of four weight categories, ranging from 0.03 to 0.25 g and 0.25 to 1 cm in length. Fragments were color coded according to size class using fine plastic-coated wire. The fragments were then placed in two different environments. One set of fragments was placed in a pan filled with sand covered with 0.5-cm plastic-coated mesh. The other set of fragments was placed in cages with walls, floors and roofs of 0.5-cm plastic-coated mesh. Both the pan and the cages were placed on the reef flat on the windward side of HIMB and left in the field for approximately 6 days.

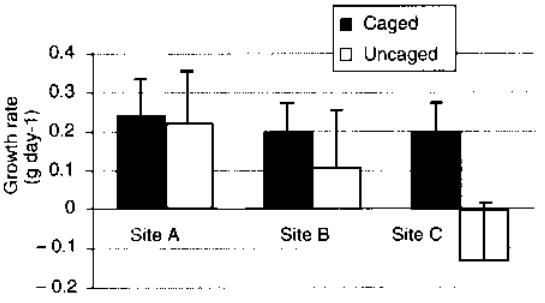

Figure 3. Average growth rates for caged and uncaged treatments at sites A, B, and C in November 1998. Error bars indicate +/- one standard deviation. Sites A and B are areas of high *K. striatum* abundance and site C is an area of low *K. striatum* abundance.

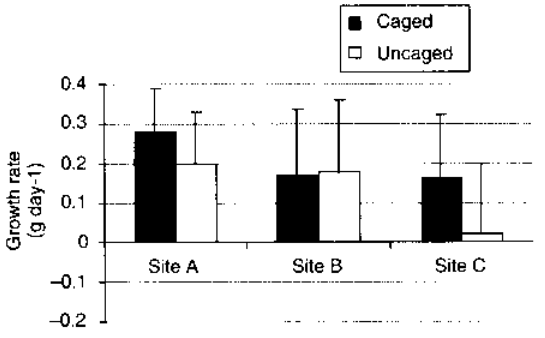

Figure 4. Average growth rates for caged and uncaged treatments at sites A, B, and C in December 1998. Error bars indicate +/- one standard deviation. Sites A and B are areas of high *K. striatum* abundance and site C is an area of low *K. striatum* abundance.

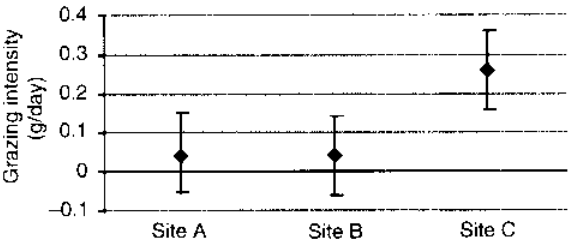

Figure 5. Grazing intensity calculated by average differences between paired caged and uncaged thalli at sites A, B, and C. Error bars indicate +/- one standard deviation. Growth rates measured in November and December 1998. Sites A and B are areas of high *K. striatum* abundance and site C is an area of low *K. striatum* abundance.

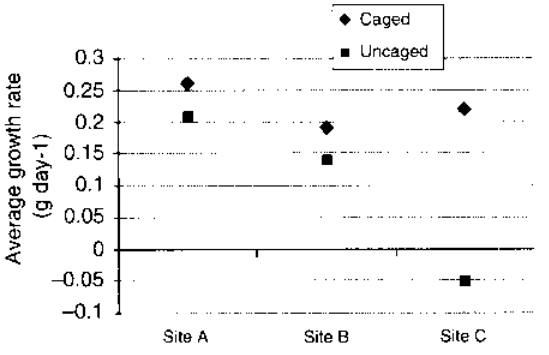

Figure 6. Average growth rates of caged and uncaged treatments in November and December 1998 at sites A, B, and C.

At that time, all fragments were brought back from the field and reweighed.

Coral and Algae Interaction

To monitor the effects of algal encroachment on live coral, four photostations were constructed in the back reef area of Kane'ohe Bay (Figure 1, site A). Metal stakes used to mark these permanent sites and act as a camera mount in order to minimize changes in the photographic area through time. The stakes were pounded deeply into the sandy substrate. The camera was mounted on a platform constructed from acrylic and PVC piping that slid over the stake. Nondestructive markers were placed on the coral head to indicate lines for orientation. Photographs were taken monthly.

Results

Growth Potential

Average growth rates of all caged plants were compared. Growth rates were found to be significantly different ($P < 0.004$) among sites (Figures 3, 4, and 6). Site A was significantly different from site B and site C ($P < 0.05$). There was no significant difference between sites B and C.

Herbivory

The average growth rates of both the caged and uncaged treatments at sites A, B, and C in both November and December are shown in Figures 3 and 4. The paired design of the experiment allowed for a measure of grazing intensity to be calculated by subtracting the uncaged growth rates from the caged growth rates. Results indicated that there was a significant difference ($P < 0.0001$) in grazing intensity among sites (Figure 5). There was no significant difference between grazing intensity and date ($P < 0.712$). As shown in Figure 5, site C, the area of low macroalgal abundance, had a higher grazing intensity than in sites B and C. The Tukey multiple comparisons test resulted in a significant difference between sites C and A, as well as between sites C and B ($P < 0.05$). There was no significant difference between

Table 1. Table of initial and final weights of fragment size classes in caged and sand treatments.

Treatment	Initial Wt. Range (g)	Final Wt. Range (g)
Caged	.05–.08	.11–.15
	.11–.15	.18–.24
	.20–.25	.40–.56
	.30–.35	.43–.70
Sand	.05–.08	.08–.09
	.11–.15	.11–.16
	.20–.25	.21–.26
	.30–.35	.27–.44

sites A and B. Average growth rates of all uncaged plants can be seen in Figure 6, with negative average growth rate at site C.

Fragment Growth

Results of the fragment growth experiments are shown in Table 1. The fragments placed in cages exhibited positive growth or maintained initial weight in all size classes. Fragments placed in the sand maintained or lost biomass in all size classes.

Coral and Algae Interaction

Photographs taken in July show an increase in the algal cover of live coral compared to the photographs taken in January. One of the four photostations is shown in Figure 7.

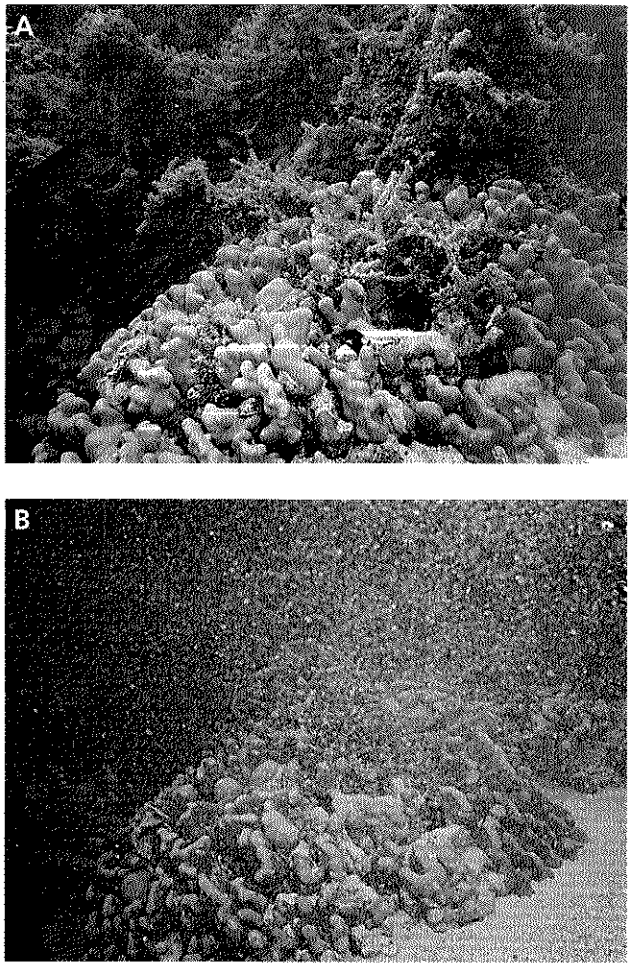

Figure 7. Photographs taken at photostation in the back reef area of Kane'ohe Bay to monitor algal overgrowth. (A) July 1997, (B) January 1998.

Discussion

Abundance of an alga on tropical reefs is a function of plant growth ability, nutrient availability, and grazing pressures. Previous to this study, the ability of *K. striatum* to spread and grow in different areas of the bay was a point of some confusion (Russell 1981). The varying environmental conditions across the bay could possibly limit the distribution of *K. striatum* and play a role in its patchy distribution. Results from this study, however, have demonstrated that *K. striatum* is capable of net growth within cages at the three sites sampled. Comparison among sites of the growth rates in cages demonstrated a significantly higher growth rate at site A. This high growth rate may in part explain the success of *K. striatum* at site A, but at site B, which also has dense algal coverage, a lower growth rate was observed. The lack of significant difference between site C, with low coverage, and site B, with dense coverage, further emphasizes the role of herbivory. Caged growth rates between sites were similar, yet at site B, *K. striatum* was abundant and in site C, *K. striatum* was absent. These results suggest that varying environmental conditions and nutrient availability underlie the distribution of *K. striatum*, and other factors such as herbivory and dispersal may be more important regulators of the current distribution of *K. striatum*.

The results of this study suggest that grazing intensity, as measured through average growth rate differences between paired caged and uncaged thalli, is low at sites with high *K. striatum* abundance (Figure 5). Grazing intensity values at high abundance sites A and B were consistently close to zero for both dates. An explanation of the low grazing intensity values could be that, because of the presence of large amounts of *K. striatum* in the area, grazers may have

not encountered the uncaged thalli. In addition, the presence of alternate food sources at sites A and B may result in lower grazing intensity. It is apparent, that *K. striatum* is growing faster at these sites than grazers can consume it. This may arise from several factors such as low grazer density, lack of sea urchins in the bay, and low food preference in comparison to other algae (Stimson, unpublished data). In spite of the low grazing intensity results of the predator exclosures at sites A and B, significant grazing scars have been observed in the field. A study conducted by Russell (1983) demonstrated that fish selectively grazed on the branch tips of *K. striatum*. Thus, the ability of the alga to form dense, smooth masses over the coral can be viewed as a morphological defense and an additional explanation for the invasive success of *K. striatum*.

Site C, Coconut Island, is a marine protected area where fishing and collection are regulated. With a presumed higher abundance and diversity of grazers, one would expect grazing intensity to be higher as well. This high grazing intensity may also arise from the lack of alternate food sources in the area. Coconut Island was the original site of the *K. striatum* introduction and once supported a large population of *K. striatum* that has drastically declined. This situation lends support to the hypothesis that grazers can regulate the abundance of *K. striatum* in an area. At Coconut Island, growth rates outside the cages were consistently equal to or below zero, suggesting that the algae could not establish in the presence of the current herbivore assemblage.

With few precise data about the original planting of the alga, it is difficult to assess dispersal capabilities of *K. striatum*. Unlike other introduced algae that spread to the five main Hawaiian islands within a few years, *K. striatum* appears to have spread at a much slower rate. Although *K. striatum* is able to reproduce vegetatively, fragments may sink too quickly before being transported long distances. With fragments as small as 0.05 g capable of doubling their weight in less than one week, however, this alga could disperse more widely given sufficient time. Caged fragments grew or maintained initial weight, unlike fragments placed in sand, paralleling the observed success of the alga on live and dead coral and its absence on sandy reef flats.

Monitoring the overgrowth of coral by algae through monthly photographs has allowed several observations. As hypothesized, *K. striatum* overgrows live coral. Over the six months that photostations have been monitored, however, the changes in algal cover were not readily observed. This slow but persistent growth of *K. striatum* is consistent with its history, however, as it has gradually spread throughout Kane'ohe Bay over the last three decades.

Although *K. striatum* has not exhibited the swift dispersal of other invasive species, it has demonstrated that if given enough time, it can settle and establish in certain areas. Low grazing intensity in areas of high abundance, vegetative reproduction, and phenotypic plasticity are each important components that contribute to the abundance of *K. striatum* in Kane'ohe Bay. Additional studies are needed to further examine the loss of biomass observed in the uncaged treatments at site C, the protected area. Predator exclosures will be placed at additional sites of low *K. striatum* abundance in marine unprotected areas in order to compare grazing intensity. In addition, sites may be included that have high abundances of macroalgae other than *K. striatum*. Palatability testing among herbivores and identification of grazers will also be useful in understanding the current distribution of *K. striatum* in the bay. The possibility of remediation will require great care and energy to contain small fragments. With a slower growing species such as *K. striatum*, however, it may be possible to apply occasional, and careful, large-scale harvests to crop the alga back in the habitat.

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Source of Unpublished Material

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Natural History and Biology of the Asian Shore Crab *Hemigrapsus sanguineus* in the Western Atlantic: A Review, with New Information

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ABSTRACT: **In the 1980s, the western Pacific grapsid brachyuran, *Hemigrapsus sanguineus* (De Haan 1835), entered an open niche in the middle to upper rocky intertidal zone along the mid-Atlantic coast of the United States. Several aspects of its biology and ecology in Atlantic waters are briefly updated here. Recent studies show that the crab's northern geographical range now extends to the coast of New Hampshire. Additional information on the length of its reproductive season in New Jersey has verified that reproduction at that latitude ends in September. Molted exoskeletons of *H. sanguineus* (n = 325) and *Carcinus maenas* (n = 270), its chief potential competitor, were collected over two months during the fall of 1998, from beach wrack adjacent to the crabs' rocky habitat in New Jersey. Carapace width (CW) frequency distribution of juvenile and mature *Hemigrapsus* exoskeletons showed similarities to the population living among the rocks. *Carcinus* molts were nearly all juveniles with a mean CW of 21.7 ± 7.5 mm, slightly greater than molts of *Hemigrapsus*, 16.8 ± 6.4 mm. These and other observations indicate that both species continue their sympatric relationship in New Jersey. Laboratory observations showed that adult *H. sanguineus* survive salinity reduction down to 10‰.**

Key words: *Carcinus maenas*, molting, New Jersey, reproduction, salinity tolerance

INTRODUCTION AND REVIEW

Hemigrapsus sanguineus, a native of rocky intertidal habitats in the western Pacific Ocean (from Sakhalin Island south to Hong Kong), was found for the first time in the Atlantic Ocean in 1988, along the coast of New Jersey, where it had established a breeding population (Williams and McDermott 1990; McDermott 1991). One purpose of this review is to summarize briefly our present knowledge of the biology of the Atlantic population, derived primarily from McDermott's (1998a, 1998b, 1999) studies in New Jersey, but with additional information mainly from Lohrer and Whitlach (1997) and Epifanio *et al.* (1998). Other contributions to the biology of *H. sanguineus* are found in this Proceedings.

The present geographical distribution of *H. sanguineus* in the western Atlantic extends from North Hampton, New Hampshire (McDermott, recent data discussed later) to Oregon Inlet, North Carolina (McDermott 1998a). The largest populations appear to be located from Long Island Sound southward to Delaware. In New Jersey the crab is concentrated in the middle to upper rocky intertidal area, but may be located at times throughout the intertidal and into the subtidal. It is the only species of brachyuran crab north of Virginia that inhabits the drier upper intertidal, occupying a niche that was unoccupied prior to its establishment in the mid-Atlantic. In Long Island Sound, however, Lohrer and Whitlach (1997) have shown conclusively that populations of *H. sanguineus* are most prevalent in the lower rather than the upper intertidal.

The chief brachyurans sympatric with *H. sanguineus* are the green crab, *Carcinus maenas* (Portunidae) and the mud crabs, *Dyspanopeus sayi*, *Eurypanopeus depressus*, and *Panopeus herbstii* (Xanthidae). The xanthids occupy only the lower intertidal and subtidal zones, while *Carcinus* distinctly overlaps *Hemigrapsus*' habitat, but does not exist in the highest intertidal portions of the latter's habitat. It is generally agreed that in the Asian shore crab's newly exploited Atlantic habitat, *Carcinus* may be the most direct competitor both spatially and trophically (Lohrer and Whitlach 1997; McDermott 1999). In New Jersey, the *H. sanguineus* populations compete primarily with juvenile green crabs having average carapace widths (CW) slightly greater than those of adult *Hemigrapsus*. *H. sanguineus* males have wider carapaces than females and they are also heavier, due

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primarily to their larger chelipeds. The largest male recorded in the literature was from New Jersey (43.9 mm CW).

As *H. sanguineus* increases in carapace width, the linear dimensions of the homochelous chelae (length, height, width) in both sexes increase in distinct proportion to one another (McDermott 1999). In males, an upward inflection in each parameter at 15 mm CW, not present in female chelae, may relate to their maturation size. The larger chelae of males, plus their greater mechanical advantage ratio (L_1/L_2; Warner and Jones 1976) and wider gape, make them more formidable for feeding and defense than those of females (McDermott 1999).

H. sanguineus is an omnivorous and apparently opportunistic feeder, as shown both by the analysis of stomach contents and by laboratory observations (Lohrer and Whitlach 1997; McDermott 1998a, 1999). Various species of micro- and macroalgae, *Mytilus edulis, Semibalanus balanoides,* and a variety of other crustaceans have been identified in stomach contents, and, in southern New England, Lohrer and Whitlach (1997) noted that the relatively small littorinid gastropods, *Littorina obtusata* and *L. saxatilis* also are important food items. Laboratory experiments have further suggested that the abundant mid- to upper-intertidal amphipod, *Hyale plumulosa,* may be a particularly good food source in rocky areas (McDermott 1999).

H. sanguineus reproduces from late April through September in New Jersey (McDermott 1998b), whereas a shorter breeding season, June to September, was recorded for Long Island Sound (Lohrer and Whitlach 1997). Crabs may become ovigerous at 12 mm CW, but based on an abdominal width (AW)/CW ratio of ≥ 0.60 indicating maturity, female crabs up to 17 mm CW are often not mature (McDermott 1998b). There is a positive correlation between the numbers of embryos in a brood and the CW. Large females from New Jersey produced broods of > 40,000 embryos as determined by direct counts.

In the laboratory, embryonic development to hatching of zoeae takes 22.3±1.8 d at 19-20°C (McDermott 1998b). Epifanio *et al.* (1998) found that laboratory development at 25°C required 14 d. These data agree basically with those of Kurata (1968) and Fukui (1988) for *H. sanguineus* in the Pacific. Data of Kurata (1968) and Hwang *et al.* (1993), relating water temperature and larval developmental times, suggested that at the latitude of New Jersey about 40 d may be required for metamorphosis from the first zoea to the first crab stage (McDermott 1998b). Thus, it is estimated that along the mid-Atlantic states the cycle from oviposition to the first crab may be approximately two months, more than a month of which represent larval stages that may be dispersed great distances by tides and currents.

In Korea, Hwang *et al.* (1993) described the five zoeal stages and megalopa of *H. sanguineus* (laboratory-reared at 25°C) and distinguished these stages from those of other members of the genus. They recorded mean times for development from hatching to the megalopa and first crab at 18 and 31 d, respectively. In America, Epifanio *et al.* (1998) also successfully raised all of the larval stages as well as the first five crab stages of *H. sanguineus* in the laboratory as part of a study directed primarily to temperature-salinity requirements for development. Approximately 16 d at 25°C and 15‰ salinity were required for zoeae to reach the megalopa, but survival was only 8% compared to 60% for those reared at 20‰. At lower temperatures, salinities > 20‰ were required for this same development. Megalopae, however, developed only at salinities ≥ 25‰. These data suggest that larval sensitivity to low salinities might limit the establishment of adult populations in the mesohaline portions of estuaries. Epifanio *et al.* (1998) found that it took 25 d from hatching to the first crab stage at 25°C, a duration in relatively close agreement with the development time for *H. sanguineus* recorded by Hwang *et al.* (1993). Thirty-five days were required for first crabs to reach the fifth juvenile stage (Epifanio *et al.* 1998). Both Saigusa and Kawagoye (1997) (with Pacific crabs) and Epifanio *et al.* (1998) found that the release of first zoeae occurred throughout spring and neap tides.

Recruitment to the *H. sanguineus* population in New Jersey begins in early June and continues into the winter. By 1994 this population had become so large that fishermen obtained crabs from among the rocks and used them as bait for tautog (*Tautoga onitis*). Recently (June 1997), some fishermen were observed collecting and filling one-gallon containers with *H. sanguineus* (McDermott, pers. obs.). Interestingly, these fishermen were Japanese, who recognized this species as an inhabitant of the waters in their homeland.

New information on the biology of *H. sanguineus* is presented and discussed in the remainder of this paper: the latest geographic range of the species,

further delimitation of the reproductive season in New Jersey, molting in the field, and salinity tolerance in mature crabs.

Materials and Methods

The north-south geographic range of *H. sanguineus* in the Atlantic Ocean was reevaluated in 1998. Trips were made to New England and North Carolina to check for crabs in locations where they were not found in 1995 and 1996, respectively.

In order to verify and expand my previous report (McDermott 1998b) that the reproductive season for *H. sanguineus* in New Jersey was from late April through September, mature females were collected during April, September, and October 1998 at Townsends and Hereford Inlets. Gonads of these females were examined and ovigers (= ovigerous females (see Ryan 1956) were recorded).

In the early years of its ocurrence in New Jersey, molted exoskeletons of *H. sanguineus* were found infrequently among the rocks at the main sampling site at Townsends Inlet. By 1996, molts of crabs (carapaces or whole molts) were being found in increasing numbers in the high-water beach wrack near rocky intertidal locations, suggesting an increase in these grapsid populations in southern New Jersey. In the Fall of 1998, an attempt was made to monitor molting at the Townsends Inlet site. From 22 September to 24 November, 11 collections of molted exoskeletons of *H. sanguineus* (n = 325) and green crabs, *Carcinus maenas* (n = 270), were made from the high-water beach wrack along a 100-m transect of a sheltered sandy beach adjacent to the crabs' rocky habitat. The first three collections (22 September to 8 October) were approximately one week apart, and the second three were obtained on consecutive days (18 to 20 October). The seventh to eleventh collections were on 2, 3, 8, 23, and 24 November. Only molted carapaces and whole molts were collected and measured (CW).

In May 1992 an experiment was performed to test the ability of *H. sanguineus* to tolerate reduced salinities. The crabs used in this experiment were collected at Townsends Inlet, and had been individually maintained prior to the experiment at a salinity of 32‰.

They were kept at ≈ 19°C in a dark incubator with *Ulva* as food. Mean carapace width of males was 20.8 ± 2.2 mm (range 17.5 to 24.8 mm) and of females was 20.4 ± 3.0 mm (range 15.6 to 27.9 mm). Three crabs of each sex were subjected to salinities of 32, 25, 20, 15, 10, and 5‰. They were placed immediately into the lower salinities. Crabs were held individually in fingerbowls with 165 ml of the seawater dilutions at 19°C in a dark incubator. Times of exposure varied, but two of the salinity groups were exposed for the entire length of the experiment, *i.e.*, 199 h. In order to conserve the relatively few crabs available at the time, the six crabs used at 32‰ for 48 h were used again at 15‰. Water was changed every 48 h.

Results

Geographic Distribution

Hemigrapsus sanguineus was reported previously to range from Green Harbor, Massachusetts (north of the Cape Cod Canal and south of Boston) to Oregon Inlet, North Carolina (McDermott 1998a). No crabs were recovered in 1995 at three locations north of Green Harbor (Magnolia Bay on Cape Ann, Massachusetts; Odiorne Point State Park, Rye, New Hampshire, and York Harbor, Maine), or in 1996 at one location south of Oregon Inlet (Beaufort, NC). Some of these same sites on either end of the geographic range were reinvestigated in 1998. In North Carolina (19-20 March), *H. sanguineus* was again not found either at Beaufort (salinity 28.6‰) or at Harkers Island (salinity 17.7‰), both approximately 158 km south of Oregon Inlet and located inside of Shackelford Banks north of Cape Lookout. In New England, while again failing to recover this crab at Magnolia Bay and York Harbor, one immature female (13.5 mm CW; AW/CW ratio = 0.55) was found on 31 October among beach rocks at North Hampton, New Hampshire, approximately 10 km south of Odiorne State Park. Juvenile *Carcinus maenas* (mean CW 12.6 ± 8.0 mm, range 4.3 to 36.3 mm, n = 38) outnumbered *H. sanguineus* at North Hampton by at least 50 to 1. W. W. Lull and S. J. Miller (pers. comm.) have had no reports of *H. sanguineus* being recovered in their area. However, M. C. Tyrrell (pers. comm.), found an 11.2-mm male *H. sanguineus* at Dover Point in Great Bay, New Hampshire, and noted that colleagues at the University detected these crabs in October 1999 at Rye Harbor. Thus, the invader has now reached New Hampshire approximately 100 km north of Green Harbor, Massachusetts, but its southern limit has not been extended.

Delimiting the reproductive season

From 1988 to 1997, only one oviger was present among 39 mature female *H. sanguineus* collected in New Jersey during April. This oviger, found 22 April 1997, had embryos not yet undergoing cleavage, *i.e.*, the brood was only recently oviposited. In the month of October, no ovigers were found among 32 mature females, but might have been found during this month if larger numbers of females were examined (McDermott 1998b).

In the spring of 1998, further collections were made in New Jersey. On 22 April, three of nine mature females were ovigerous; two had broods in which cleavage had not taken place and in the other cleavage had reached the 8-cell stage. The mean CW of the nine crabs was 23.3 ± 2.7 mm (range 19.9 to 29.3 mm); the mean CW of the ovigers was 23.4 ± 1.6 mm (range 22.2 to 25.2 mm). All of the ovigers had greatly depleted, light yellow, stringy gonads with poorly developed oocytes, whereas the six other mature crabs had broad, dark brown gonads with mature oocytes. All except two (nonovigers) had full receptacles. Three immature crabs (13.9 to 15.1 mm CW), collected at the same time, had undeveloped gonads. One of the ovigers (22.2 mm CW) had an AW/CW ratio of 0.57, which was an abnormally low value. Of four mature crabs collected on 22 May (mean CW 19.7 ± 3.1 mm, range 16.6 to 23.7 mm), two were ovigerous (16.6 and 18.0 mm), and of these, one had broad, dark brown gonads. Two nonovigers (20.4 and 23.7 mm) also had similar dark brown gonads. These observations indicate that some, but not all ovigers, retain adequate gonads to produce further broods.

Near the end of the reproductive season of 1998, two collections of mature females were made at the Hereford Inlet monitoring site (23 September and 3 October) in order to detect ovigers and examine gonads (Table 1). Three of the 25 crabs (12%) in September were ovigerous with late-stage eyed embryos, whereas none was ovigerous in October. The gonads of the October group were all depleted. Seven of 27 (25.9%) had molted recently and were in paper-shell stages, and five were ready to molt; in the September group there were no recent molts and no signs of imminent molting. The complete gonad depletion and increase in female molting in October are indicative of reproductive cessation. Nine of 26 mature crabs examined in October still had egg membranes attached to their pleopods, which indicated that these late brooders had yet to undergo their reproductive season-ending molts.

Table 1. The number of ovigers, gonad condition and recent molting of mature female *Hemigrapsus sanguineus* from New Jersey observed on two occasions near the end of the reproductive season of 1998.

Date	Number of crabs	Carapace width (mm)	Number of ovigers	Condition of gonads	Number recently molted
23 Sep.	25	19.9 ± 2.5	3	—	0
3 Oct.	27	18.9 ± 2.1	0	depleted *	7

* gonads colorless, stringy and without visible oocytes.

Molting of Hemigrapsus and Carcinus in the Field

The CW frequency distribution of molts for *H. sanguineus* (Figure 1) closely resembles the size frequency among the living crabs in the rocky intertidal population, except for fewer young of the year (y.o.y.) molts in the <10 mm CW category (McDermott 1998a, and unpublished data). The mean CW was 16.8 ± 6.4 mm, with the majority of crabs in the 10 to 21 mm range. Of the 325 molts, 197 (60.6%) were whole crabs, 112 being males and 85 females. Young of the year molts (n = 49) were more numerous in the November collections (n = 40). The CW frequency distribution of male molts was comparable to that of the 128 unsexed carapace molts, but the largest female molt was only 24.1 mm. Perhaps the female population had not reached its peak in molting so soon after the end of the reproductive season. However, some of the unsexed molts ≥ 24 mm CW (n = 53) were likely females. For example, it was calculated that a female molt found without a carapace, but with an AW of 20.9 mm collected in late November, would have had a CW of about 30 mm (regression formula, McDermott 1998b). Only 26.3% (20 of 76) of female molts were from mature crabs.

The majority of *Carcinus maenas* molts (Figure 1) were from immature crabs. The mean CW was 21.7 ± 7.5 mm. The largest sexed molt was from a 51.1-mm male, judged to be probably mature, since females of *C. maenas* become ovigerous at approximately this size in New Jersey (McDermott, unpublished data). One hundred ninety-six of the 270 *C. maenas* molts (72.6%) were sexed (91 males and 105 females). The

CW frequency distributions of both sexes were similar to the frequency distribution of the unsexed exoskeletons.

As water temperatures decreased from 16.5°C in the middle of October to 10°C near the end of November 1998, the number of molts deposited in the beach wrack decreased significantly. Collections on 19 and 20 October yielded 23 and 30 molts of *H. sanguineus* and 14 and 19 molts of *C. maenas*, respectively, whereas on 24 November only four molts of the former and one of the latter were collected.

Salinity tolerance

Table 2 reveals no adverse effects on *H. sanguineus* at salinities reduced suddenly to as low as 10‰ for over 4 d. At 5‰, one of six crabs became sluggish during the 24-h exposure.

Table 2. Tolerance of mature *Hemigrapsus sanguineus* collected at Townsends Inlet, New Jersey, and exposed to diluted seawater in the laboratory. Three males and three females were tested at each salinity.

Salinity ‰	Hours exposed	Percent surviving	Remarks
32	48	100	
25	199	100	
20	199	100	1 female molted in first 24 h
15[a]	151	100	1 female molted in first 24 h
10	103	100	
5	24	100	1 male weak

[a] Same crabs subjected previously to 32‰ for 48 h.

Discussion

The first North Atlantic specimen of *H. sanguineus* was found in southern New Jersey in 1988 (Williams and McDermott 1990). Through my efforts and those of numerous correspondents over the past ten years, we have been able to delimit its present geographic distribution in the Atlantic from New Hampshire to North Carolina. At the extremes of this range the species is not abundant, and it was suggested (McDermott 1998a) that it may have been introduced in the early 1980's somewhere between the New York harbor region and the Delaware Bay estuary, an area where it was most abundant. Based on its Pacific distribution, *H. sanguineus* has the potential to spread beyond its present Atlantic coast range, and will likely do so. Its temperature tolerance is broad and it is omnivorous, but the availability of suitable, rocky intertidal habitats and the presence of spatial competitors may be limiting factors in its final distribution in the North Atlantic. In natural rocky locations, such as New England, there typically is an abundance of frequently recruited food sources, *e.g.*, various algal species, *Mytilus edulis*, and barnacles. Hence, in such situations the food source should not be a major factor in the competition among sympatric brachyurans (McDermott 1999).

The April-through-September reproductive season that has been recorded for *H. sanguineus* in New Jersey might be shortened in the colder waters to the north, and possibly expanded in warmer waters beyond North Carolina. However, on 6 February 1997, an ovigerous *H. sanguineus* was found at Weekapang Point, Rhode Island by a student of J. T. Carlton (pers. comm.), but the specimen, approximately 20-mm CW, no longer exists. This record of reproduction in the middle of winter, certainly predates the beginning of the reproductive period (late April) recorded for the waters of New Jersey (McDermott 1998b). Thus, more intense collecting of mature females during the winter and early spring may help to clarify this possibly aberrant record. More than the three broods per season suggested for New Jersey (McDermott1998b), may occur in warmer Atlantic waters as found in the Pacific by Fukui (1988) in Tanabe Bay where the mean annual water temperature is 14°C (equivalent to coastal Atlantic temperature at a latitude south of Cape Lookout, North Carolina).

In Japan, the majority of reports indicate that *H. sanguineus* is most common in rocky locations on open coasts or the lower reaches of estuaries (Fukui 1988). Most crab collections over the years have been around inlets where salinities were usually >30‰. However, at my collection sites in Sandy Hook Bay in northern New Jersey, salinities ranged from 25 to 26‰, and in the Arthur Kill, between New Jersey and Staten Island, New York, crabs were found where the salinities ranged from 21 to 25‰ (F. Steimle, pers. comm.). At collection sites at Cape Charles, Virginia (Lower Chesapeake Bay) and Oregon Inlet, North Carolina, salinities measured near low water were 19 and 15‰, respectively. While the laboratory salinity experiment reported here showed that adult crabs were very tolerant to low salinities, the intolerance of megalopae to water below 25‰ (Epifanio *et*

al. 1998) might minimize population sizes in the mesohaline parts of estuaries.

The CW frequency of molts of *H. sanguineus*, recovered over a 64-d period from beach wrack at Townsends Inlet, New Jersey, gives a fair representation of the existing population of crabs living among the nearby rocks. Although a distinct cohort of y.o.y. crabs having CW <10mm is seen clearly in the molt population (Figure 1), the y.o.y. numbers are fewer than expected at this time of year (McDermott 1998a). Perhaps some of the smaller, thinner exoskeletons of y.o.y. crabs are more easily damaged. Small size of molts, however, is probably not an important factor in their detection, because in a large collection obtained in June 1999, molts as small as 3.2 mm were recovered (McDermott, unpublished data).

Molts of *C. maenas* were about equal in numbers to those of *Hemigrapsus* in the beach wrack at Townsends Inlet, which indicates that in this location juvenile green crabs have not been displaced from their shared habitat by the Asian invader. Personal correspondence with collectors in southern New England and the findings of Lohrer and Whitlach (1997), however, suggest that green crabs in some locations are being displaced by *Hemigrapsus*. M. G. Tyrrell (pers. comm.) reported that by 1998, *H. sanguineus* had almost completely displaced *C. maenas* at Little Harbor, Buzzards Bay, Massachusetts. In New Hampshire, where the population of *H. sanguineus* is very meager, the *Carcinus* population is still dominant.

The data on *Carcinus* also confirm previous information from 1990 to 1994, that most of the green crabs living with *Hemigrapsus* at Townsends Inlet were juveniles (McDermott 1998a). The similar CW distributions would tend to minimize reciprocal predation as compared to a situation where mature rather than juvenile green crabs were abundant in the intertidal with *Hemigrapsus*.

As *H. sanguineus* becomes more abundant along Atlantic shores, it is likely that their utilization will go beyond the bait fishery. Their ease of capture, maintenance, and relatively small size should make them excellent representative brachyurans for teaching, as test animals for toxicity assays, and for other research purposes. For example, in Asia these abundant decapods have been employed for many years for basic research, *e.g.*, as models for studying rhythmic changes in rhabdom structure and function in

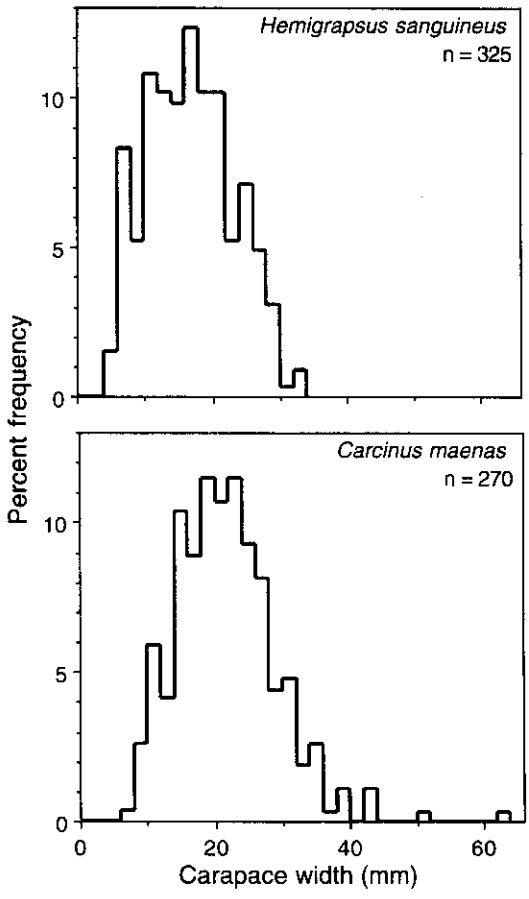

Figure 1. Percent frequencies of carapace widths (2 mm classes) of molts from *Hemigrapsus sanguineus* and *Carcinus maenas* collected from beach wrack, 22 September to 24 November 1998, at Townsends Inlet, New Jersey.

the compound eye (Arikawa *et al.* 1987, 1988; Eguchi *et al.* 1991; Matsushita and Arikawa 1997). It remains to be seen whether *H. sanguineus* will become a serious predator of native commercial shellfish.

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Food Preference Studies of the Asiatic Shore Crab *(Hemigrapsus sanguineus)* from Western Long Island Sound

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ABSTRACT: The Asiatic shore crab *(Hemigrapsus sanguineus)*, a recent introduction to the Atlantic coastline, has been extending its range since its first recorded appearance in Cape May, New Jersey in 1988 (Williams and McDermott 1990). Breeding populations of *H. sanguineus* are now well established in western Long Island Sound (Brousseau, pers. obs.) and its appearance has been documented at least as far north as Cape Ann, Massachusetts (Brousseau, pers. obs.). Very little is known about the ecological impact this invader is having on the indigenous biota, but indications are that it could be significant. This study presents preliminary information on the food preferences of the Asiatic shore crab in western Long Island Sound based on gut content analyses and food choice experiments conducted in the laboratory. Pairwise choice experiments on four of the most common intertidal macroalgae in western Long Island Sound indicate the following preference hierarchy: *Enteromorpha* spp. = *Chondrus crispus* >*Ulva lactuca* >*Fucus vesiculosus*. Crab algal consumption rates do not appear to be a function of crab size (carapace length). Experiments designed to investigate possible predatory habits of the Asiatic shore crab demonstrated that in addition to feeding on macroalgae, *H. sanguineus* will prey on a variety of bivalve seed, including *Crassostrea virginica, Mercenaria mercenaria, Mya arenaria* and *Mytilus edulis*. These findings support the conclusion that the Asiatic shore crab is an active predator and may represent the latest addition to a growing list of bivalve-seed predators along the east coast of the United States. Gut content analyses of wild-caught crabs were also performed on *H. sanguineus* and the two most common co-occurring species of mud crabs (*Eurypanopeus depressus* and *Panopeus herbstii*) to determine degree of dietary overlap with the Japanese shore crab. Macroalgae were found to make up a large part of the gut contents of both *H. sanguineus* and *E. depressus*, suggesting that these two species may be competing for similar food resources, whereas *P. herbstii* guts contained larger amounts of barnacle and mussel shell fragments indicating that they focus primarily on animal food items.

Key words: Asiatic shore crab, feeding preference, predator, Long Island Sound

INTRODUCTION

The Asiatic shore crab, *Hemigrapsus sanguineus*, is a relatively recent introduction to the east coast of the United States. It has been undergoing range expansion since its first recorded appearance in Cape May, New Jersey, in 1988 (Williams and McDermott 1990) and now has well-established breeding populations throughout Long Island Sound (Lohrer and Whitlatch 1997; Brousseau, pers. obs.). In western Long Island Sound, *H. sanguineus* is very abundant in rocky intertidal areas where substrates range from large boulders to sediments of rock, cobble and broken shell. It is reported to prefer the upper intertidal in Japan (Fukui 1988; Takada and Kikuchi 1991) but studies of its distribution along the coast of New Jersey (Celestino and Hales 1998) and in eastern Long Island Sound (Lohrer and Whitlatch 1997) indicate that it is more abundant in the lower half of the intertidal zone.

According to Lohrer and Whitlatch (1997) this species has been considered a strict herbivore by some, reported to feed exclusively on intertidal macroalgae. Recent information about the diet of this crab in introduced habitats along the Atlantic coast suggests that it is omnivorous, feeding on a variety of resident organisms, including macroalgae, salt marsh grasses (*Spartina* spp.), and small invertebrates (Lohrer and Whitlatch 1997). It is unclear, however, if this species is an active predator, since much of the information relating to its feeding ecology has come from gut content investigations,

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making it impossible to rule out incidental ingestion as the source of animal food items in its diet.

Nonindigenous species can cause ecological harm in introduced environments by preying on or competing with resident species for food and space (Elton 1958; Williamson 1996; Grosholz and Ruiz 1996). It is difficult to assess the potential impact of the Asiatic shore crab invasion because of the limited information available about the ecological requirements of the invader. However, some general characteristics of *H. sanguineus,* such as its broad distribution in the intertidal and its reported omnivorous feeding habits, suggest that with time it may alter the community structure of east coast intertidal environments.

To better understand the food preferences of the Asiatic shore crab in western Long Island Sound, we conducted a series of food-choice experiments in the laboratory. The results of macroalgae choice experiments and experiments designed to investigate possible predatory habits of the Asiatic shore crab are presented here. Those findings are compared with gut content data for three species of wild-caught crabs *(H. sanguineus, Eurypanopeus depressus,* and *Panopeus herbstii).*

Materials and Methods

Stomach Content Anaysis

Three species of crabs, *H. sanguineus, E. depressus,* and *P. herbstii,* were collected from the intertidal zone at two sites, Black Rock Harbor, Bridgeport, CT, and Burying Hill Beach, Westport, CT. Dates of collection, sample sizes, and carapace widths (CW) of the crabs are given in Table 1. Both sites consisted of boulder jetties with adjacent areas of small rocks and mud. The live crabs were returned to the laboratory and placed in a cold room until they were dissected (not longer than 24 hr). Stomachs were removed and the contents examined under dissecting and compound microscopes. Methods used for stomach content analyses are described in Ropes (1989).

Macroalgal Choice Experiments

Crabs to be used in the experiments were collected from Black Rock Harbor, Bridgeport, CT, and placed in individual 3.8-L aquaria on a running seawater table at the National Marine Fisheries Service Laboratory in Milford, CT. Four of the most common species of intertidal macroalgae, *Enteromorpha* spp. (green filamentous), *Ulva lactuca* (green sheet), *Fucus vesiculosus* (brown branched), and *Chondrus crispus* (red branched) were also collected from the same area.

Table 1. Collection dates, sample sizes (N), and carapace widths (CW) of the three species of crabs, *Hemigrapsus sanguineus, Eurypanopeus depressus,* and *Panopeus herbsti* used in the gut content analysis studies.

Crab species	Collection date	Sample size (N)	CW (mm) Mean	CW (mm) Range
H. sanguineus	April 1998	16	19.1	11.3 – 23.8
	May 1998	11	20.0	10.0 – 25.5
	June 1998	34	22.4	15.7 – 28.7
	September 1998	30	24.0	16.9 – 31.3
	October 1998	17	22.8	17.7 – 32.7
	November 1998	32	20.9	13.9 – 32.5
	December 1998	10	25.8	21.2 – 31.6
E. depressus	May 1998	5	16.2	13.9 – 20.3
	June 1998	6	19.2	15.6 – 21.7
	July 1998	8	25.6	18.7 – 29.4
	September 1998	13	21.0	18.3 – 28.6
	October 1998	10	22.7	15.9 – 19.2
	November 1998	28	21.0	18.3 – 25.2
	December 1998	7	20.7	19.3 – 21.8
P. herbstii	July 1998	17	31.2	22.8 – 43.4
	September 1998	4	25.9	18.4 – 41.2

In the laboratory, individually numbered "macroalgal turf tiles" were prepared by epoxying macroalgae to 4 cm × 9 cm pieces of plexiglass. Two turf tiles containing different species of macroalgae were placed with each crab. The aquaria were covered with nylon mesh to contain the crabs and prevent the escape of any pieces of macroalgae that might become unattached from the turf tile, and then were submerged in running seawater. A total of six pairwise choice experiments *(Enteromorpha* vs. *Ulva, Enteromorpha* vs. *Fucus, Enteromorpha* vs. *Chondrus, Ulva* vs. *Fucus, Ulva* vs. *Chondrus, Fucus* vs. *Chondrus*) with eight to ten replicates each were run. All crabs were starved for at least 24 hr before experimentation. Each experiment was terminated either after all macroalgae had been consumed from one turf tile, or after the experiment had run for at least ten days.

To determine the wet weight of macroalgae consumed, the turf tiles were blotted and weighed both before and after each experiment. Preweighed samples of each of the four species of macroalgae were oven-dried at 50°C and reweighed to determine the relationship between wet and dry weight. The follow-

Table 2. Stomach content analysis results for all wild-caught crabs collected with food in their stomachs during the study period (April 1998 through December 1998). Percent occurrence refers to the percent of crab stomachs containing the food item listed. NP = not present.

	Percent Occurrence		
Food Item	*H. sanguineus*	*E. depressus*	*P. herbstii*
Algae*			
Brown	58.0	31.7	36.4
Green	55.5	16.9	9.1
Red	11.0	NP	NP
Barnacles	24.3	39.1	40.9
Amphipods	0.8	NP	NP
Mussels	3.4	4.9	13.6
Worms	0.8	NP	NP
Spartina spp.	16.8	NP	9.1
Other†	16.0	29.9	4.5

* Algae includes all macroalgal species regardless of color or thallus form.
† Other = detrital, unidentified animal tissue.

ing equations were used to determine the dry weight amounts of macroalgae consumed:

Chondrus crispus: $Y = 6.6977e^{-3} + 0.15567\ X$ ($r^2 = 0.965$)
Enteromorpha spp.: $Y = 5.2210e^{-3} + 0.09398\ X$ ($r^2 = 0.957$)
Ulva lactuca: $Y = 1.3075e^{-2} + 0.10171\ X$ ($r^2 = 0.923$)
Fucus vesiculosus: $Y = 3.1063e^{-2} + 0.16124\ X$ ($r^2 = 0.858$).

Samples of fresh macroalgae were cleaned of macroscopic epibiota, rinsed in distilled water, oven-dried at 50°C for 48 hr, powdered, and analyzed in triplicate. Total carbon (C) and nitrogen(N) were determined using a Perkin-Elmer Series 2400 CHN analyzer.

Predation Experiments

Crabs were collected from Black Rock Harbor, Bridgeport, CT, and held in individual 3.8-L aquaria on a running seawater table until experimentation began. Hatchery-reared *Crassostrea virginica* (oyster) and *Mercenaria mercenaria* (hard-shell clam) were obtained from F.M. Flower & Sons, Bayville, NY, and hatchery-reared *Mya arenaria* (soft-shell clam) were supplied by the Beals Island Regional Shellfish Hatchery, Brewer, ME, and are indicated as hatchery-reared (H). Wild *C. virginica* seed were collected at Southport Beach, Fairfield, CT, and wild *Mytilus edulis* (blue mussel) seed were collected at Pebble Beach, Rockport, MA, and are indicated as wild-caught (W).

In the laboratory, five experiments with eight to ten replicates each were conducted to determine if the Asiatic shore crab is a bivalve-seed predator. *M. mercenaria* (H), *Mya arenaria* (H), *C. virginica* (H), and *Crassostrea virginica* (W) seed were placed in gravel at the bottom of the test aquaria and *Mytilus edulis* (W) seed were epoxied to plexiglass tiles before being introduced to the crab. No experiment ran for longer than 6 d but some were terminated sooner if all the prey had been consumed. At the end of the experiment, the numbers of live prey remaining were counted and the stomachs were removed from each crab and analyzed for stomach contents.

Results

Stomach Content Analysis

Gut content analyses revealed that the wild-caught crabs ate a variety of food items (Table 2). Macroalgae were found to be a more common food item in *H. sanguineus* than in either of the mud crabs; the majority of the Asiatic shore crabs examined had either brown or green macroalgae in their guts. Red macroalgae was present in only 11% of the *H. sanguineus* stomachs and did not appear to be an important food item for either species of mud crab. *H. sanguineus* also consume barnacles and mussels, but such prey appear to be more important in the diet of mud crabs, especially *P. herbstii*, in which almost half of the stomachs of the wild-caught crabs contained barnacle fragments.

Macroalgal Choice Experiments

Results of the macroalgae choice experiments are shown in Figures 1 and 2. Preference for a particular species of macroalgae was based on the amount (wet or dry weight) of macroalgae consumed by *H. sanguineus* in each experiment. *Enteromorpha* spp. was preferred in three of the four pairings. It was preferred over *F. vesiculosus* by six of the eight crabs tested, and over *U. lactuca* by nine out of ten crabs, based on both wet- and dry-weight amounts. When paired with *C. crispus*, it was preferred five out of eight times based on wet-weight results and four out of eight times based on dry-weight amounts. *Chondrus crispus* was the clear favorite when paired with either *F. vesiculosus* or *U. lactuca* and *U. lactuca* was preferred only when paired with *F. vesiculosus*.

When the results of all pairings are combined, *H. sanguineus* shows a nearly equal preference for *Enteromorpha* spp. (76% based on wet weight; 73%

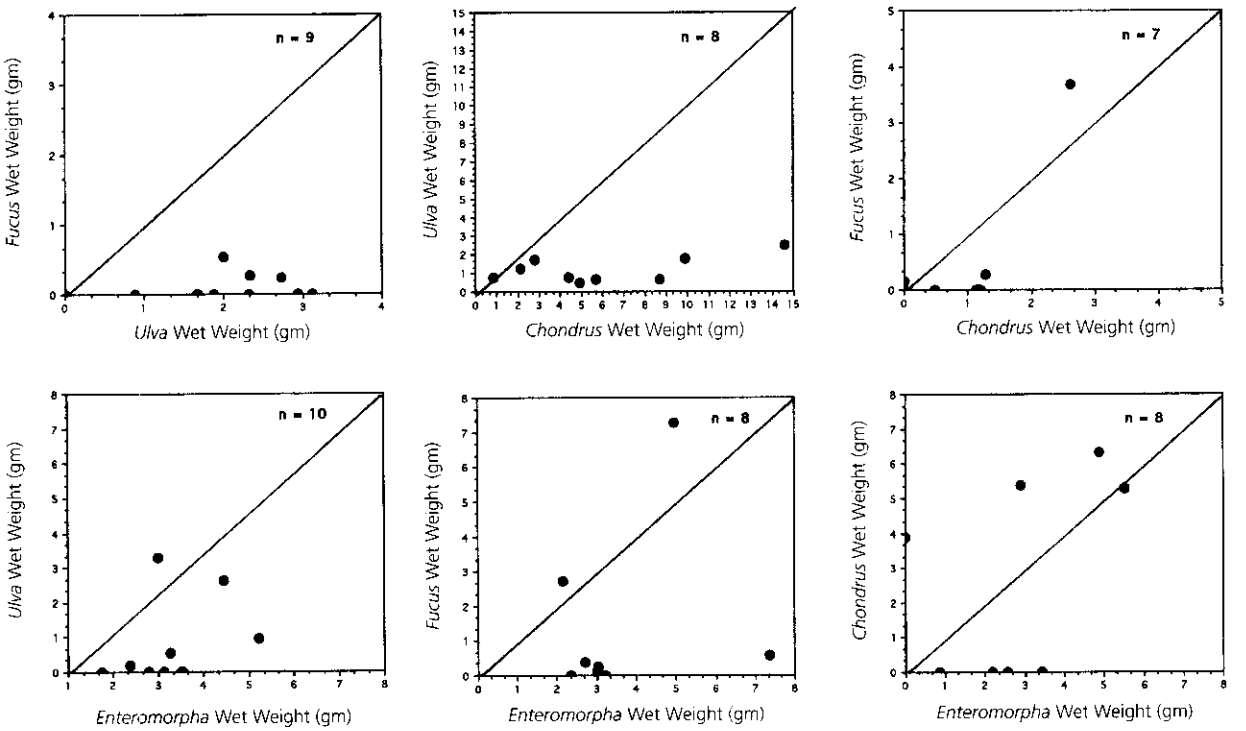

Figure 1. Graphs showing the total wet weight of macroalgae consumed in each of the pairwise choice experiments. Points falling below the line indicate a preference for the species on the x-axis, those falling above the line indicate a preference for the species on the y-axis. (n = sample size).

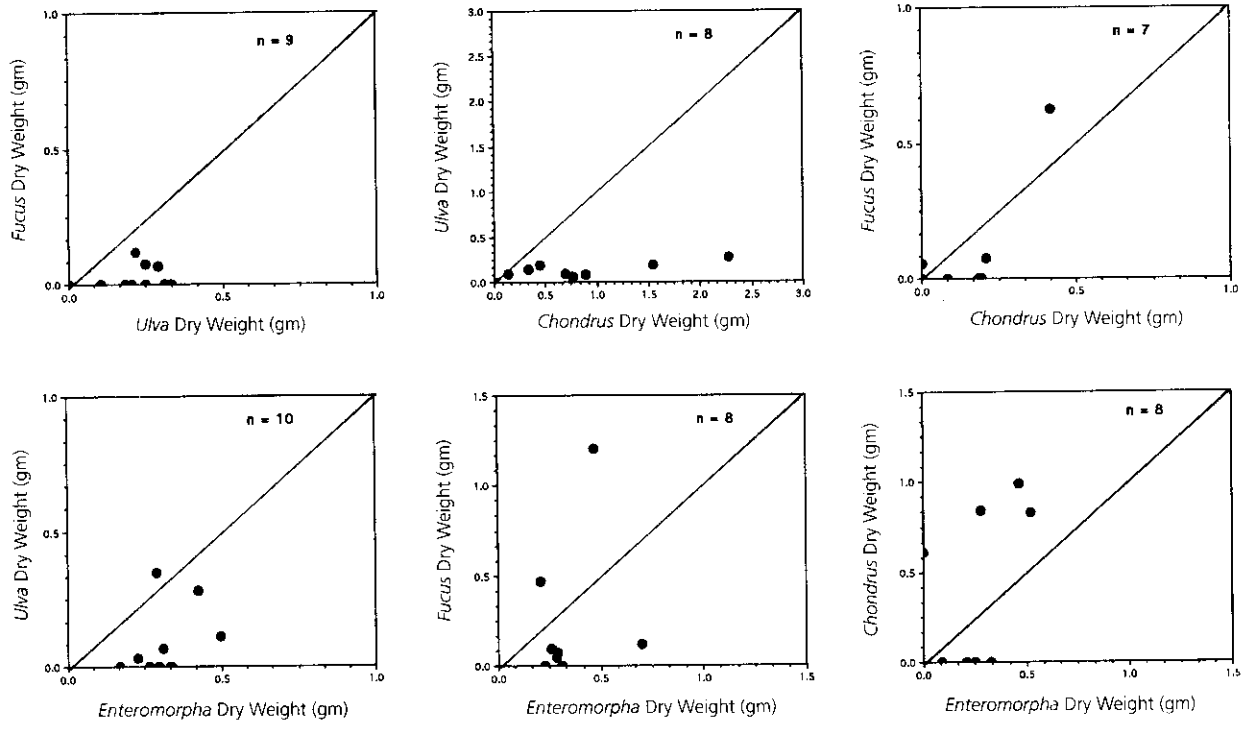

Figure 2. Graphs showing the total dry weight of macroalgae consumed in each of the pairwise choice experiments. Points falling below the line indicate a preference for the species on the x-axis, those falling above the line indicate a preference for the species on the y-axis. (n = sample size).

based on dry weight) and *C. crispus* (74% based on wet weight; 78% based on dry weight). Of the two remaining species, *U. lactuca* was preferred in only 37% of the tests, whereas *F. vesiculosus* was chosen preferentially only 14% of the time. Based on these results, the following preference hierarchy was established: *Enteromorpha* spp. = *C. crispus* > *U. lactuca* > *F. vesiculosus*.

Crab macroalgal consumption rates do not appear to be a function of crab size. Comparisons of the total wet and dry weights of macroalgae consumed versus carapace length did not show a significant trend (Figure 3: N = 53, $r^2 = 0.030$ wet weight; $r^2 = 0.083$ dry weight).

Mean tissue levels of %N were similar in all four species of macroalgae, but mean tissue levels of %C were significantly different in each of the species (ANOVA; $P<0.01$). The highest %C occurred in *F. vesiculosus* and the lowest in *Enteromorpha* spp.

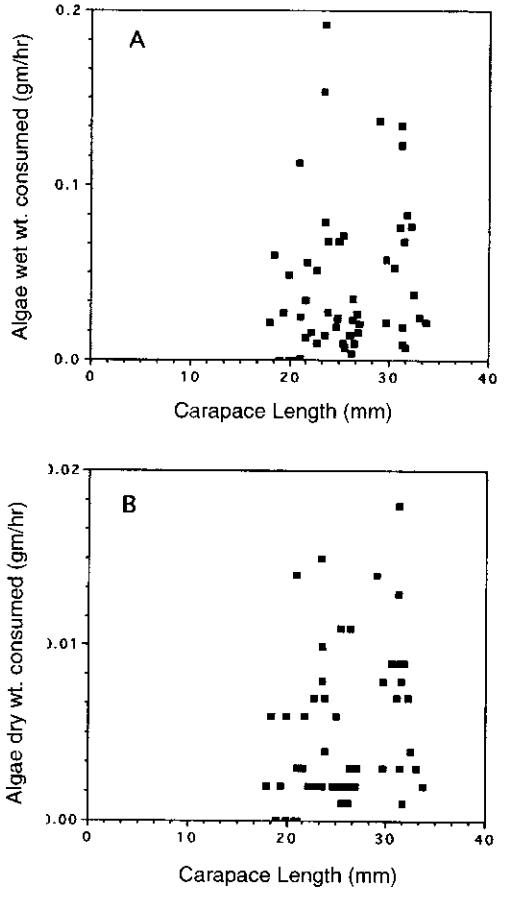

Figure 3. Relationship between crab carapace length (CL) and the total amount of macroalgae consumed per hour in the choice experiments (CL not converted to CW).

Table 3. Tissue levels (% dry weight) of carbon (C) and nitrogen (N) and molar C:N ratios in four species of macroalgae, *Chondrus crispus*, *Enteromorpha* spp., *Fucus vesiculosus*, and *Ulva lactuca*, collected from Black Rock Harbor, Bridgeport, CT.

Species	% C Mean±S.E.)	%N (Mean±S.E.)	C:N (Mean±S.E.)
Enteromorpha spp.	23.50 ± 0.80	3.96 ± 0.40	7.09 ± 0.90
Fucus vesiculosus	37.57 ± 0.30	4.41 ± 1.00	10.93 ± 2.30
Chondrus crispus	30.53 ± 0.00	6.04 ± 0.60	5.99 ± 0.50
Ulva lactuca	33.23 ± 0.10	6.09 ± 0.70	6.59 ± 0.90

Table 4. Percent of *Hemigrapsus sanguineus* preying on *Crassostrea virginica*, *Mercenaria mercenaria*, *Mya arenaria*, and *Mytilus edulis* for each of the predation experiments. Numbers of crabs tested (N) and prey shell length (mm) are given.

Prey species	N	Prey shell length (mm)	% Predators
Crassostrea virginica (H)	9	8.0 - 17.0	89
Crassostrea virginica (W)	8	10.0 - 20.0	0
Mercenaria mercenaria (H)	8	3.0 - 5.0	75
Mya arenaria (H)	10	6.0 - 9.0	90
Mytilus edulis (W)	10	3.0 - 15.0	100

H = Hatchery seed, W = Wild set.

The higher C:N ratios in *F. vesiculosus* are probably due to the high amounts of tissue C (Table 3).

Predation experiments

In the laboratory, *H. sanguineus* preyed on all species of bivalves tested, but predation was limited to hatchery-reared seed in the case of *C. virginica* (Table 4). In the experimental groups where at least some predation occurred (*C. virginica* (H), *M. mercenaria* (H), *M. arenaria* (H), and *M. edulis* (W)), at least 75% of all crabs tested were found to prey on bivalve seed, and 100% of the crabs fed *M. edulis* (W) ate them.

The number of bivalve seed consumed varied among crabs (Table 5). Among the crab predators, nearly all ate at least 50% of the prey presented, and in most cases more than 90% of the prey were eaten. Of four cases where 0–4% of the prey were consumed, one crab was a gravid female and two had molted during the test period.

Stomach analyses of the crabs exhibiting predation revealed that many crabs contained few or no shell fragments, despite being fed a diet exclusively

composed of bivalve seed (Table 6). In fact, 56% of the crabs had empty stomachs and fully three-quarters of the stomachs were either empty or contained fewer than five shell fragments.

Discussion

Macroalgal Food Preferences

Hemigrapsus sanguineus has been known to include large amounts of macroalgae in its diet (McDermott 1991; Lohrer and Whitlatch 1997). According to Lohrer and Whitlach (1997), this species has even been considered by some to be a strict herbivore, feeding exclusively on intertidal macroalgae. In this study, *H. sanguineus* overwhelmingly preferred the green alga, *Enteromorpha* spp., and the red alga, *C. crispus,* over either the green alga, *U. lactuca,* or the brown alga, *F. vesiculosus*. These four species of macroalgae are among the most abundant of the intertidal algae in western Long Island Sound from spring to fall (Taylor 1962), the period when the crabs are actively feeding.

Many macroalgae on rocky shores have evolved a variety of physical and chemical defenses against herbivores (Duffy and Hay 1990). Fucoids produce phenolic compounds (Lobban and Harrison 1994), substances known to deter invertebrate herbivores (Geiselman and McConnell 1981). It is not surprising then, that *H. sanguineus* avoided *F. vesiculosus*, preferring instead *Enteromorpha* and *Chondrus*, species which lack secondary metabolites and calcification and have been shown to be highly preferred by other crustaceans (Shacklock and Croft 1981) and herbivorous fish, *Zebrasoma flavescens* (Wylie and Paul 1988).

The nutrient content of algae also varies among algal species. The most nitrogen-poor alga among the four species tested was *F. vesiculosus*; C:N ratios were similar in the other three species. This finding is consistent with previous reports that phenol production and N availability are inversely related in *F. vesiculosus* (Ilvessalo and Tuomi 1989). In general, however, algae have been shown to be an inadequate source of N for many herbivorous animals (Mattson 1980; Wolcott and O'Connor 1992). Some herbivorous crabs have been shown to supplement their diets with animal protein, thereby enhancing growth (Wolcott and Wolcott 1984; O'Connor 1992).

The physical ability of the crab to eat certain algae appears to be among the most important factors in determining food choice. Female *H. sanguineus* have narrow, elongate, monomorphic claws, whereas the male chelae are generally more robust with different dentition patterns. Nonetheless, in both sexes the claws have blunt tips that abut and appear well adapted for removing filaments off hard substrates. *H. sanguineus* feeds on algae by picking it from the rock surface (Brousseau, pers. obs.). The filamentous *Enteromorpha* spp. and the highly branched *C. crispus* are algal types that can probably be easily handled by *H. sanguineus*. Large foliose forms such as *U. lactuca*, on the other hand, may be too difficult for the crab to manipulate.

Despite the fact that *H. sanguineus* exhibited an overwhelming preference for *Enteromorpha* spp. and *C. crispus* over *F. vesiculosus* in the lab, brown and green algae food items were found to be equally abundant in the stomachs of the wild-caught crabs. The avoidance of *Fucus* in the laboratory suggests

Table 5. Results of predation experiments showing the percent of *Crassostrea virginica* (W), *C. virginica* (H), *Mercenaria mercenaria* (H), *Mya arenaria* (H), and *Mytilus edulis* (W) consumed by *Hemigrapsus sanguineus* in each replicate. Mean crab carapace width (CW), range of carapace widths and number of prey tested per crab are given.

Species	# of Crabs	CW (mm) Mean±S.E.	CW Range (mm)	# Prey/ Crab	% Prey eaten (Mean±S.E.)
Crassostrea virginica (W)	8	25.4 ± 0.6	23.5 – 27.8	25	0
Crassostrea virginica (H)	9	29.0 ± 1.2	23.9 – 34.7	25	87.1 ± 11.0
Mercenaria mercenaria (H)	8†*	28.5 ± 1.8	21.1 – 35.7	75	65.8 ± 14.6
Mya arenaria (H)	10†	27.5 ± 0.9	23.3 – 32.6	50	87.4 ± 9.5
Mytilus edulis (W)	10†	25.2 ± 0.8	21.5 – 30.0	100	87.8 ± 5.5

† 1 Crab molted during experiment.
* 1 Gravid female among sample.

Table 6. Results of a stomach content analysis of *H. sanguineus* used in the bivalve predation experiments. Categories of stomach content include: (1) no shell fragments, (2) < 5 shell fragments, and (3) > 5 shell fragments.

Species	Percent 1	2	3
Crassostrea virginica (H)	100.0	0.0	0.0
Mercenaria mercenaria (H)	75.0	25.0	0.0
Mya arenaria (H)	40.0	40.0	20.0
Mytilus edulis (W)	11.1	22.2	67.7
$\bar{X}$ =	55.6	22.2	22.2

that species of brown macroalgae other than *F. vesiculosus* are being utilized in the field. More work is needed to determine patterns of brown algae utilization by the Asiatic shore crab in order to better assess the importance of this group in the overall diet of this crab.

The degree to which *H. sanguineus* is competing with two co-occuring species of mud crabs (Xanthidae) is difficult to evaluate. Gut content analyses indicate that all three species appear to be eating the same food items (Table 2), but they are eating them in different proportions. Caution must be applied when using stomach content analyses to determine diet, however, since food in an advanced stage of digestion is often unidentifiable and there is always the possiblity that some food items may have been incidentally ingested. Nevertheless, preliminary data suggest a greater dietary overlap exists between *H. sanguineus* and *E. depressus* than between *H. sanguineus* and *P. herbstii*.

Bivalve Seed Predation

Hemigrapsus sanguineus has been known to include large amounts of macroalgae in its diet (see above discussion). Despite the fact that feeding studies have shown that macroalgae consistently make up the largest component of the diet of the Asiatic shore crab, the experimental results presented here support previous reports that *H. sanguineus* is also a predator on blue mussels (Lohrer and Whitlatch 1997) and that it will eat the seed (juveniles) of a variety of bivalves including *C. virginica*, *M. mercenaria*, and *M. arenaria*.

Although crabs that feed on hard-shelled molluscs usually possess dimorphic claws, with one powerful crusher claw to open the prey and one smaller cutter claw, sometimes complex shell-opening behavior may compensate for limited crushing ability. Lau (1987) divides decapod predatory tactics into three categories: (1) those for which a claw is not required, *e.g.*, swallowing the prey whole or chipping or biting the edges of the shell using the mandibles, (2) those which involve wedging open the operculum or shell valves, leaving the shells intact, and (3) those which require a claw for crushing, chipping, or peeling the edges of the shell.

The Asiatic shore crab, which lacks the characteristic crusher claw common to specialized molluscivores, likely falls into Lau's category 3. The scarcity of shell fragments in the stomachs of the predatory crabs suggests that they do not swallow their prey whole or use feeding mouthparts to grind open the shells of their prey. Residues of chipped and broken shell fragments in the experimental aquaria at the end of the experiment rule out category 2, and suggest that *H. sanguineus* manipulates and opens bivalve food items by crushing the umbo or chipping the edge by using its non-specialized claws. Because it lacks a well-developed crusher claw, however, it is likely that it may be limited to eating relatively small, thin-shelled prey. Limitations imposed by shell thickness may be one reason why the crabs ate thin-shelled hatchery-reared oysters, but rejected the wild oyster seed.

Estimated Ideal Mechanical Advantage (IMA) values for the claws of this crab (McDermott 1999) further support the conclusion that although unspecialized, the chelae of the Asiatic crab can exert sufficient force to chip/crush the shells of small bivalves. These values fall within the range reported for other molluscivorous crabs (Seed and Hughes 1995) and are similar to those reported for two congeneric species, *H. nudus* and *H. oregonensis* (Behrens Yamada and Boulding 1998). The greater IMA, heavier teeth, and more robust claws of male *H. sanguineus* imply that they may be better adapted for crushing mollusc prey than are the females, and could be utilizing prey of a different size range. We are currently investigating the degree to which this type of resource partitioning could be occurring within populations of this crab.

The predatory behavior used by these crabs also makes the use of gut content analyses for estimating the importance of shelled food items such as molluscs and crustaceans in the diet unreliable. Shell fragments are needed for positive prey species identification, since partially digested animal soft tissue cannot be identified. Fully 75% of the crabs that ate bivalves in the laboratory had five or fewer shell fragments in their stomachs, and over 50% had no shell fragments at all, despite feeding exclusively on bivalves. Caution must therefore be used when interpreting the results of stomach content analyses of *H. sanguineus* because the significance of bivalve food items in the diet of this crab might be underestimated.

The impact of the Asiatic shore crab on resident intertidal bivalve stocks along the east coast remains to be determined. Past experience with crab predators (*e.g.*, green crab predation on soft-shell clams) has shown that they can have a devastating effect on

shellfish stocks by depleting the numbers of surface-dwelling juveniles in the population (Glude 1955; Griffiths *et al.* 1992). Blue mussel populations that co-occur with *H. sanguineus* in rocky intertidal habitats or along boulder jetties are probably most threatened; however, these crabs also frequent soft-sediment environments in Japan (Sakai 1976). It is very likely that they may forage for food on tidal flats during high tide, thereby placing juvenile soft-shell and hard-shell clams at risk. In addition to possible impacts on natural bivalve populations, larval crab settlement into shellfish growout trays could result in seed predation problems for the aquaculture industry as well. More information is needed about foraging behavior and food selection in the field before an accurate assessment of the impact of this invader can be made.

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Potential Impact of the Introduced Asian Shore Crab, *Hemigrapsus sanguineus*, in Northern New England: Diet, Feeding Preferences, and Overlap with the Green Crab, *Carcinus maenas*

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ABSTRACT: **The Asian shore crab, *Hemigrapsus sanguineus*, is currently established in Massachusetts and four specimens have been found in New Hampshire. To predict the potential impacts of an invasion by *H. sanguineus* in northern New England, various aspects of its feeding ecology were examined. Stomach contents of crabs collected from Massachusetts indicate a primarily herbivorous diet, with some evidence of omnivory. Investigation of the algal feeding preferences of *H. sanguineus* elucidated which species are most likely to be negatively impacted due to herbivory by this crab. *H. sanguineus* prefers *Enteromorpha intestinalis* in multichoice feeding preference trials, but will consume all species tested when presented in isolation. Feeding preferences of both *H. sanguineus* and *Carcinus maenas* (another introduced crab) on molluscs were assessed to determine potential competitive overlap. Both crab species preferred *Mytilus edulis, Littorina obtusata* and *L. saxatilis* (in descending order) and avoided *L. littorea*. The pre-invasion community structure of two sites that are likely to be impacted by populations of *H. sanguineus* was also examined. Quantitative sampling documented the abundance of potential prey (algae, molluscs, barnacles) and potential competitors (*C. maenas*). Models are presented that illustrate predicted consequences of this crab's invasion into northern New England.**

Keywords: *Hemigrapsus sanguineus, Carcinus maenas,* feeding preference, stomach content, community structure

INTRODUCTION

Human impacts on marine ecosystems are increasing in frequency and severity. Declining habitat quality and associated biodiversity often accompany human-mediated species introductions. The New England intertidal zone has been impacted by several conspicuous species introductions including the common periwinkle, *Littorina littorea,* and the green crab, *Carcinus maenas*. The ecology of the New England intertidal zone is well known (Menge 1976; Lubchenco 1978, 1983; Lubchenco and Menge 1978; Bertness 1984; Mathieson *et al.* 1991), which makes it an ideal system in which to examine the impacts of introduced species. Previous studies have described the role of introduced species in New England (*C. maenas*: Glude 1955; Ropes 1968; Vermeij 1982; Seeley 1986, *L. littorea*: Lubchenco 1978; Bertness 1984), but no studies have documented what the community was like before an introduction occurred. Another introduced crab species that is likely to become established in northern New England within a short period of time is *Hemigrapsus sanguineus*.

H. sanguineus, a small intertidal crab native to Asia, was first documented in New Jersey in 1988 (Williams and McDermott 1990). Within less than 10 years, its range expanded northwards to the north side of Cape Cod and south to North Carolina (McDermott 1998a). This crab tolerates a wide range of environmental conditions, and it is likely to continue to expand its range to include New Hampshire and Maine (McDermott 1998a). In October 1998, the first specimen of *H. sanguineus* in New Hampshire was found by John McDermott at Hampton Harbor (J.J. McDermott, pers. comm.) and the following year we found four additional *H. sanguineus* at Dover Point (an estuarine site) and Rye Harbor.

The overall objective of this research is to determine potential impacts of establishment of *H. sanguineus* in northern New England. The specific focus of this project was to assess the feeding ecology of *H. sanguineus* in order to predict changes in intertidal community structure that may result from predation by this crab. In addition, some aspects of the feeding ecology of the European green crab, *Carcinus maenas*, were also examined to assess potential competitive

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overlap between these two introduced crab species. In northern New England, *C. maenas* is the only crab that is typically found in the rocky intertidal zone; two native crab species, the rock crab, *Cancer irroratus,* and the Jonah crab, *C. borealis*, are primarily found in the subtidal zone (Gibeault 1995).

Several different approaches were used to assess the feeding ecology of *H. sanguineus*. The diet of *H. sanguineus* from Quissett (Falmouth), Massachusetts (41°32' N, 70°39' W), a location where it is already well established, was analyzed to provide some indication of its potential diet if it becomes established in northern New England. The general dietary trends were inferred from the stomach contents of crabs collected during fall and spring. The stomach contents from the fall collection were also compared to the composition of the specific community to assess the degree to which *H. sanguineus* selects its food based on its availability.

The feeding preferences of *H. sanguineus* for several common algal and mollusc species of northern New England were assessed to predict which species are most likely to experience increased predation pressure if this crab becomes established in this region. In addition, the mollusc feeding preferences of *C. maenas* were also assessed to determine potential competitive overlap between these two introduced crab species. The intertidal community structure of two New Hampshire locations that are likely to be inhabited by *H. sanguineus* was also documented. The densities and size distributions of potential prey and competitors of *H. sanguineus* were obtained to provide baseline information on the pre-invasion (by *H. sanguineus*) community. Finally, information from laboratory feeding experiments and field observations was synthesized into predictions of the impact of *H. sanguineus* on rocky intertidal community structure in northern New England. Two models are presented which detail some of the potential impacts of this crab on community structure and on its main potential competitor, *C. maenas*.

Materials and Methods

Stomach Contents

Field Collections

H. sanguineus used for stomach content analysis were collected from throughout the intertidal zone of a sheltered cobble shore in Quissett, MA. This site is close to Woods Hole, MA where *H. sanguineus* has been established since 1992 (McDermott 1998a). Because *H. sanguineus* has been established in this region for several years and is present in high densities at this site (at least 25 m^{-2}; M.C. Tyrrell, pers. obs.), it is likely that the impact of this crab has affected the community.

Two collections of crabs were made from throughout the intertidal zone, one in October 1996 and one in April 1997. Mature male *H. sanguineus* have much larger chelipeds than females. McDermott (1998b) documented sex-related differences in consumption of prey by *H. sanguineus*; a larger size range of mussels was consumed by males. We collected only adult male *H. sanguineus* because they have the greatest potential for feeding on the widest range of food items. For both collection periods, approximately 35 large (15-28 mm carapace width (CW)) male *H. sanguineus* were fixed in the field. In October, the carapace of each crab was pierced and the whole crab was placed in either a buffered formalin solution or alcohol; in April, the crabs were injected with a syringe containing formalin and the body was placed in alcohol. There were no observable differences in the condition of the stomach contents between the alcohol or formalin preservation methods.

To document general patterns of community composition, color slides of 32 0.06-m2 quadrats were taken at the collection site 5 da after the October collection. The quadrat was placed at the low-water mark and photographed in 0.5-m increments up to the upper limit of the intertidal zone. Slides were analyzed by identifying the organism or substrate under 25 randomly placed points. Using photographic slides to determine community composition is useful to describe general patterns of relative abundance of conspicuous species but is not suitable to document small species and those that occur in cryptic habitats (*i.e.*, in crevices or under rocks). We felt that the community sampling was not detailed enough to justify applying quantitative techniques (*i.e.*, electivity indices) to compare the community composition and the stomach contents of *H. sanguineus*.

Laboratory Analyses

Williams (1981) examined the gut contents of four species of portunid crabs and suggested that a sample size of 30 gastric mills that are >50% full is sufficient to describe the natural diet of crabs. The cardiac stomach was extracted from *H. sanguineus* and only those stomachs that were >50% full were

included in the analysis. The percent composition of different foods was determined by spreading the stomach contents evenly on a 50-point grid and using a dissecting microscope to identify food items on each point.

Feeding Preferences and Rates

Algae

Five algal species, *Ascophyllum nodosum, Fucus spiralis, F. vesiculosus, Enteromorpha intestinalis*, and *Mastocarpus stellatus*, were collected from Newcastle, New Hampshire for use in algal preference and feeding rate trials. These algal species were chosen for feeding trials because they are some of the most commonly encountered species on sheltered rocky coasts in northern New England (Menge 1976; Lubchenco 1983; Mathieson *et al.* 1991; Tyrrell 1999). Algae were spun in a salad spinner to remove excess water (30 rotations, approx. 1 rotation per sec) and equal amounts (2 +/- 0.1 g damp weight) of all five algal species were then placed into a small round container (17 cm diam, 12 cm height) with approximately 1.5 L of sea water. This experiment was performed in two separate trials, in January and April 1998. Ten containers were prepared for the January trials and 16 containers were used for the April trials.

Only adult male *H. sanguineus* (20–28 mm CW) were utilized to minimize differences in preference due to sex and age of crabs. Experimental animals were starved for 24–48 hr before each feeding trial, and individual crabs were not used for more than one algal feeding preference trial. Crabs were randomly placed into half of the containers. Control containers had algae but not crabs and served to account for autogenic changes in weight of algae. Containers were kept covered and in a dark temperature control room (15°C) to minimize algal growth and to encourage the crabs to eat (*H. sanguineus* forage most actively at night, Lohrer and Whitlatch 1997). After 12 hr, crabs were removed from experimental treatments, and algae in both experimental and control containers were separated by species, spun in a salad spinner and weighed.

The change in weight of each algal species was calculated by subtracting the weight at 12 hr from initial weight. Changes in weight that occurred in controls (autogenic) were compared to mean changes in weight in experimental containers (autogenic and herbivory) to determine the effect of herbivory by the crabs. Results were analyzed using a split plot design ANOVA with experiment (January or April) as a blocking factor in Systat 5.2 (SPSS, Inc.).

To compare differences in feeding rates on various species of algae in multichoice trials and in single-species trials, feeding rates of *H. sanguineus* were examined with only one species of algae in the container at a time. The same procedure was followed for feeding rate trials as in the multichoice trials, but this time there were only 2 +/-0.1 g (damp weight) of algae in the containers (equivalent to the amount of each species in the multichoice preference experiments). For each species of algae, a total of 20 containers were prepared (10 with crabs and 10 without crabs). The change in weight of each algal species was calculated by subtracting the weight at 12 hr from initial weight. A non-parametric Welch's t-test was used to analyze the differences in weight change between experimental and control containers after 12 hr.

Molluscs

In order to reduce variability due to ability to obtain prey, only adult male *H. sanguineus* were utilized in feeding preference trials because male *H. sanguineus* have much larger chelae than do females. The CW of *H. sanguineus* used in experimental trials ranged between 20 and 27 mm. It was difficult to obtain sufficient numbers of *C. maenas* for feeding preference experiments; therefore, a larger size range of CW (21–51 mm), and both male and female *C. maenas* were utilized. All crabs were maintained on algal diets prior to experimentation and crabs were starved between trials. Each crab was given all possible paired combinations of prey species but was not used for the same combination of prey more than once. Each crab species was kept in separate aquaria to eliminate the possibility of convergence of feeding preferences between crab species due to chemosensory perception.

The gastropods, *Littorina littorea, L. obtusata*, and *L. saxatilis*, and the mussel, *Mytilus edulis*, were offered in all possible paired combinations to both species of crabs. To minimize differences in shell thickness due to exposure, gastropods were collected from only one site in Newcastle, NH. *M. edulis* were collected from a nearby fouling community.

Feeding capabilities. Prior to the experimental trials, individual crabs were given gastropods and mussels of varying sizes to determine their ability to consume different sizes of prey. These animals were not

used in subsequent mollusc feeding preference trials. Adults of both crab species demonstrated the ability to consume snails with a shell length as great as their CW minus 5 mm. For *M. edulis*, crabs were capable of preying on mussels with shell lengths up to 0.6 mm shorter than their CW.

Feeding preferences. Crabs were maintained in individual containers and pairs of prey were presented randomly. Prey sizes were matched as closely as possible (within 0.5-mm shell length for gastropods), but because both species of crabs have the ability to consume a large range of sizes of *M. edulis*, and because very small mussels were difficult to obtain, size ranges of mussels offered differed by as much as 5 mm from gastropods. In all cases, prey offered in feeding preference trials were well within size ranges available to the crab.

Containers were checked frequently (at least daily) for signs of predation (removal of the operculum in snails and/or consumption of all soft tissue) on either prey species. In almost all of the trials, the crab consumed the preferred prey within the first 24 hr. In the case of *M. edulis*, the crabs usually consumed these molluscs within the first hour of the experiment. When a crab had either wholly or partially consumed one of the prey species and left the other prey unharmed, it was considered to have indicated a preference between the prey species. There were at least 20 (range 20–25) successful trials for each prey species with *H. sanguineus* and 10 (range 10–13) with *C. maenas*. Contingency tables and a chi-square goodness-of-fit test were used to test each crab species' preference between prey. These contingency tables were then subdivided; high chi-square values were dropped and the analysis was repeated. To determine if feeding preferences of both crab species overlapped, a chi-square test of independence was utilized to test whether the distributions of feeding preferences were the same.

Pre-Invasion Community Structure

Two New Hampshire locations were examined for this pre-invasion community assessment: Ft. Stark in Newcastle and Odiorne State Park in Rye. Both sites are relatively sheltered with a thick fucoid canopy and *C. maenas* is abundant at both locations, indicating that each site has suitable habitat for intertidal crabs. The mean tidal range at these sites is about 2.6 m, with a spring tidal range of 3 m. Four tidal heights (0.5, 1.0, 1.5, and 2.0 m) above mean lower low water

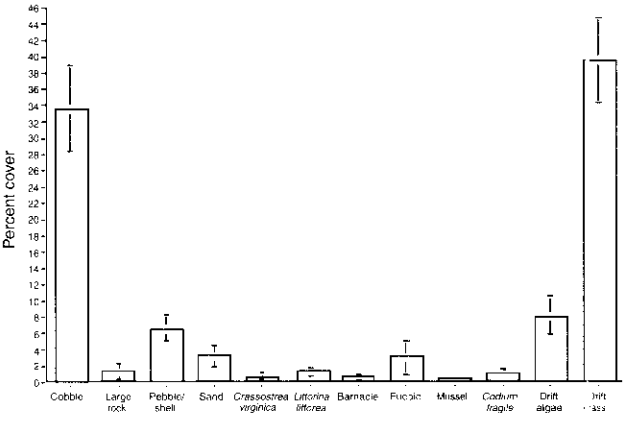

Figure 1. Mean percent cover and standard error of the community at Quissett, MA, where *Hemigrapsus sanguineus* were collected for stomach contents analysis. Drift grass, *Codium fragile*, ssp. *tomentosoides*, and drift algae were in the wrack line and represent an ephemeral, but important, part of the community at this site.

were determined using tidal data from Harbormaster (version 3, Zihua Software, Marlborough, Connecticut). At least ten (range 10–12) 0.25-m^2 quadrats were placed at random distances along a transect at each tidal height. Quadrat sampling took place from 20 August to 7 September 1997 at Fort Stark and from 14 to 30 September 1997 at Odiorne. There were no changes in patterns of abundance of any of the dominant species between August and September, but because high densities of *C. maenas* recruits were found in recruitment samplers at these locations at this time (M.C. Tyrrell, unpub. data), only *C. maenas* that were larger than 5-mm CW were included in analyses.

Canopy cover in the quadrat (mostly fucoid algae) was determined under 25 intersections of monofilament line. In areas where the algal canopy was incomplete, the type of substrate (rock bench, cobble, pebbles, etc.) was recorded. There was a consistently thick algal canopy at Ft. Stark. These algae were displaced outside the quadrat to obtain subcanopy (substrate and understory algae) data. Algal canopy cover at Odiorne was patchier than at Ft. Stark, and separate subcanopy data were not necessary to characterize the community. Densities and size of potential prey species were obtained (M.C. Tyrrell, unpubl. data). Because they occur in high densities, barnacles and mussels were counted in four 100-cm^2 squares, while numbers of gastropods (littorinids, *Nucella lapillus*, and the limpet *Notoacmea testudinalis*) were obtained from the entire quadrat. Crabs were also collected from the entire

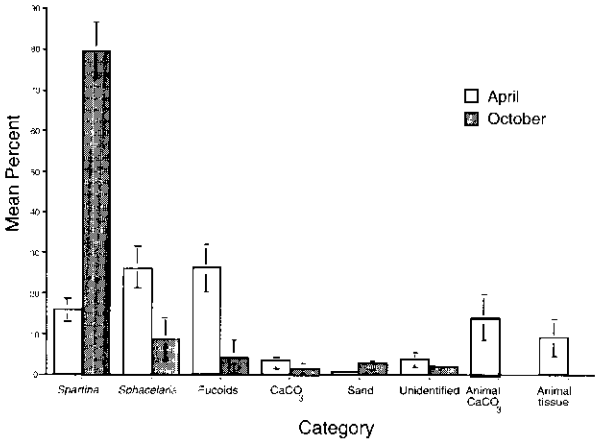

Figure 2. Mean percent composition and standard error of different categories of food found in the stomach contents *Hemigrapsus sanguineus* collected in October 1996 and April 1997 at Quissett, MA.

quadrat by overturning and sifting through all rocks and substrate by hand. Each crab was measured (CW) using dial calipers. Results from individual quadrats were pooled at each tidal height from each sampling location.

Results

Stomach Contents

Community Composition

In contrast to the rocky shores of New Hampshire, there was little live algae in the community at Quissett; drift grass and drift algae composed almost half of the community at the time of sampling (Figure 1). Most of the drift algae was fucoid with some red filamentous algae and the green alga, *Codium fragile* ssp. *tomentosoides*. It appears that there were low densities of juveniles of the common periwinkle, *L. littorea*, the mussels, *Geukensia demissa* and *M. edulis*, and the oyster, *Crassostrea virginica* (all of these potential prey visible in the slides were adults), but disruptive quantitative sampling would be necessary to verify this observation. The rock barnacle, *Semibalanus balanoides*, also potential prey for *H. sanguineus*, was found among the cobble substrate, but in low numbers (Figure 1).

Stomach Analysis

The cardiac stomach was dissected out of 62 crabs, but only 20 from the October collections and 16 from the April collections contained sufficient amounts of food for analysis. Evidence of a primarily herbivorous diet was found for crabs collected in both April and October. The diet of *H. sanguineus* in October was heavily dominated by *Spartina* spp. and algae (Figure 2). The availability of drift grass is reflected in the stomach contents of crabs collected at this time. In April, herbivory was still important: *Spartina* spp. and algae comprised 69% of the diet, and animals contributed 23% of the diet of these crabs (Figure 2). Much of the animal material was shell fragments and other hard parts representing the indigestible remains of different animal groups. A majority of the animal-derived calcium carbonate in April collections appeared to be fragments of barnacle test. Other recognizable animal material included a fish jawbone, barnacle exoskeletons, a polychaete cuticle, and bivalve shell fragments. Thick fibrous material, which may have been hydroid perisarc, constituted a majority of the animal tissue category.

Table 1. Feeding perferences of *Hemigrapsus sanguineus* for different algal species, based on multichoice experiments. Average change in wet weight (and standard error) of five algal species after 12 hours of herbivory by *H. sanguineus* or controls (autogenic changes in algae). Value in bold is statistically significant.

Algal species	Average wt. change: controls	Average wt. change: *H. sanguineus*	F-ratio	Consumption
Ascophyllum nodosum	0.01 (0.03)	−0.08 (0.04)	2.71	P=0.114
Enteromorpha intestinalis	0.07 (0.04)	−0.49 (0.11)	19.42	**P<0.001**
Fucus spiralis	0.08 (0.02)	0.04 (0.03)	0.95	p=0.341
Fucus vesiculosus	0.07 (0.02)	0.01 (0.03)	1.86	P=0.187
Mastocarpus stellatus	0.06 (0.01)	0.03 (0.02)	0.75	P=0.396

Table 2. Feeding rates of *Hemigrapsus sanguineus* on different algal species, based on single-species experiments. Average change in wet weight (and standard error) of algae after 12 hours of herbivory by *H. sanguineus* or controls (autogenic changes in algae). Values in bold are statistically significant.

Algal species	Average wt. change: controls	Average wt. change: *H. sanguineus*	Consumption
Ascophyllum nodosum	0.11 (0.01)	−0.15 (0.08)	**t=3.3203, P=0.0089**
Enteromorpha intestinalis	0.53 (0.08)	−0.21 (0.13)	**t=4.9920, P=0.0002**
Fucus spiralis	0.20 (0.03)	0.04 (0.05)	**t=2.7212, P=0.0151**
Fucus vesiculosus	0.14 (0.01)	−0.07 (0.04)	**t=4.7158, P=0.0006**
Mastocarpus stellatus	0.09 (0.01)	−0.12 (0.03)	**t=7.3949, P<0.0001**

Feeding Preferences and Rates

Algae

Results of multichoice algal preference tests indicate that *H. sanguineus* strongly prefers some algal

Table 3A. Chi-square goodness-of-fit for mollusc feeding preferences of *Hemigrapsus sanguineus*. Values in bold indicate statistically significant preferences among prey species.

	Mytilus edulis	*L. obtusata*	*L. littorea*	*L. saxatilis*	Overall
Chi-square value	24.30000	1.6333	28.0333	0.8333	54.8000
Degrees of freedom					3
P value					**<0.0001**
Preference between *M. edulis*, *L. obtusata*, and *L. saxatilis*					
Chi-square value	26.7667				
Degrees of freedom	2				
P value	**<0.0001**				
Preference between *L.obtusata* and *L. saxatilis*					
Chi-square value	2.4667				
Degrees of freedom	1				
P values	**0.1163**				

Table 3B. Chi-square goodness-of-fit for mollusc feeding preferences of *Carcinus maenas*. Values in bold indicate statistically significant preferences among prey species.

	Mytilus edulis	*L. obtusata*	*L. littorea*	*L. saxatilis*	Overall
Chi-square value	6.2336	0.2008	8.2992	0.0041	14.7377
Degrees of freedom					3
P value					**0.0021**
Preference between *M. edulis*, *L obtusata*, and *L. saxatilis*					
Chi-square value	6.4385				
Degrees of freedom	2				
P value	**0.0400**				
Preference between *L. obtusata* and *L. saxatilis*					
Chi-square value	0.2049				
Degrees of freedom	1				
P values	**0.6508**				

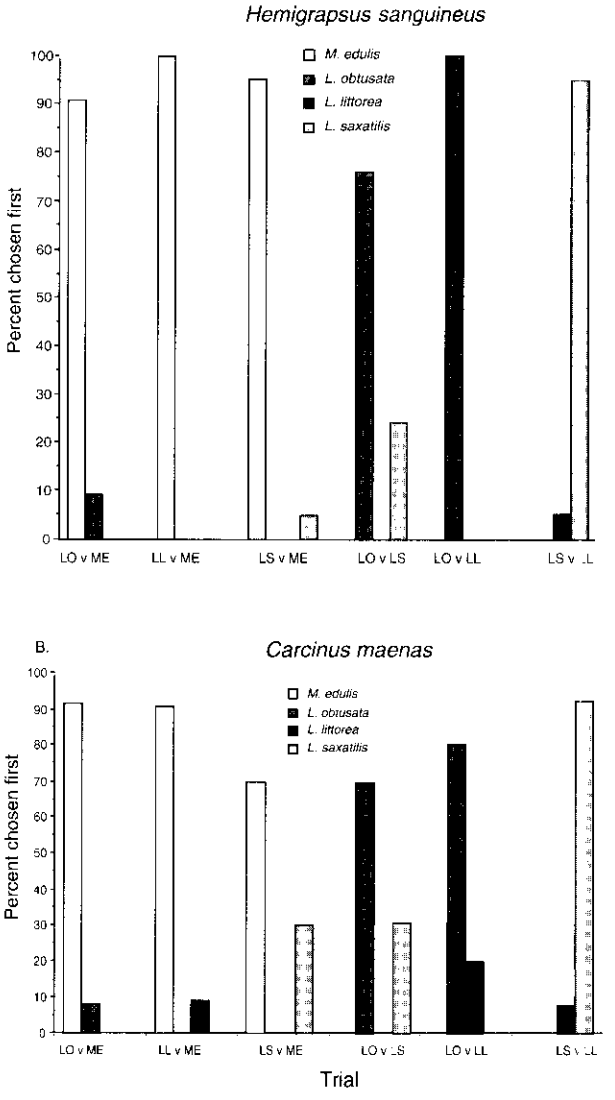

Figure 3. Percentage a molluscan prey species was chosen over another in a feeding preference trial with *Hemigrapsus sanguineus* (A) and *Carcinus maenas* (B). Prey species were *Mytilus edulis*, *Littorina obtusata*, *L. littorea*, and *L. saxatilis*.

species over others. Preference for *E. intestinalis* compared to other algal species was obvious; crabs would begin eating this species immediately upon being placed in the container. In addition, blades of *F. vesiculosus* also appeared to have been nibbled by crabs. Weight of algae in control containers increased for all species over time, perhaps due to absorption of water. In general, algae in containers with crabs either lost weight over time or gained less weight than algae in control containers. Weight loss in experimental containers over time was statistically significant for *E. intestinalis* (Table 1).

In single-species trials, weight change due to consumption was significantly higher than weight change in control containers for all algal species (Table 2). This result indicates that each of these species is a potential, if not preferred, food for *H. sanguineus*.

Molluscs

Both *C. maenas* and *H. sanguineus* expressed preferences for some prey species over others, and these preferences were consistent between different pairings of prey (Figure 3). There are strong overlaps in feeding preferences between the two crabs: *M. edulis* was strongly preferred by both crab species in all trials, followed by *L. obtusata* and *L. saxatilis*. *L. littorea* was consistently avoided by *H. sanguineus* (Figure 3A). *C. maenas* occasionally chose *L. littorea*

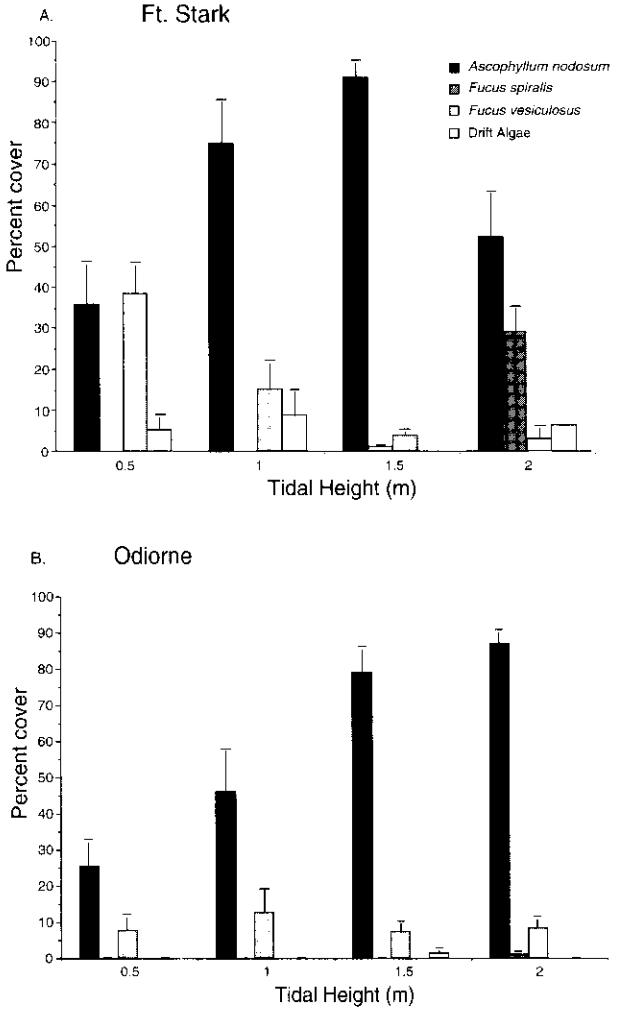

Figure 4. Mean percent cover and standard error of the algal canopy in 0.25-m^2 quadrats at Ft. Stark (A) and Odiorne, NH (B). Cover types represent major algal canopy species, drift algae is not identified to species because of its highly variable composition and abundance. Tidal height is meters above mean lower low water.

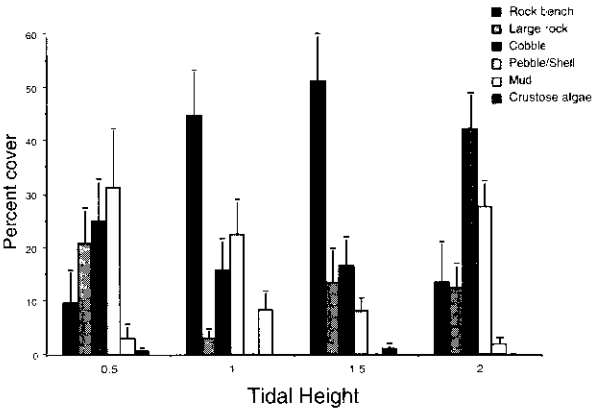

Figure 5. Mean percent cover and standard error of substrate types in 0.25-m^2 quadrats at Ft. Stark. Tidal height is meters above mean lower low water

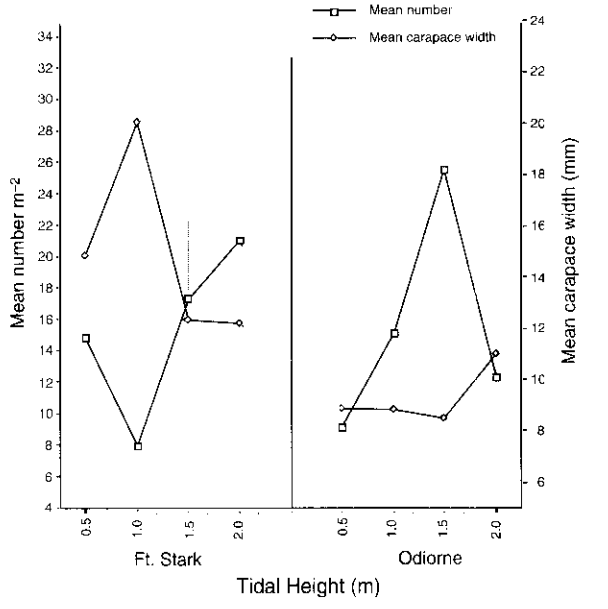

Figure 6. Population structure of *Carcinus maenas* at study sites.

over alternate prey, but its overall preference in all cases was for other prey species (Figure 3B).

Tables 3A and 3B show results of chi-square tests of goodness-of-fit for preferences among prey species. High chi-square values indicate either strong preference for or against a type of prey. The overall chi-square value for *H. sanguineus* is much higher than that of *C. maenas*, indicating that *H. sanguineus* was more consistent in its feeding preferences (Table 3A, B). Preferences between all prey species and between the three most preferred species (*M. edulis*, *L. obtusata*, and *L. saxatilis*) were significant for both *C. maenas* and *H. sanguineus*, which was due to avoidance of *L. littorea* and strong preference for *M. edulis* (Table 3A, B). Although both crabs preferred *L. obtusata* over *L. saxatilis*, this preference was not statistically significant for either crab species (Table 3A, B). Results of the chi-square test of independence indicate that there is no significant difference in feeding preferences between *C. maenas* and *H. sanguineus* (P= 0.135).

Pre-Invasion Community Structure

Figure 4 illustrates the mean percent cover for different types of algal canopy cover at both sampling locations at four tidal heights. All algae that compose the canopy at these sites are potential food for *H. sanguineus*. *A. nodosum* constitutes the majority of the canopy cover at most tidal heights at both sampling locations, and *F. spiralis* and *F. vesiculosus* are also rela-

Table 4. Density per m^2 and standard error of common intertidal animals at each sampling location. Tidal height is meters above mean lower low water.

Location	Tidal Height (m)	*Littorina littorea*	*L. obtusata*	*L. saxatilis*	*Nucella lapillus*	*Notoacmea testudinalis*	*Mytilus edulis*	*Semibalanus balanoides*
Ft. Stark	0.5	226.2 ± 74.5	28.7 ± 4.2	0.0	16.7 ± 3.5	3.3 ± 1.2	168.7 ± 64.7	5854.6 ± 1135.8
	1	52.4 ± 18.0	32.8 ± 11.7	0.4 ± 0.4	0.4 ± 0.4	0.0	182.2 ± 65.5	9045.0 ± 2119.0
	1.5	14.7 ± 3.6	16.0 ± 2.9	0.3 ± 0.3	3.0 ± 1.3	0.3 ± 0.3	37.5 ± 16.7	9893.8 ± 1837.7
	2	188.4 ± 72.4	10.9 ± 3.2	25.3 ± 15.2	0.0	0.0	0.0	1513.6 ± 629.3
	mean	119.6 ± 28.8	21.7 ±3.2	5.6 ± 3.7	5.1 ± 1.4	0.9 ± 0.4	93.8 ± 24.7	6596.0 ± 895.1
Odiorne	0.5	146.4 ±16.4	22.4 ± 9.4	3.6 ± 1.4	8.8 ± 2.3	2.4 ± 1.6	0.0	852.5 ± 555.6
	1	374.4 ± 102.2	32.0 ± 8.2	4.8 ± 3.6	4.8 ± 1.3	2.0 ± 1.2	197.5 ± 107.0	1777.5 ± 660.0
	1.5	244.8 ± 72.2	52.0 ± 11.1	0.4 ± 0.4	3.2 ± 2.4	0.0	90.0 ± 28.4	2897.5 ± 744.9
	2	56.0 ± 14.5	43.6 ± 7.1	1.2 ± 0.9	0.4 ± 0.4	0.0	127.5 ± 63.2	3540.0 ± 1196.4
	mean	205.4 ± 35.9	37.5 ± 4.7	2.5 ± 1.0	4.3 ± 1.0	1.1 ± 0.5	103.8 ± 32.7	2266.9 ± 429.9
	Overall mean	160.4 ± 23.2	29.2 ± 2.9	4.1 ± 2.0	4.7 ± 0.9	1.0 ± 0.3	98.6 ± 20.1	2534.5 ± 561.4

tively important in the community as evidenced by their high percent cover in quadrats (Figure 4).

Figure 5 illustrates the amount of different substrates at Ft. Stark. In general, substrates such as large rock, cobble, and pebbles provide the best shelter for crabs and there is a relatively high proportion of all three of these categories at all tidal heights. The population structure of *C. maenas* is shown in Figure 6. Overall, mean crab densities were very similar between Odiorne and Ft. Stark (15.6 vs. 16 m^{-2}), but crabs were smaller at Odiorne than at Ft. Stark (9.2 vs. 13.8 mm CW). Neither crab density nor size was consistently affected by tidal height between sampling locations (Figure 6). Size of *C. maenas* was more variable at Ft. Stark than at Odiorne. At Ft. Stark, the overall trend was of decreasing size with increasing density of crabs (Figure 6). *C. maenas* that were less than 5-mm CW were excluded from analysis because recruitment was taking place at the same time that quadrat sampling was occurring. A total of 737 *C. maenas* found in the quadrats were <5 mm: 156 at Ft. Stark and 581 at Odiorne.

Table 4 shows densities of common molluscs and barnacles at each tidal height and sampling location. In general, only barnacle densities increased with increasing tidal height. Both *L. obtusata* and *L. littorea* were much more abundant at Odiorne than at Ft. Stark, with unclear relationships between densities of these two snail species and tidal height. All of these organisms are potential prey of *H. sanguineus* when they are juveniles, but adult *L. littorea, N. lapillus*, and *M. edulis* are generally too large to be susceptible to crab predation.

Discussion

Our results indicate that *Hemigrapsus sanguineus* has the potential to impact the rocky intertidal of northern New England as an omnivorous predator and by causing increased competitive interactions with *Carcinus maenas.*

Stomach Contents

There are marked differences in percent composition of *Spartina* spp. and algae between the fall and spring collections of crabs (Figure 2). These results could be due to a decreased availability of *Spartina* spp. in the spring, or to the increased relative availability of algae (*Sphacelaria* and fucoids). Unfortunately, there is no quantitative information on community composition in the spring, but it is likely that the decreased importance of *Spartina* spp. in the diet reflects the increased availability of algae. The high percentage of *Spartina* spp. in guts of *H. sanguineus* in the fall corresponds with availability of this food as drift grass at this time (Figure 1). *Spartina* spp. breaks down in the fall (Wijte and Gallagher 1991) and the dead stalks of this grass become a significant portion of the drift material (Bertness and Ellison 1987). Vascular plants such as *Spartina* spp. are likely to have a longer residence time in the stomach than algae and therefore its importance in the diet of *H. sanguineus* could be over

represented. However, by only analyzing only guts that were at least half full (*i.e.*, crabs that had been feeding recently), some of this bias could have been eliminated (Williams 1981). Based on the observations in this study, it appears that the diet of *H. sanguineus* generally reflects the availability of different food sources in the environment.

The diet of *H. sanguineus* as indicated by this study and by that of Lohrer and Whitlatch (1997) demonstrate the broad feeding capabilities of this introduced crab. This generalist diet translates to a potentially wide niche where the only important limiting factors may be predation and availability of shelter. Although *H. sanguineus* has been recorded as a rocky shore species (Fukui 1988), its ability to consume *Spartina* spp. and a wide variety of algae and animal prey suggest that it will be able to become established in a variety of habitats (*H. sanguineus* was found under rocks in mud flats at Yarmouth, MA, M.C. Tyrrell, pers. obs.).

Feeding Preferences and Rates

Algae

Results of the multichoice trials indicate that *E. intestinalis* is the most preferred algal species of the five species offered because it was consumed in the highest levels (Table 1). Lubchenco (1978) also found that *L. littorea* preferred *E. intestinalis* over other algae and the fact that both of these introduced species share this preference may indicate that they could compete for food. The result that *H. sanguineus* will consume all five species of algae when it is not given a choice between foods indicates that all five species tested are potential, if not preferred, food items.

Newly recruited algae are generally more susceptible to herbivory than mature conspecifics (Lubchenco, 1983). Like other herbivores, *H. sanguineus* may consume proportionately more germlings than mature conspecifics, thereby inhibiting replacement of algae that is removed by storms or physical damage. It is possible that herbivory by *H. sanguineus* on fucoids and other perennial algae will be most conspicuous by reduced recruitment of these species, rather than by drastic reductions in established cover.

Molluscs

All of the crabs were used in each pairwise prey preference trials, which allowed us to see if any of the crabs had specialized on a particular prey species. We found that the preferences of both crab species were very consistent both between individuals and between the two species. The observations that both introduced crab species preferred *M. edulis* over other molluscan prey is consistent with studies of stomach contents in *C. maenas* in this region (Ropes 1968; Elner 1981). Both authors found that these bivalves were very important in the diet of the green crab. The result from this study that both *H. sanguineus* and *C. maenas* preferred *L. obtusata* over *L. littorea* is not surprising, considering that *L. obtusata* underwent a rapid morphological transition following the establishment of *C. maenas* (Seeley 1986). The consistent avoidance of *L. littorea* by both crab species may provide additional explanation for the lack of a morphological response in this species. Lubchenco (1978) observed that *C. maenas* may control recruitment of *L. littorea* by consuming very small individuals as they settle into tide pools, but she also found that medium and large *L. littorea* are not consumed by *C. maenas* in laboratory experiments. Both *L. littorea* and *C. maenas* are introduced to this area from Europe (Cohen *et al.* 1995 for *C. maenas*; and Gosner 1978 for *L. littorea*). As they grow, it is possible that *L. littorea* may develop behavioral adaptations or acquire toxic compounds that make them less susceptible (or palatable) to crab predators.

Feeding preferences of *H. sanguineus* and *C. maenas* closely overlapped and statistical significance and orders of preference were the same between crab species (Table 3A, B). The strong overlap in feeding preferences of these two introduced crabs has several implications for the effects of their introductions on community structure. Further range expansion of *H. sanguineus* north of Cape Cod may place additional pressure on the molluscs preferred by both introduced crabs. Declines in preferred prey species, as well as increased competitive interactions between these two intertidal crabs, will be likely in areas where the two species co-occur.

Pre-Invasion Community Structure

The two study locations were selected on the basis of their accessibility (for sampling purposes) and similarity (for replication). Therefore, one would not expect large differences in the intertidal communities between these two sites. However, some notable differences do exist. The algal canopy is almost exclusively *A. nodosum* at Odiorne, while at Ft. Stark, *F. spiralis* and *F. vesiculosus* are also impor-

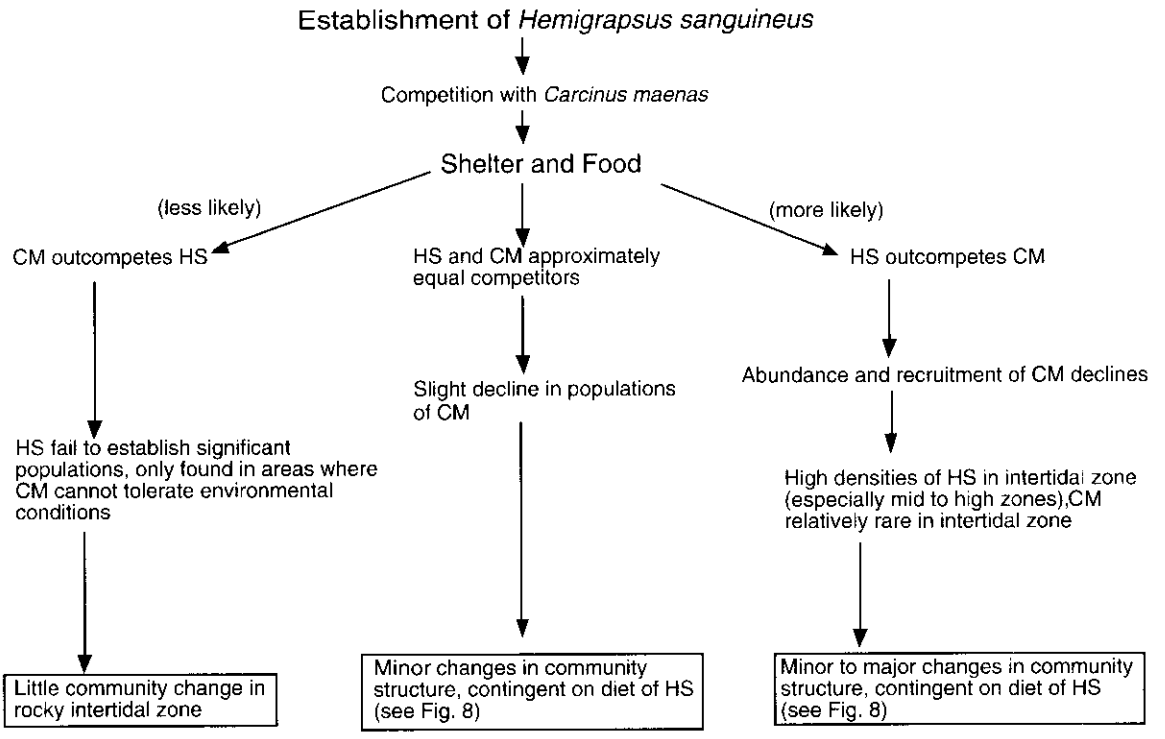

Figure 7. Predicted impacts of the establishment of *Hemigrapsus sanguineus* in the New Hampshire and Maine rocky intertidal zone, emphasizing effects of potential competition with *Carcinus maenas*. CM= *Carcinus maenas*, HS= *Hemigrapsus sanguineus*.

tant (Figure 4). Odiorne has much higher numbers of *L. littorea* and *L. obtusata* than Ft. Stark (Table 4). In contrast, barnacles are nearly three times more abundant at Ft. Stark than at Odiorne (Table 4).

The abundance of *C. maenas* in relation to tidal height did not vary consistently between Odiorne and Ft. Stark. In general, the pattern of decreasing size of crabs with increasing tidal height at Ft. Stark is consistent with the findings of other researchers working in Wales (Edwards 1958). The trend of generally higher densities of crabs with increasing tidal height that was found in this study contradicts the findings of other researchers (Atkinson and Parsons 1973). However, Atkinson and Parsons (1973) only worked with *C. maenas* that were greater than 20 mm CW, a size much greater than the mean size of crabs found in this study. From the results of this study and of the other studies of *C. maenas* distribution, it is apparent that small crabs seek refuge in the upper intertidal zone and occur in higher densities than adult crabs.

In general, shelter may be more important to crabs than tidal height; crabs may seek shelter under any suitable substrate (cobble, pebble, etc.) wherever it occurs in the intertidal zone. Lohrer and Whitlatch (1997) found that the three species of crabs in their study (*H. sanguineus, C. maenas*, and *Eurypanopeus depressus*) did not partition space according to tidal height. The suggestion of Lafferty and Kuris (1996) that *H. sanguineus* may not have a strong affect on established crabs because of its upper intertidal distribution may be incorrect. Our study illustrates that *C. maenas* are found throughout the intertidal zone in high densities. One of the most important impacts of *H. sanguineus* on *C. maenas* may be increased competition for shelter at all tidal heights and life history stages.

C. maenas usually moves to subtidal zones after it reaches 30-35 mm CW (a size at which it is much less susceptible to predation) and migrates to the intertidal zone to feed (Edwards 1958; Warman *et al.* 1993). In contrast, *H. sanguineus* adults (up to 43 mm CW), are commonly found throughout the intertidal zone (McDermott 1998a). In competition-for-shelter experiments, *H. sanguineus* is able to exclude *C. maenas* that are as much as 5 mm CW larger (M.C. Tyrrell and B. Hull, unpubl. data). Based on these laboratory observations, one would expect that *H. sanguineus* would tend to displace *C. maenas* from shelters in areas where they co-occur (Figure 7).

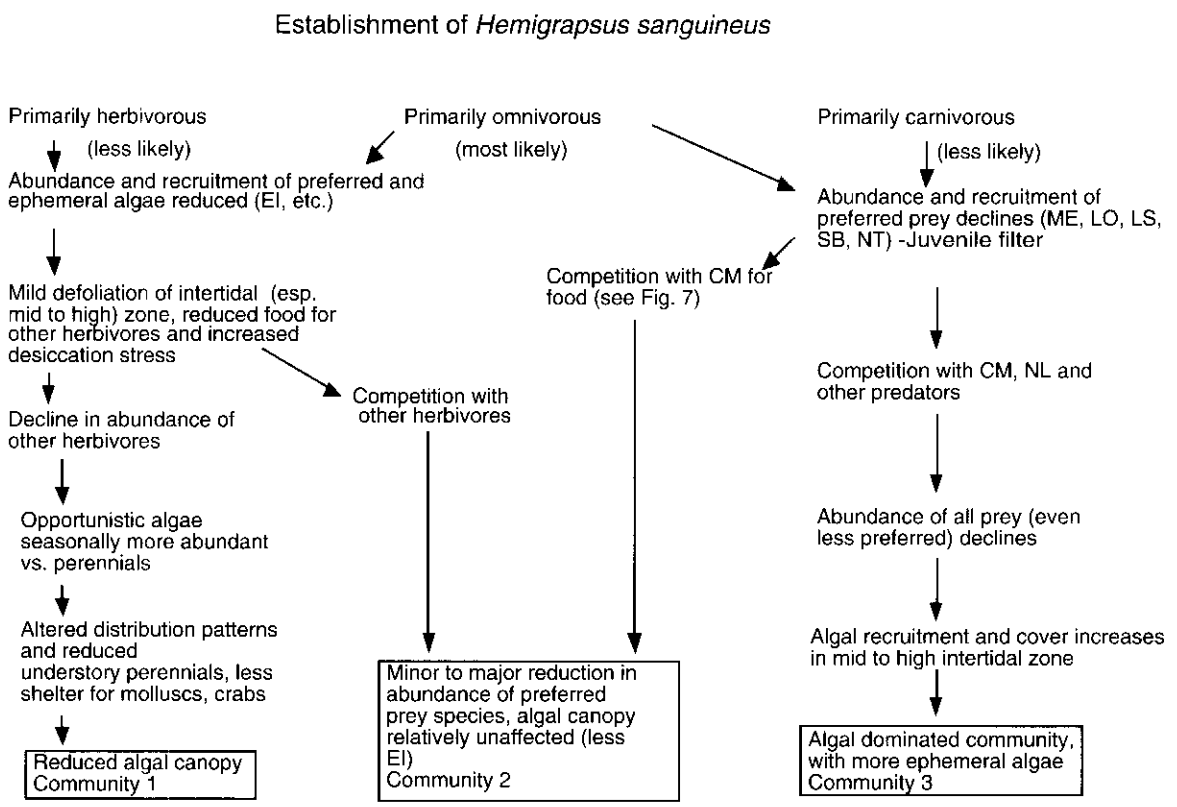

Figure 8: Predicted impacts of the establishment of *Hemigrapsus sanguineus* in the New Hampshire and Maine rocky intertidal zone emphasizing effects of *H. sanguineus* as a predator. Note the pre-invasion community is characterized by an algal-dominated shore with a high proportion of perennial algae versus ephemeral algae. CM= *Carcinus maenas*, ME= *Mytilus edulis*, LO= *Littorina obtusata*, LS= *L. saxatilis*, NT= *Notoacmea testudinalis*, SB= *Semibalanus balanoides*, NL= *Nucella lapillus*, EI= *Enteromorpha intestinalis*.

Littorinids, other molluscs, and barnacles are distributed throughout the intertidal zone at both sampling locations (Table 4). All of the species listed in Table 4 are potential prey of *H. sanguineus* (*N. testudinalis* and *N. Lapillus*, M.C. Tyrrell, unpublished data; barnacles, Lohrer and Whitlatch (1997); M.C. Tyrrell, unpubl. data). Again, the effects of predation by *H. sanguineus* on all of these potential prey is expected to be more severe on the juveniles of these species. Most of the species listed in Table 4 are herbivores (littorinids and *N. testudinalis*). If *H. sanguineus* consumes primarily molluscs, it may reduce the predation pressure on intertidal algae (Figure 8), but because it is more herbivorous than *C. maenas*, we expect a mix of herbivory and herbivore removal.

In conclusion, it appears that none of the tidal heights utilized in this study were strongly or consistently preferred by *L. littorea*, *L. obtusata*, *L. saxatilis*, and the potential competitor, *C. maenas*. In contrast, the distributions of other potential prey species (*N. lapillus*, *N. testudinalis*, *M. edulis*, and *S. balanoides*) were more consistently affected by tidal height, with varying preferences among these species. It is likely that *H. sanguineus* will be distributed throughout the intertidal zone and its presence will modify the entire community to some extent. Some of the most obvious effects of *H. sanguineus* may be a decline in preferred prey species and a decline in numbers of *C. maenas* because of increased competition for food and shelter. These sites will continue to be monitored in the same way for several years to determine if the predicted changes in community structure occur if *H. sanguineus* becomes established.

General conclusions and predictions

The range extension of *H. sanguineus* along the Atlantic coast has taken place very rapidly: from 1988 to 1995, it spread approximately 800 km (McDermott 1998a). The recent discoveries of this species in both coastal (Hampton and Rye Harbors) and estuarine (Dover Point) locations indicate that it may continue to establish populations in a variety of

habitats. This invasive species has a remarkable ability to become established in a variety of habitats: rocky and muddy shores, sheltered and exposed locations, and estuaries (McDermott 1998a; M.C. Tyrrell, pers. obs.). Its wide physiological tolerance is combined with an ability to utilize a wide variety of food types (as indicated by its omnivorous diet). Many of the factors that make *H. sanguineus* a successful invader contribute to its disruptive potential for the communities it invades. Some projected consequences of the establishment of *H. sanguineus* on New Hampshire and Maine rocky shores are summarized in Figures 7 and 8.

H. sanguineus has the potential to cause changes in the resident intertidal community by both direct (predation, competition, etc.) and indirect (exploitation, competition etc., see Menge 1995 for further examples) effects. Even adults of this crab are found throughout the intertidal zone and, therefore, the refuge value of the upper intertidal zone for small molluscs and young *C. maenas* may be diminished by *H. sanguineus*. *H. sanguineus* may act as a juvenile filter for all species (both algal and animal) that inhabit the intertidal zone during their life cycle (Figure 8).

Feeding preference trials conducted under laboratory conditions indicate which species are most likely to be affected by predation by *H. sanguineus*. However, the accessibility of preferred prey will differ among prey species and time of year. Juvenile *M. edulis* are often nestled in byssal threads between adult conspecifics and may be difficult for crabs to extract. In addition, small *L. obtusata* that take refuge in the hollow vesicles of *A. nodosum* may be floating out of reach of crabs during high tide (when crabs are most likely to be foraging). Despite the many issues surrounding accessibility of food items, it is expected that the negative impacts of *H. sanguineus* will be disproportionately concentrated on preferred food items (both algal and animal), leading to a decline in their density and relative abundance as compared with less preferred food items (Figure 8).

Several authors have recognized competition with *C. maenas* as a major impact of the establishment of *H. sanguineus* (McDermott 1991, 1998a; Lohrer and Whitlatch 1997). *H. sanguineus* is more aggressive than similar-sized *C. maenas* and can consume *C. maenas* when starved (M.C. Tyrrell and B. Hull unpub. data). Competition between the two introduced crab species can occur for both food and shelter, with varying consequences in each situation (food competition, see Figure 8; shelter competition, see Figure 7). In areas where *H. sanguineus* is already established, it is the numerically dominant crab (Lohrer and Whitlatch 1997) and it has been suggested that it may inhibit recruitment of *C. maenas* (A. M. Lohrer, pers. comm.). If this occurs in northern New England, populations of *C. maenas* could be severely reduced (Figure 7).

It is exceedingly difficult to predict the exact ecological consequences of an invasion of a generalized predator such as *H. sanguineus*. However, the effects of *H. sanguineus* along the east coast of the United States may be comparable to those predicted for *C. maenas* on the west coast. In a review of the possible impacts of the introduction of *C. maenas* in San Francisco Bay, Cohen *et al.* (1995) stated 104 families and 158 genera are potential prey items for this generalized predator. In addition, they listed displacement of competitors, increased availability of food for crab predators, and economic losses due to predation on shellfish as other potential impacts of *C. maenas* (Cohen *et al.* 1995).

It is likely that northern New England will soon experience a second invasion by an intertidal crab, *H. sanguineus*, and this potential invasion provides an excellent opportunity to examine the ecological impacts of both introduced crab species. Further research into the effects of *H. sanguineus* on the northern New England rocky intertidal community is currently being conducted using microcosms. The results of these experiments will be compared with the potential impacts of *H. sanguineus* that are listed in Figures 7 and 8 and will contribute to our understanding of the effects of introduced species.

Acknowledgments

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Reestablishment of a Native Oyster, *Ostrea conchaphila*, Following a Natural Local Extinction

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ABSTRACT: **Populations of the oyster, *Ostrea conchaphila*, occur only in isolated estuaries with stable euryhaline zones (22-28 ppt) between Washington and central California. These estuaries have formed since the Pleistocene, and in some, subsequent natural sedimentation eliminated both the polyhaline zone and *O. conchaphila* populations. In Coos Bay, Oregon, *O. conchaphila* thrived in the late Holocene, but went locally extinct prior to European settlement. In 1988, following years of accidental inoculations via shellfish culture activities, *O. conchaphila* reestablished in Coos Bay, and has maintained a large population since then. The distribution of *O. conchaphila* in Coos Bay is clearly correlated with salinity patterns typical of a stratified estuary. Navigational dredging has more than doubled the main channel depth, permitting high salinity to intrude, and again producing a stable euryhaline habitat suitable to *O. conchaphila*. The depth increase has also increased proportional water retention during tidal fluctuation, which could reduce flushing pressure on estuarine larvae of *O. conchaphila*. Natural events created, and then apparently destroyed, a habitat for a native species. Human activities of a destructive nature (dredging) have apparently restored this habitat for *O. conchaphila*.**

Key words: indigenous species, human disturbance, oyster, *Ostrea conchaphila*

INTRODUCTION

The Olympia oyster, *Ostrea conchaphila* Carpenter, 1859 (= *lurida*), is the only oyster native to the eastern Pacific, north of Mexico. The biology, ecology, and distribution of *O. conchaphila* was reviewed by Couch and Hassler (1989) and Baker (1995). The primary habitat is estuaries with stable polyhaline regions (20-28 ppt). Individual *O. conchaphila* are seldom, if ever, found along the open coast between estuaries north of Point Conception, California. Estuaries that support *O. conchaphila* populations are usually isolated from each other along the outer coasts of Washington, Oregon, and California. In the absence of molecular genetic data, the term population is here used to mean all individuals living in an isolated estuary.

Since the arrival of Europeans in the region, some *O. conchaphila* populations have declined or gone extinct (Baker 1995). There is evidence, however, that at least some populations were declining prior to the arrival of Europeans. The best documented occurrence of this phenomenon is in Coos Bay, Oregon, where large deposits of recent fossil *O. conchaphila* occur in both dredge spoils and in American Indian shell middens (Dall, 1897; Baker *et al.*, unpubl. data). By the time European settlers arrived, however, *O. conchaphila* had gone extinct in Coos Bay (Dall 1897), for reasons unknown.

Another pre-European extinction of *O. conchaphila* appears to have occurred in Big Lagoon, California–evidence that local extinction is not unique to Coos Bay. There is no extant population of

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O. conchaphila in Big Lagoon, but this species is abundant in nearby Indian shell middens (Elsasser and Heizer 1966). Big Lagoon is currently blocked from a regular marine connection by migrating coastal sand dunes–a fate which has befallen many small estuaries in this region within the past few thousand years (Johnson *et al.* 1985).

Dall (1897) was the first to report both the abundance of recent *O. conchaphila* shells in Coos Bay, and the current local absence of this species. Based on the distribution of fossil shells, *O. conchaphila* was restricted almost exclusively to the East Arm of Coos Bay (Figure 1). The subsequent history of *O. conchaphila* in Coos Bay until 1986 is known from various federal and state government reports. In 1917, local residents attempted to reestablish *O. conchaphila* in Coos Bay, importing large numbers of adults from Willapa Bay, Washington. These specimens were spread across about 5.7 ha of intertidal ground in Coos Bay, and strong recruitment was reported for two years (Edmondson 1923). By 1927, however, all traces of this inoculation appeared to have vanished, and local shellfish harvesters seemed unaware that *O. conchaphila* ever had been present, indicating that the population had been deceased for several years (Galtsoff 1929). Subsequent benthic surveys using a variety of sampling methods reported many bivalve species in Coos Bay, but no living *O. conchaphila* (Day 1971; Gaumer *et al.* 1973; Jefferts 1977; Hancock *et al.* 1979; Roye 1979). Thus, a major inoculation of Coos Bay in 1917, by clearly viable *O. conchaphila*, failed to establish more than an ephemeral population.

There is a significant oyster culture industry in Coos Bay, based on the nonindigenous Pacific oyster, *Crassostrea gigas*. This industry has been locally continuous since about 1934, and until the last several years, has been supported almost entirely by the importation of juvenile *C. gigas* attached to adult shells, which were reared in either Willapa Bay or Puget Sound, in Washington State (Qualman 1983). To the south of Coos Bay is Humbolt Bay, which, like Puget Sound and Willapa Bay, supports *C. gigas* culture and a large population of *O. conchaphila* (Baker 1995). In 1987, Coos Bay oyster culturists observed that living oysters they believed to be *O. conchaphila* were occasionally imported as fouling organisms with *C. gigas* shells and juveniles (M. Clausen and L. Qualman, pers. comm.). Nonetheless, as of 1986, there was no historic record of an established population of *O. conchaphila* in Coos Bay.

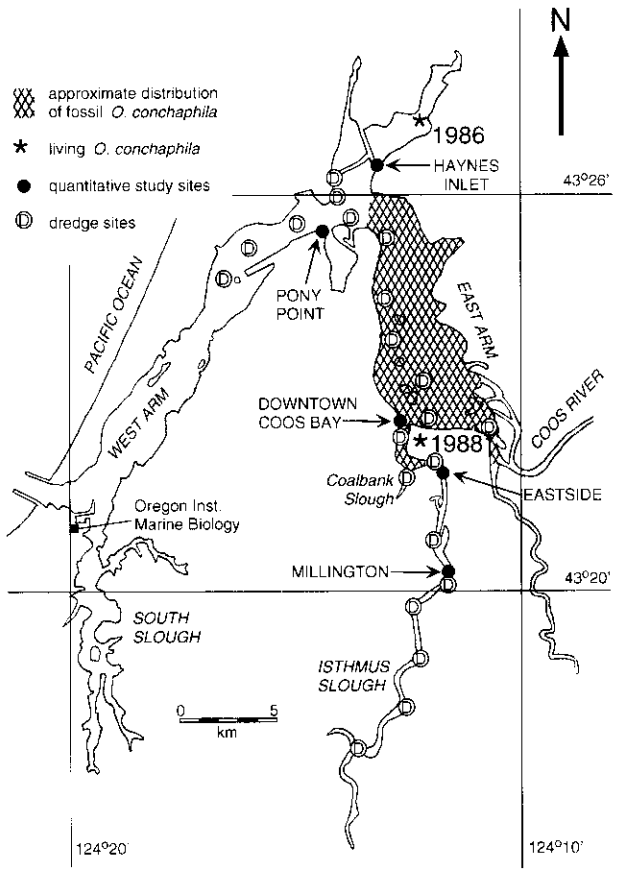

Figure 1. Coos Bay, Oregon, showing *Ostrea conchaphila* distribution to 1988, and quantitative survey sites.

In 1988, however, several living juvenile *O. conchaphila* were collected during a benthic survey of Isthmus Slough, a subestuary of Coos Bay (Baker *et al.*, unpubl. data). In subsequent years, this species became abundant in Isthmus Slough and adjoining portions of Coos Bay (Oregon Department of Fish and Wildlife, unpubl. data). Additional *O. conchaphila* juveniles, from a population in Yaquina Bay, Oregon, were released into Coos Bay by researchers in 1994 (Robinson and Johnson 1997), but the population detected in 1988 had already become large (Hewitt 1993). Hewitt (1993) reported that *O. conchaphila* was introduced to Coos Bay in 1992, but this contradicts unpublished data by fishery officials (Oregon Department of Fish and Wildlife), and data presented in this study, supporting an earlier date.

Other historical populations of *O. conchaphila* in Oregon are extinct or declining (Baker 1995). The reappearance of this species in Coos Bay is thus of interest: why is *O. conchaphila* currently thriving in Coos Bay, when it was could not previously be estab-

lished in 1917? Alternately, *is* this species thriving in Coos Bay, or are recent reports of the reestablishment of this relatively long-lived species (Baker 1995) based on a single cohort that has not subsequently reproduced?

Qualitative and quantitative surveys were used to address the second question posed above. The initial question–why is *O. conchaphila* established now, when a deliberate attempt in 1917 failed–cannot be tested experimentally, because both extinction and reestablishment were historic and unique events. Comparisons of the Coos Bay physical environment between 1917 and 1988 can be made, however, and correlated with current distribution patterns of *O. conchaphila* in Coos Bay to generate a hypothesis regarding this species' local population history.

Materials and Methods

Study Site

The research area was Coos Bay, Oregon, 43°25' N, 124°12' W. Coos Bay is a geologically recent estuary, with the dendritic shape of drowned river valleys (Roye 1979). At mean high water, the surface area of all tidal portions is about 4500 ha (estimated from U.S. Geological Survey data). Mean tidal flux is about 2 m at the mouth of Coos Bay (National Oceanic and Atmospheric Administration, co-ops.nos.noaa.gov), and at mean low low water, surface area is only about 2200 ha.

For purposes of this manuscript, Coos Bay is divided into a West Arm and an East Arm (Figure 1). Major subsidiary estuaries include South Slough, Haynes Inlet, and Isthmus Slough. The only major freshwater input, Coos River, flows into the south end of the East Arm. Marshfield Channel, the deepest undredged channel within the main portion of Coos Bay, is 2-4 m at low water, with occasional soundings to 6 m. A shipping channel maintained at 36 ft (11 m) at low water runs the length of both the West Arm and the East Arm, and about 2.5 km into Isthmus Slough (Figure 2). Isthmus Slough is 6-9 m in depth for an additional 8 km upstream of the currently maintained channel, but this is an artifact of historic channel maintenance.

1986-88 Qualitative Surveys

Qualitative surveys have the advantage, over quantitative surveys, of sampling a large area in a relatively short time. They are particularly useful for presence or absence of relatively large organisms (such as oysters). Furthermore, such surveys become quantitative if none of the study species are present: zero is a precise quantity.

From November 1986 to June 1987, we surveyed most of the shoreline of Coos Bay and subsidiary estuaries for the presence of *O. conchaphila*. Surveys were conducted on foot at low tide (0 m or lower). All oysters were counted and recorded as either adults (>20 mm in shell height, for *O. conchaphila*) or juveniles. This size division for *O. conchaphila* is based on the sizes of apparent cohorts, reported in this manuscript. Because of the abundance of recent fossil material, dead *O. conchaphila* specimens were not recorded unless both shells were still attached by the ligament, indicating a relatively recent demise.

A Wildco biological dredge (50-cm mouth, 2-cm mesh) was used to take qualitative benthic samples at representative locations in the main channel, from the mouth of Coos Bay into Isthmus Slough. Dredges were 5 min long each, towed at minimal speed, and were towed from the edge of the main channel into shallower water. The tow distance was 50-80 m, for a total area of 25-40 m^2.

In April to August 1988, some shoreline and benthic sites were re-surveyed specifically for the presence or absence of *O. conchaphila*, using the same methods and criteria as above.

1996-98 Qualitative Surveys

The approximate distribution of the current *O. conchaphila* population in Coos Bay was estimated by qualitative shoreline and benthic surveys in 1996, 1997, and 1998. Surveys for the presence or absence and relative abundance of *O. conchaphila* were made throughout known or suspected habitat, as well as in regions both seaward and landward (with respect to the salinity gradient) of the suspected distribution of this species. Shoreline qualitative surveys were conducted as for the 1986-88 surveys (above).

Benthic dredge surveys were conducted, using the same equipment and technique as in 1986-88. 1996-98 dredge sample sites are shown in Figure 1. In no instance did the dredge collection bag become entirely filled with material unable to pass through the cod end of the collection bag. All hard substrate collected in each dredge sample, and the proportions of each type of substrate, by mass, were recorded.

All oysters were counted, identified, and measured (shell height). The presence or absence of some suspected *O. conchaphila* predators, including crabs

(*Cancer* spp.), predatory snails (*Nucella* spp.), and sea stars (*Pisaster* spp.) (Baker 1995), was noted at all shoreline and dredge survey sites.

Quantitative Surveys

Based upon relative *O. conchaphila* abundance estimates from 1996 and 1997 qualitative surveys (above), five sites were selected for quantitative surveys in 1997. There was an apparent region of *O. conchaphila* maximum density in Isthmus Slough and the southwest portion of the East Arm of Coos Bay (Figure 2). The Downtown site (20.7 km from the mouth of Coos Bay) and Millington site (25.5 km) were selected because they bracketed this zone seaward and landward, respectively. The Eastside site (23.6 km) was between the prior two. Two additional sites, Pony Point (13.4 km) and Haynes Inlet (15.5 km, but not along the main channel axis), were selected to examine an apparently more modest density of *O. conchaphila* in the north part of Coos Bay (Figure 1).

Three strata were sampled: shallow (mean low low water, or approximately 0 m), mid-depth (4-5 m) and deep (9-10 m). All three depths were surveyed at Eastside and Millington sites. At the Downtown site, only low tide and mid-depth strata were surveyed, because navigational dredging immediately prior to sampling had removed all substrate and fauna below 6 m. At the Haynes Inlet site, the channel was only 5 m deep at low tide. At the Pony Point site, qualitative dredge sampling indicated no *O. conchaphila* at either mid-depth or deep strata, and only the shallow (0 m) stratum was sampled.

At each site and stratum, a metered line was tethered up-current, and allowed to trail along the stratum. Six 0.25-m^2 quadrats (Miller *et al.* 1993) were placed at random intervals (1-10 m) along the survey line. Scuba divers collected all oysters within each quadrat. Except in quadrats where substrate was homogeneous (*e.g.*, 100% cover by sandstone cobble and boulder), or where the substrate was too large to collect (large boulder or log), all hard substrate over 1 cm in diameter was also collected. In cases where substrate was not collected, all oysters were collected from the substrate. All living oysters were identified, and the shell height (hinge to opposite margin) of each specimen was recorded. Each site was also examined qualitatively by divers for the presence of individuals of *Cancer*, *Nucella*, and *Pisaster* species, as for the qualitative surveys (above).

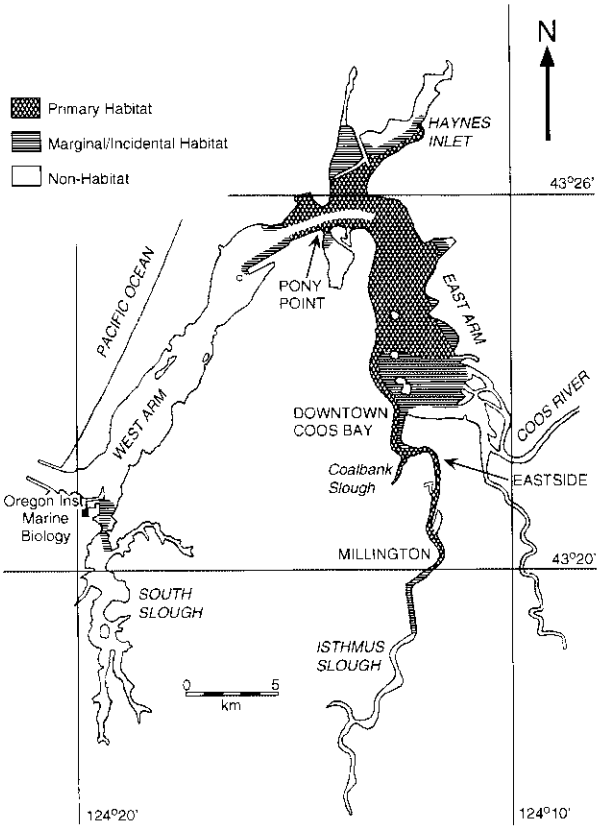

Figure 2. Generalized 1997 distribution of *Ostrea conchaphila* in Coos Bay.

Specimens were divided into 0-year-class individuals (juveniles) and adults (> 20 mm shell height). One-factor analysis of variance (Zar 1996) was used to test the null hypothesis that adult density did not vary between depth strata, within sites, for those sites where adults occurred at more than one depth. Regression analysis (Zar 1996) was used to test the null hypothesis of no dependence of the depth at which maximum *O. conchaphila* adult density occurred, on sample site distance from the mouth of Coos Bay.

Salinity Data

Salinity data were obtained from two sources which matched seasons and sites. Baptista (1989) reprinted various historical salinity survey data, including data for spring (May) and late summer/early autumn (September), in bottom waters of northern Isthmus Slough, for 1930 and 1931. Data were also provided by an on-going study by the Oregon Department of Fish and Wildlife (unpubl. data) for bottom salinity at approximately the same site, also in May, and in August. May is normally a

Table 1. Mean densities (number per m^2) of large (> 20 mm shell height) and all *Ostrea conchaphila* at each of three sample depths (low tide level, mid-depth or 5m, and deep or 10m), at each site. Standard deviations (as +/–) are shown in parentheses. Within sites, treatments that do not differ significantly are indicated by superscripts. See text for explanations for no deep samples at Downtown or Haynes Inlet sites.

Depth	Millington	Eastside	Downtown Coos Bay	Haynes Inlet	Pony Point
low tide: large	0	0.7 (1.6)	6.7 (3.9)	0.7 (1.6)	5.3 (6.0)
low tide: all	0	23 (30)	201 (309)	77 (35)	21 (28)
% large	NA	0.52	2.00	0.86	22.9
mid-depth: large	0	0.7 (1.6)	10 (16)	0	0*
mid-depth: all	12 (20)	5.3 (9.6)	81 (164)	9	0*
% large	0	11.1	12.4	NA	NA
deep: large	10 (10)	57 (68)	0*	max depth 5 m	0*
deep: all	48 (31)	394 (378)	0*	max depth 5 m	0*
% large	20.8	14.4	NA	NA	NA
distance from mouth of Coos Bay	26.5 km	23.6 km	20.5 km	15.5 km	13.4 km

*from qualitative dredge samples

period of relatively high freshwater input, and August/September is usually at the end of the driest part of the year, so these should represent contrasting freshwater input regimes for Isthmus Slough. Baptista (1989) noted that the 1930 and 1931 were drought years, with much lower than normal freshwater input to Coos Bay (about 114 cm and 153 cm, respectively). Rainfall in 1996 and 1997 was 219 and 137 cm, respectively, with peak rainfall in winter months (Oregon Climate Service climate data, www.ocs.orst.edu).

Historic Coos Bay shipping channel depth records were provided by the Oregon International Port of Coos Bay. Estimates of pre-dredging depths were made from historic charts (National Oceanic and Atmospheric Administration, chartmaker.ncd.noaa/ocs). The null hypothesis of no effect of channel depth on salinity data in Isthmus Slough was analyzed with regression analysis (Zar 1996), for May and August/September, separately.

Results

Adult *O. conchaphila* occurred in shoreline surveys from the west end of the North Bend Municipal Airport (10.5 km from the mouth of Coos Bay) to Eastside, in Isthmus Slough (23.3 km). Adult *O. conchaphila* from benthic dredge samples occurred from the U.S. Hwy 101 Bridge (15.6 km) in North Bend to Millington, in Isthmus Slough (25.0 km). Juvenile *O. conchaphila* were found further upstream in both the main axis (Isthmus Slough), as well as in side channels (North Slough, Haynes Inlet, and Marshfield Channel). Results of shoreline and dredge qualitative samples are summarized in Figure 2. No *O. conchaphila* were found in the remainder of Coos Bay or South Slough, with the exception of three adults attached to floating piers in the Charleston small boat harbor, near the Oregon Institute of Marine Biology.

Several sites merit additional comments. The mouth of Coalbank Slough, a qualitative dredge site (Figure 1), was highly depositional, yet supported high densities of *O. conchaphila*. The Coalbank sample consisted of living or recently dead (valves still connected by a ligament) *O. conchaphila*, attached in clusters. At most Isthmus Slough sites, a dominant substrate was a soft sandstone, easily broken or abraded by hand, but *O. conchaphila* were found attached directly to this material. At the middle stratum of the Downtown Coos Bay site, many *O. conchaphila* were found living on broken lead-acid battery cases.

Results of quantitative benthic surveys are summarized in Table 1. At all quantitative sites, the majority of living *O. conchaphila* were under 20 mm in shell height, often with an obvious and large distribution peak between 2 and 10 mm (Figures 3, 4). The majority of the remaining specimens were 22-44 mm in shell height, with no significant peak based on Kolmogorov-Smirnov goodness-of-fit analysis (Zar 1996) of adults (22-44 mm) pooled from all sites ($d_{max} = 4.67$). Maximum shell height was 58 mm, from the deep stratum at Eastside. Size distribution pooled for all sites is shown in Figure 4.

At only two sites, Eastside and Downtown Coos Bay, did adult *O. conchaphila* occur at more than one depth. At Eastside, the number of adults at mid-depths (5 m) did not differ from the expected ratio found in deep water (10 m) ($\chi^2 = 0.0$), but the number of adults in shallow water (0 m) was distinctly lower than expected, even based on the number of juveniles ($\chi^2 = 21.04$). The same was true of shallow water at the Downtown Coos Bay site, compared to the ratio of adults at 5 m ($\chi^2 = 17.36$).

The depth of maximum density of adult (>20 mm shell height) *O. conchaphila* varied, depending on site (Table 1). At the Eastside site, adult density was

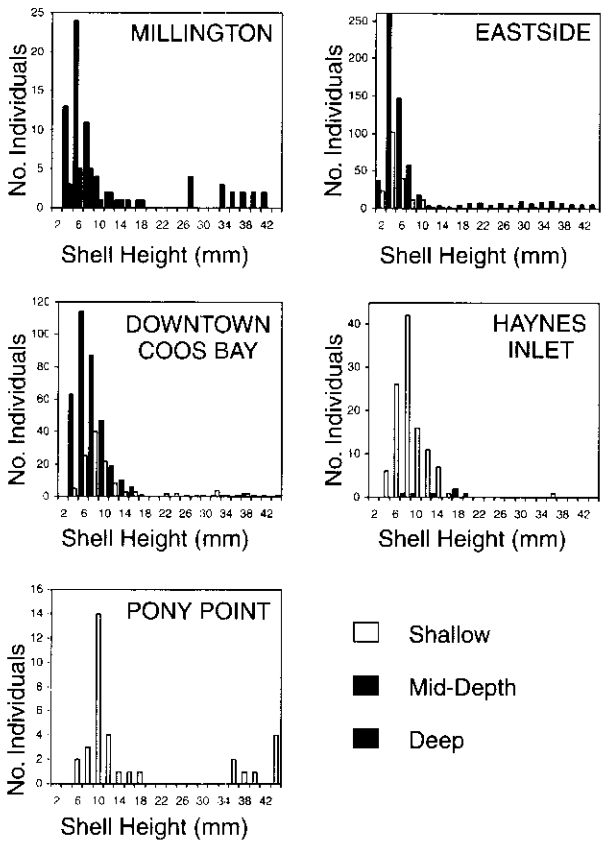

Figure 3. Size distribution of *Ostrea conchaphila* from selected quantitative study sites. All individuals over 44 mm (n = 9), and individuals from the Middle stratum at Eastside (n = 8) are omitted for graphical clarity.

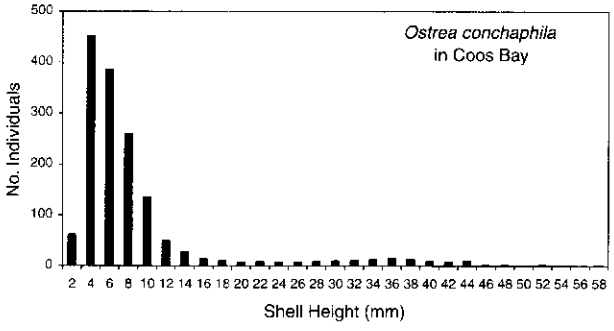

Figure 4. Size distribution of *Ostrea conchaphila* from all quantitative survey sites in Coos Bay, pooled.

significantly greater in deep water (10 m) than at other depths ($p = 0.0398$). At the Downtown Coos Bay site, there was no significant difference in adult density between the shallow and mid-depth sampling levels ($p = 0.434$), so the depth of maximum adult density was taken as the mean of those two depths (2.5 m). At other sites, adults were found at one depth only. The dependence of the depth of maximum density on distance from the entrance of Coos Bay was strong ($r^2 = 0.82$, $p = 0.0001$), with greater depths of maximum density occurring further from the ocean.

Bottom salinity in northern Isthmus Slough appeared to be much higher in 1996-97 than in 1930-31. In both May ($r^2=0.76$, $p < 0.00005$), and in August/September ($r^2=0.70$, $p = 0.0002$), the higher salinities were significantly related to channel depth (Figure 5A and B).

Native crabs, *Cancer magister*, and nonindigenous green crabs, *Carcinus maenas*, occurred at sites throughout Coos Bay. *C. maenas*, a recent invader, is predicted to be a significant bivalve predator in Pacific coast estuaries (Cohen *et al.* 1995; Grozholz and Ruiz 1995). Other known predators, including sea stars (*Pisaster brevispinus* and *P. ochraceous*), and gastropods (primarily *Nucella lamellosa*) occurred mainly in the western arm of Coos Bay, largely outside of the main region of *O. conchaphila* distribution.

Discussion

Is Ostrea conchaphila reestablished in Coos Bay?

Ostrea conchaphila has been present continuously for more than ten years in Coos Bay. By itself, this does not clearly demonstrate establishment of the species, because oysters are long-lived, and a single cohort could probably persist for that long (Baker 1995). Examination of the size/age distribution in Coos Bay, however, suggests that this species is reproducing regularly.

The *O. conchaphila* population in Coos Bay was clearly represented by more than one cohort, including a strong zero year-class cohort (Figure 4). Assuming size is related to age, this distribution is thought to be typical of a population with a Type III survival curve, in which most of the large number of larvae and early juveniles die, but older individuals have a good chance of living several years (Deevey 1947). This type of a life cycle is typical of marine bivalves, including *O. conchaphila* (Baker 1995). No distinct older size class could be detected, and apart from a fairly distinct break in size class abundance at 20 mm, sizes up to 46 mm were nearly equally represented. Nearly the full normal size range for this species was represented in Coos Bay, and most of the adults were comparable to Puget Sound specimens of 2-5 yr of age (Baker 1995). The data

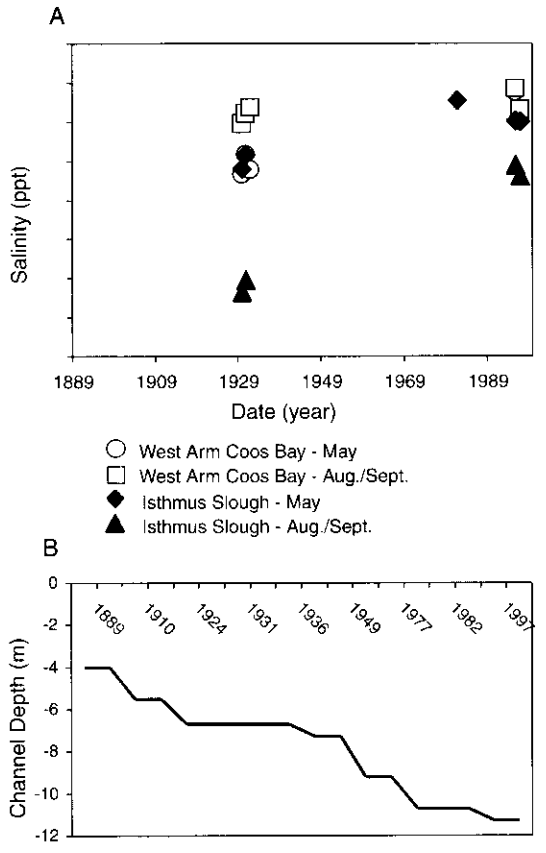

Figure 5A and B. Bottom salinity (A) for two sites at two times of the year, and shipping channel depth (B) in Coos Bay, plotted against time.

were consistent with the hypothesis that this species had reproduced in large numbers, and on a regular basis, in Coos Bay.

Distribution patterns of Ostrea conchaphila

Salinity is a key environmental correlate with *O. conchaphila* distribution, where it has been studied previously, with main populations restricted to a modally intermediate salinity range. The three-dimensional distribution of *O. conchaphila* in Coos Bay could be matched to the generalized salinity patterns of a mixed estuary. Most of Coos Bay is marine in summer months, but in winter and spring, salinity becomes progressively lower inland (Roye 1979, Baptista 1989). Coos Bay also becomes stratified in winter and spring, however, so that high salinity extends further inland in deep water than in surface water (Baptista 1989). This is a characteristic of most stratified estuaries (Dyer 1997). For part of the year, therefore, optimal salinity for *O. conchaphila* occurs in shallow water towards the entrance to Coos Bay, and in deep water inland. The distribution patterns of adult *O. conchaphila* in Coos Bay matched this, with adults in deep water occurring further inland than adults in shallow water (Table 1).

The low-salinity boundary is probably set by the osmotic tolerance of *O. conchaphila*, although this seems unlikely for high salinity (Baker 1995). Young-of-the-year juveniles, produced in summer, have yet to experience winter and spring salinity declines, and may thus occur inland of the adult distribution. The fact that they did suggests that adult distribution is set in part by differential mortality during high freshwater input periods.

The high-salinity population limit for *O. conchaphila* may also be set by salinity, indirectly through its effects on predators. Although crabs (*C. magister* and *C. maenas*) occurred in all areas *O. conchaphila* did, some other suspected predators did not. The gastropods (*N. lamellosa*) and sea stars (*Pisaster* spp.) in Coos Bay were largely restricted to seaward of *O. conchaphila*, and were hence less affected by low salinity in winter and spring. Unfortunately, most work on *O. conchaphila* predation has focused on nonindigenous predators such as the prosobranch gastropod *Ocinebrinellus inornatus (=Ceratostoma inornatum)* (Chew 1960) and the polyclad flatworm *Pseudostylochus ostreophagus* (Woelke 1956); no quantitative research has been conducted on native predators. Some parasites of *O. conchaphila* have been reported (Mix and Sprague 1970; Bradley and Seibert 1978), but none appear to be important at a population level. Thus, much remains to be done on the biotic hypothesis for high-salinity limitations on *O. conchaphila* populations.

There was little evidence to suggest that other factors strongly affected *O. conchaphila* population distributions. Two of the sites (Eastside and Downtown Coos Bay) were in the heart of the industrial and shipping zone, and at the Downtown Coos Bay site, many of the *O. conchaphila* in the middle stratum were attached to broken lead-acid battery casings. Current levels of pollution, therefore, did not seem to be affecting population distribution. Most hard substrata in Coos Bay appeared suitable for *O. conchaphila*, based on presence of attached individuals. Even in some highly depositional sites (Coalbank Slough, Figure 2), this species could become abundant simply by growing on shells of conspecifics. In Puget Sound, large populations of *O. conchaphila* occur in soft mud areas by growing on each other (Baker 1995). Thus, although the above factors may

still affect population density, they did not appear to affect *O. conchaphila* distribution, except on a very small scale.

How did Ostrea conchaphila re-invade Coos Bay?

As mentioned previously, one possible vector for the reinvasion of Coos Bay by *O. conchaphila* was the transfer of cultured Pacific oysters, *C. gigas*. Based on personal communications with Coos Bay oyster culturists in 1987, it is virtually certain that adult *O. conchaphila* occurred as fouling organisms on *C. gigas* transferred from other estuaries (especially Willapa Bay, Washington) to Coos Bay. It is not known how often this occurred, and because similar-sized specimens of the two species are similar in appearance, many inoculation of Coos Bay with *O. conchaphila* could have gone unreported. The introduction of a single adult female could, in theory, establish a new population, because larvae are brooded ovoviviparously (Baker 1995). This could produce scattered individuals on other substrates, such as those found in this study in 1987 in Haynes Inlet (Figure 1), but subsequent establishment of the species would require that these F1 individuals were in high enough density to overcome sperm dilution problems (Levitan and Petersen 1995).

We favor oyster culture as a reestablishment vector, not only because of recent evidence (the "smoking gun" of *O. conchaphila* occurring on *C. gigas* from other estuaries), but also because of recorded historical patterns of species introductions. Oyster shells present nearly ideal substrate for many settling invertebrates, including other oysters, and oyster transfers have been implicated in numerous biological invasions (Carlton 1979). Two alternate hypotheses for reestablishment will also be considered briefly, however: larval transport and rafting of adults.

A planktonic larval stage is the normal dispersal mechanism for marine benthic organisms, and *O. conchaphila* has planktonic larvae for up to two weeks (Baker 1995). A population of *O. conchaphila* has existed continuously, however, about 100 km north of Coos Bay, in Yaquina Bay, and failed to establish in Coos Bay prior to 1987. Other populations exist further away, both to the north and south (Baker 1995). This suggests both that planktonic larval transport is, at best, an unlikely mechanism for reestablishment, and that many estuaries with this species represent isolated populations. There is evidence from studies of the American oyster, *Crossoserea virginica*, that larvae can be retained in estuaries, possibly by vertical migration at different tidal phases (Mann 1988). *O. conchaphila* larvae may undergo a similar process in Coos Bay.

Adult rafting, on logs or on intracoastal shipping, is another possible invasion vector. Evidence from this study suggests that *O. conchaphila* attach readily to wood and bark, and a single floating log, in theory, could harbor tens of thousands of adults. In the past, drifting logs may well have been a natural vector for intracoastal invasion, but the current price of timber, plus the hazard that logs present to shipping, ensure that most stray logs are quickly harvested before they reach the ocean or linger in the water long enough for oysters to grow to maturity. Intracoastal shipping is poorly documented, even by port authorities, but Carlton and Hodder (1995) showed that fouling organisms on some vessel hulls (*e.g.*, barges) could present an intracoastal invasion vector. At present, this invasion by intracoastal shipping remains largely unstudied.

Human Alterations to Coos Bay

Throughout this century, the relatively small Coos Bay estuary has been heavily modified by humans. Some effect of two factors–dredging and filling–will be discussed here, along with their implications for *O. conchaphila* reestablishment and survival.

Dredging alters bathymetry, and bathymetry affects salinity in estuaries. In deep estuaries, low-salinity water flows seaward over a layer of deeper, relatively marine water. The thickness of the layer of reduced-salinity water remains constant, even as it entrains some of the deeper high-salinity water along the boundary of the two zones. This characteristic of estuarine mixing ensures a net landward flow of high-salinity water in deep zones–a phenomenon known as the salt wedge. Only a large increase in fresh water can entirely flush out the salt wedge, and then only if the estuary is shallow enough (Dyer 1997).

Coos Bay is geologically recent, and has been filling with both river and marine sediments since its formation following Holocene sea level rise (Roye 1979). Reducing the depth of the estuary would have reduced or eliminated the zone in which the salt wedge occurred. Navigational dredging projects since 1910 (Figure 5) reversed that trend, by increasing the depth of the main channel to 10.7 m at mean low

low tidal level (MLLW) by 1978, and to 11.3 m by 1997, for the entire length of the main portion of Coos Bay. This channel occupies a significant portion of Coos Bay, and permits deep, high-salinity water to penetrate far into the estuary.

The limited historic salinity data for northern Isthmus Slough, at the center of current *O. conchaphila* distribution, are consistent with the hypothesis that bottom salinity has increased as the main channel of Coos Bay has become deeper (Figure 5A and B). Baptista (1989) criticized the 1930-1931 data because they came from a drought period. If anything, however, this would increase salinity above normal historic levels, and strengthen our current argument. Rainfall in 1996 was above average, and about normal in 1997, so patterns of freshwater input cannot account for reduced salinities in 1996-97. Despite this, salinity was significantly higher in both spring and later summer, in Isthmus Slough, in 1996-97, compared to 1930-31 (Figure 5A and B). These observations, although consistent with predictions from channel deepening, are sparse, and further study is warranted.

The above trends suggest that human alterations have set back the geological clock for Coos Bay, and restored habitat for *O. conchaphila*. Dredging is normally considered an extreme form of disturbance. It appears likely, however, that dredging is also responsible for habitat "restoration," however accidentally, for a desirable native species.

Dredging also alters the tidal flushing regime of Coos Bay, as does filling and draining of intertidal areas. Proportional tidal flushing is the percent of estuarine water which drains to the open ocean with each tidal exchange. An unknown quantity of this water returns on the next incoming tide, but longshore currents ensure that a large part does not. All water over intertidal areas is, by definition, flushed at low tide, but in deep areas, a volume equal only to the tidal range is flushed. Filling intertidal areas, therefore, decreases the proportion of the water that will be removed at low tide, and deepening the channels increases the proportion of water that will be retained at low tide.

Tidal flushing is important to estuarine invertebrates with planktonic larvae, because larvae may also be flushed out of the estuary. Even if larvae have a mechanism for estuarine retention, as reviewed by Mann (1988), this mechanism is unlikely to be absolutely reliable. Flushing, therefore, represents a pressure on the population, and more flushing results in more larval loss.

The surface area of Coos Bay in 1890 was approximately 7706 ha at high tide and 2362 ha at low tide, with high tide and low tide volumes of approximately 181×10^6 m^3 and 91×10^6 m^3, respectively (calculated from NOAA Image Archives of Historic Map and Chart Collection, chartmaker.ncd.noaa/ocs). Mean tidal range at the mouth is about 2 m, resulting in a nearly twice-a-day flushing of 50.5% in 1890. In 1997, in contrast, high tide surface area had been reduced (by filling) to 4990 ha, but high tide volume had remained about the same (181×10^6 m^3), because the main channel was dredged deeper. Low tide surface area had remained the same, but low tide volume had increased to 111×10^6 m^3 (calculated from NOAA and U.S. Geological Survey charts), for a current mean tidal exchange of 60.7%.

A 10% difference in tidal flushing may not seem significant, but in a typical planktonic larval period of *O. conchaphila*, there will be about 40 such semidiurnal tidal exchanges (Baker, 1995), and the effects are multiplicative. In crude terms, this means that while only 2.12×10^{-7} % of the original water remains after 40 tidal cycles at present (discounting the unknown proportion of estuarine water which returns on each incoming tide), even less (1.35×10^{-10} %) remained in 1890.

Both of the above flushing rates appear effectively 100% over the larval cycle of *O. conchaphila*, unless there is, in fact, a mechanism whereby larvae are retained in estuaries. In this case, the difference between flushing rates, which could also be thought of as larval loss pressure, is over three orders of magnitude lower currently than in 1890. In light of this, it would be valuable to research whether *O. conchaphila* larvae resist estuarine flushing, and the mechanism involved.

Alternate Hypotheses

We would be remiss if we did not at least mention alternative hypotheses for the question: why did *O. conchaphila* invade when it did and not decades earlier? Pollution control efforts have been ongoing and progressive for several decades, and it would be gratifying if we could know that such efforts resulted in habitat restoration for a desired species. Unfortunately, there have been few toxicological studies on *O. conchaphila*, and some of those that exist have been tainted by conflict-of-interest (Baker, 1995). In any

case, based upon the distribution of metal-containing debris, *O. conchaphila* thrives in the midst of what is now the most polluted part of Coos Bay. On the other hand, decreasing pollution may act in concert with increasing salinity to improve habitat to the critical point where reestablishment can occur.

The greatest, and least testable, confounding factor in any biological invasion is the role of chance events. There must be inoculation, the inoculation must be of a sufficient number and condition of propagules, and environmental conditions in the host ecosystem must remain favorable. Each of this series of events has its own, separate probability, and can be difficult to estimate. Thus, while we believe we have presented a strong circumstantial case for dredging as the underlying cause for the return of *O. conchaphila* to Coos Bay, the fact that the species reestablished in about 1988, rather than in 1979 (immediately following the prior channel deepening), strongly suggests that a high level of stochasticity was also involved.

Conclusions

The reestablishment of the Olympia oyster, *O. conchaphila*, in Coos Bay, is a unique and historical event, not amenable (scientifically or legally) to experimentation. We believe, however, that we have assembled a reasonable case that *O. conchaphila*, a native species which had gone locally extinct through natural mechanisms, was reestablished by human vectors. Furthermore, the massive human alterations to Coos Bay have, if anything, actually improved habitat for this species. The changes caused by dredging and filling are consistent with the hypothesis that natural sedimentation of Coos Bay led to *O. conchaphila* extinction, while human alterations have partially restored *O. conchaphila* habitat lost by natural processes. This may also account for the failure of a concerted attempt to reestablish *O. conchaphila* in Coos Bay in 1917. In 1917, the channel had been only slightly deepened from natural conditions (Figure 5A and B), which would barely modify the salinity regime, and hence osmotic pressure on *O. conchaphila.*

It is not our intent to advocate either species introduction or large-scale human modifications of existing ecosystems. The example of *O. conchaphila* in Coos Bay, however, illustrates that simple rules about biological invasions and human disturbance will fall wide of the mark on many occasions. Every biological invasion and every human disturbance will result in some change to an ecosystem, but it is not always possible to measure these changes in terms of net harm.

Acknowledgements

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In Memoriam

Neil Richmond, our co-investigator, colleague, and friend, passed away in May 1999, while conducting field research off the Oregon coast. Neil had been with the Charleston branch office of the Oregon Department of Fish and Wildlife for two decades, and was intimately familiar with the Coos Bay estuary, its ecosystems, and its shellfish industries. He brought to our research a great deal of knowledge, skill, and above all, an appreciation of our natural resources. His loss is keenly felt.

P.B. and N.B.T.

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Bird Use of *Phragmites australis* in Coastal Marshes of Northern Massachusetts

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ABSTRACT: **One of the major management concerns regarding salt marshes on the east coast is the replacement of existing salt and brackish vegetation by common reed (*Phragmites australis*). This invasive grass thrives in areas where tidal flushing has been reduced by dikes, embankments, and undersized culverts. *P. australis* habitats are thought to be of substantially less wildlife value than the marsh vegetation they replace. There is, however, little documentation of this assertion. We carried out three seasons of quantitative bird censuses in *P. australis*, *Spartina* spp., and *Typha angustifolia* marshes in northern Massachusetts. Census methodologies included visual observation, passive listening, and playback techniques in 50-m-radius point count circles. Circles were selected that contained various amounts of forest, *P. australis*, *Spartina* spp., or *T. angustifolia*. The significance of correlations between the abundance of bird species detected anywhere within the circle and the plant communities present there were tested using Spearman's rank correlation coefficients. The significance of habitat preferences was also tested by comparing the availability of plant communities present in counting circles and the bird abundances detected in those plant communities. Ivlev deviations were used to test the significance of these preferences. Based on data from 1997, the number of bird species commonly encountered in each habitat differed, with *Spartina* spp. and *P. australis* marshes having the most and coastal *T. angustifolia* marshes the least number of species. The amount of *P. australis* present within each point-count circle had a positive effect on Redwing Blackbirds and had little impact on the numbers of Marsh Wrens observed in a circle. Marsh Wrens were negatively impacted by increases in the amount of either salt marsh or forest present. Saltmarsh Sharp-tailed Sparrows occurred more frequently in *Spartina* spp. marshes, and *Phragmites* had a non-significant negative effect on Virginia Rails, while cattails had a highly significant positive effect on them. Our study suggests that the most common birds in these northern Massachusetts marshes are unaffected by the presence of *P. australis*; however, future research that targets other seasons and a landscape analysis is needed.**

Key words: *Phragmites australis*, common reed, *Spartina*, *Typha angustifolia*, salt marsh, invasive plant, habitat value, bird survey, marsh bird, Ivlev deviation

INTRODUCTION

One of the major problems facing salt marshes on the east coast of the United States is the replacement of existing salt and brackish marsh vegetation by the common reed (*Phragmites australis* Cav.). This invasive grass thrives in areas where tidal flushing has been reduced by dikes, embankments, and undersized culverts. Researchers and resource managers often claim that *P. australis* habitats are of substantially less wildlife value than the marsh vegetation they replace (Roman *et al.* 1984; Jones and Lehman 1987; Hauber *et al.* 1991; Marks *et al.* 1994; Tiner 1995), however, data in support of this claim are limited. Apart from a recently published study of Connecticut salt marshes by Benoit and Askins (1999), we are aware of no quantitative work on the impact of *P. australis* invasions on marsh birds. In this paper, we report preliminary results of a study on how the presence of *P. australis* impacts the avian ecology of salt marshes in northeastern Massachusetts.

We have two goals: (1) to determine how *P. australis*-dominated habitats differ from other major vegetated coastal marsh habitats in terms of bird species composition and abundance, and (2) to predict what the effects on birds will be if this plant continues to spread. Our approach has been to compare bird abundances in coastal marsh study plots differing in their percent cover of *P. australis* and other major vegetation types. Our primary aim has been to document differences in bird abundances in four of the

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major habitats of the coastal zone; salt marsh, *P. australis* marshes, *Typha angustifolia* marshes, and adjoining woods. We have used three research strategies: (1) correlation of individual bird species' abundances with the percent cover of each of the four vegetation communities potentially found at each site, (2) use of an electivity index based on the relationship between the relative abundance of birds (by species) found within a specific plant community at each site and the availability of that plant community at that site, and (3) a comparison of the frequencies with which various stereotypical behaviors are recorded in the different plant communities.

Our study area encompassed coastal marshes on the east coast of the United States from southern New Hampshire (42° N, 71° W), through Boston Harbor (43° N, 71° W). Some of the marshes, such as Neponset Marsh near Boston, are in urban areas, often with relatively large areas of *P. australis*. Others, particularly the Parker River and Essex Bay salt marshes, the largest contiguous acreage of salt marsh north of Long Island, are still relatively undisturbed by human activity. *P. australis* occurs to some extent in all marsh systems investigated. Since *P. australis* typically invades and spreads from the oligohaline transition zone between salt marshes and upland, many of our observations were within vegetation communities at or adjacent to this transition zone.

Since increased abundance does not necessarily indicate that the habitat is of higher quality (van Horne 1983, Vickery *et al.* 1992a, b), we also carried out a preliminary assessment of those stereotypical behaviors that might indicate breeding in Red-winged Blackbirds, the only bird in our study area occurring in sufficient numbers to allow quantification of behaviors. We assume that abundance indicates the degree to which a habitat is important to the bird, but not whether that area is a source, a sink, or a "reservoir" of potential replacement breeders.

Methods

Selection of Vegetation Communities

The vegetation communities of New England salt marshes are relatively simple and easy to characterize. They are dominated by a few abundant species (primarily graminoids) that grow in dense patches (Niering and Warren 1980). The location of these communities is determined by marsh elevation in relation to tidal inundation. The four vegetation communities we compared for their avian species were *P. australis* marsh, salt marsh, cattail marsh, and adjacent forests. The first three were chosen because they are the dominant communities in terms of cover in coastal marshes in the region. The latter was chosen because many marsh birds make use of adjacent woodlands for part of their life cycle. The four communities are defined as follows:

Phragmites australis marsh contains close to 100% cover of *P. australis*. Canopy height in our study sites ranged from 1 to 2.5 m. In most cases *P. australis* grows as a monoculture with no understory; in others, some salt marsh or upland vegetation co-occurs.

Salt marsh is dominated by three salt marsh grasses, *Spartina patens* (salt marsh hay) and *Distichlis spicata* (spike grass) in high marsh (higher elevations), and *Spartina alterniflora* (salt marsh cordgrass) in low marsh. High marsh occurs immediately seaward of *P. australis* and cattail marshes and is flooded several days each month during spring tides. *Juncus gerardi* (black grass, actually a rush) grows where elevations are slightly higher. Slight depressions in the high marsh surface that are poorly drained contain a short growth form of *S. alterniflora*. Tall form *S. alterniflora* grows in areas regularly flooded during high tide, such as the banks of salt marsh creeks.

Cattail marsh in the coastal zone is almost a monoculture of *Typha angustifolia*. Since this species is restricted to lower salinities than plant species growing in the salt marsh, it occurs in higher elevations and where there are large freshwater inputs. Since the salinity range of cattails is similar to that of *P. australis*, it may be the habitat most vulnerable to *P. australis* invasion.

Forest consists of a variety of trees and shrubs growing immediately adjacent to the marsh. Major species include oaks, especially *Quercus ruber* (red oak), hickories (*e.g.*, *Carya glabra*), and *Amelanchier* spp. (shadbush). This plant community was included because a number of the bird species in this study move readily between the marsh and bordering upland.

Counting Circles

The use of counting circles is a standard way to census birds in grassland habitats (Järvinen 1978; Edwards *et al.* 1981; Hutto *et al.* 1986; Bibby *et al.* 1992; Sliwa and Sherry 1992; Ralph *et al.* 1995; Dettmers *et al.* 1999). It allows the observer to use both visual and auditory cues to determine the number of birds of each species present while also allow-

ing quantitative analysis of the habitat enclosed by each circle. The assumption is that, over a short observation period (10-30 min), a census of a circle represents the number of birds present at one point in time – hence the reference to a point count. Replication comes from periodic visits at predetermined intervals to the counting circle and by having replicate circles.

Birds were censused in 68-point count circles of 50-m radius each, with varying amounts of *P. australis*, cattails, salt marsh, and forest vegetation in each circle. Circles were selected to reflect a variety of conditions with respect to vegetation, marsh size, and soil moisture. Sites were selected without knowledge of the bird communities present, but were not selected randomly. Most salt marsh sites selected were relatively close to habitat edges, the zone of maximum potential *P. australis* invasion. No circles containing 100% forest were selected.

Bird Censuses

Each counting circle was censused every 2-3 wk (6-7 times/yr) from April to September in 1997 and 1998. Sites were surveyed from 0.5 hour before sunrise to 12 noon, and at all tides. Tides and times of day were distributed evenly among sites. Censuses of birds were carried out by a single observer from the middle of each counting circle. During each census, the observer recorded all birds seen or heard and the plant community in which they were first seen. We also recorded behaviors observed (*e.g.*, carrying nesting material or carrying food to young), distance from the observer, and the time of observation.

Because some birds are quite cryptic and the vegetation dense, playing tapes of bird songs and listening for auditory responses was included in each census. Bird songs of eight species were broadcast for a total of 16 min using a Johnny Stewart Bird and Animal Caller with booster. An initial 3 min without song broadcasts was followed by 30 sec of bird song and 30 sec of silence. This sequence was repeated twice for each species. Songs were broadcast in the following sequence: Song Sparrow (scientific names appear in Table 1), Clapper Rail, Swamp Sparrow, Sora, American Bittern, Virginia Rail, Least Bittern, and Marsh Wren. Song tapes were obtained from the Cornell Library of Natural Sounds, A Field Guide to Bird Songs (Peterson 1990), and Birding by Ear (Walton and Lawson 1989). Birds were counted as the observer approached the center of the circle, for the 3

Table 1. Common and scientific names for birds observed or mentioned in the text. 1997

Common Name	Scientific Name	Total/Yr.
European Starling	*Sturnus vulgaris*	1090
Tree Swallow	*Tachycineta bicolor*	1024
Red-winged Blackbird	*Agelaius phoeniceus*	909
Common Grackle	*Quiscalus quiscula*	163
Least Sandpiper	*Calidris minutilla*	155
Song Sparrow	*Melospiza melodia*	129
Yellow legs sp.	*Tinga* sp.	121
Saltmarsh Sharp-tailed Sparrow	*Ammodramus caudacutus*	105
Marsh Wren	*Cistothorus palustris*	104
Common Yellowthroat	*Geothlypis trichas*	68
Virginia Rail	*Rallus limicola*	44
American Goldfinch	*Carduelis tristis*	43
Eastern Kingbird	*Tryannus tyrannus*	40
Barn Swallow	*Hirundo rustica*	29
Glossy Ibis	*Plegadis falcinellus*	28
American Robin	*Turdus migratorius*	25
Cedar Waxwing	*Bombycilla cedrorum*	23
Black-capped Chickadee	*Parus atricapillus*	20
Eastern Towhee	*Pipilo erythrophthalmus*	19
American Black Duck	*Anas rubripes*	18
Baltimore Oriole	*Icterus galbula*	16
Gray Catbird	*Dumetella carolinesis*	15
Northern Mockingbird	*Mimus polyglottos*	13
Semipalmated Sandpiper	*Calidris pusilla*	12
Chipping Sparrow	*Spizella passerina*	10
Killdeer	*Charadrius vociferus*	10
Northern Flicker	*Colaptes auratus*	8
American Crow	*Corvus brachyrhynchos*	7
Mallard	*Anas platyrhynchos*	7
Mourning Dove	*Zenaida macroura*	7
Snowy Egret	*Egretta thula*	7
Tufted Titmouse	*Parus bicolor*	6
Blue Jay	*Cyanocitta cristata*	5
Canada Goose	*Branta canadensis*	5
House Sparrow	*Passer domesticus*	5
Purple Finch	*Carpodacus purpureus*	5
Yellow Warbler	*Dendroica petechia*	5
Northern Cardinal	*Cardinalis cardinalis*	4
Willet	*Catoptrophorus semipalmatus*	4
Brown Thrasher	*Toxostoma rufum*	3
Great Egret	*Ardea albus*	3
Green Heron	*Butorides virescens*	3
Semipalmated Plover	*Charadrius semipalmatus*	3
Eastern Bluebird	*Sialia sialis*	2
Seaside Sparrow	*Ammodramus maritimus*	2
Spotted Sandpiper	*Actitis macularia*	2
Belted Kingfisher	*Ceryle alcyon*	1
Black-crowned Night-Heron	*Nycticorax nycticorax*	1
Bobolink	*Dolichonyx oryzivorus*	1
Connecticut Warbler	*Oporornis agilis*	1
Red-breasted Nuthatch	*Sitta canadensis*	1
Ring-necked Pheasant	*Phasianus colchicus*	1
Scarlet Tanager	*Piranga olivacea*	1
Swamp Sparrow	*Melospiza georgiana*	1
American Bittern	*Botaurus lentiginosus*	0*
Clapper Rail	*Rallus longirostris*	0*
Least Bittern	*Ixobrychus exilis*	0*
Sora	*Porzana carolina*	0*

* Spp. song broadcast during playback. No individuals observed.

Table 2. Spearman Rank Correlation of bird abundance and plant communities – selected bird species. N=number/visit, over all sample points; r_S=Spearman's r; r_S and p are corrected for ties.

		Phragmites			Cattails			Salt Marsh			Forest		
Species	N	r_S	p		r_S	p		r_S	p		r_S	p	
Red-wing Blackbird	132	0.47	0.001	***	0.21	0.122	ns	−0.35	0.010	**	−0.12	0.368	ns
Song Sparrow	20	0.02	0.862	ns	0.03	0.837	ns	−0.07	0.601	ns	0.22	0.118	ns
Saltmarsh Sharp-tailed Sparrow	14	−0.01	0.920	ns	−0.05	0.724	ns	0.16	0.237	ns	−0.18	0.201	ns
Marsh Wren	14	0.14	0.325	ns	0.45	0.001	***	−0.46	0.001	***	−0.32	0.019	*
Common Yellowthroat	11	0.17	0.223	ns	0.11	0.413	ns	−0.40	0.004	***	0.75	0.000	***
Virginia Rail	7	−0.04	0.801	ns	0.48	0.001	***	−0.23	0.092	ns	−0.19	0.161	ns

min of silence, and for the 16 min of playbacks. Where vegetation was tall, surveys were carried out from 6 to 10-ft stepladders.

Vegetation Analysis

Vegetation analysis was carried out from mid-September to mid-November, using two separate techniques. The major plant communities (*P. australis*, salt marsh, cattail, forest) were directly mapped using a compass and a Bushnell Yardage Pro to measure distances (accuracy = ± 1 m). Distances and bearings were measured to the central stake and the resulting coordinate system was used to draw the resulting polygons in their respective places within the study plot. Polygon areas, and thus the percent of each site covered by each plant community, were calculated from these drawings. In addition to the maps, ten 0.25-m^2 quadrat frames were located randomly within each plant community using the same coordinate system as for the maps. Percent cover of all plants within the frame, stem densities, plant heights, and percent reproductives of *P. australis* and *Typha*, when present, were measured. Only the results of the mapping studies are used here.

Statistical Procedures

Bird Abundances

The effect of differences in the percent cover of each of the four plant communities within a counting circle on the abundance of birds detected in that counting circle was evaluated by the magnitude and sign of the Spearman's rank correlation coefficient (r_S), and its significance tested with the associated p-value (both corrected for ties) (Sokal and Rohlf 1981). Bivariate plots were used to assess the adequacy of a linear correlation. Both means and maxima were used to characterize species abundances (N) at each site. Means tended to be low, especially when several of the visits detected few to no birds of a given species. An alternative is to use the maximum number detected over all visits to a given site during each year (Benoit and Askins 1999; Greg Shriver, pers. comm.)– this leads to substantially higher N's. Both methods were used.

Bird Species Richness

We related bird species richness (*i.e.*, the number of species recorded) within individual counting circles to the presence of the four different vegetation communities in two ways: (1) by comparing the number of bird species present within each counting circle to the percent cover values of each vegetation community within that circle, and (2) by characterizing each counting circle as belonging to a single "site plant community" based on the extent of dominance of that community. Two definitions of dominance were tested. Sites were considered as belonging to a particular plant community if either >50% or >75% of the site was covered by that plant community. The effect on species richness was analyzed separately for each definition.

Electivity

In addition to correlating the abundance of birds detected anywhere in the circle to plant communities found there, we quantified "preference" and "avoidance" by comparing the percent of each bird species detected within each plant community to the percent of the study circle occupied by that plant community. The expectation under the null hypothesis is that the proportion of a given bird species detected in a given plant community will not differ significantly from the proportions of that plant community available in the study circle. The significance of deviations

Table 3. Spearman Rank Correlation of bird species richness and plant communities. r_s =Spearman's r; r_s and p are corrected for ties

Phragmites			Cattails			Salt Marsh			Forest		
r_s	p		r_s	p		r_s	p		r_s	p	
0.06	0.639	ns	0.21	0.126	ns	−0.30	0.028	*	0.56	>0.000	***

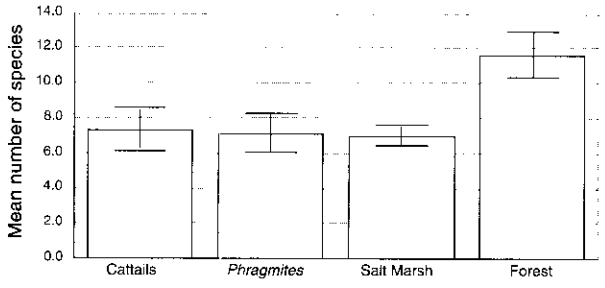

Figure 1A. Species Richness-mean number of species per sample point. Dominance defined as >50% (see Methods). Error bars=±2 SE

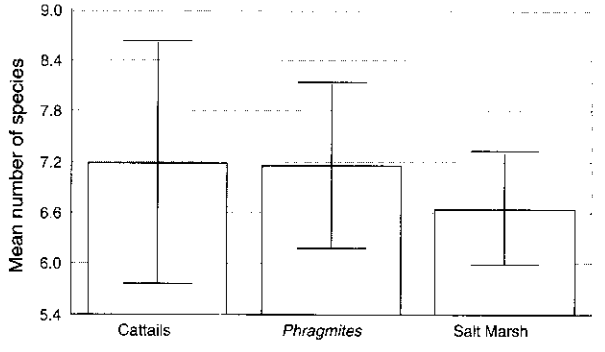

Figure 1B. Dominance defined as >75% (see Methods). In no circles did "Forest" cover >75%. Error bars=±2 SE

from expectation can be tested. The electivity index used is a modification (J. Ebersole, pers. comm.) of the Ivlev deviation (Ivlev 1961), which we have called a "weighted Ivlev deviation." The standard formula for the Ivlev Deviation (Ivlev 1961) is: (O−E)/(O+E), where O=Observed, and E=Expected.

This formula has been weighted by multiplying it by E/E_{max}, where E_{max}=maximum E/site (see also Jacobs 1974; Strauss 1979; Johnson 1980). Significant positive deviations are evidence of active selection or preference, significant negative deviations, of avoidance. Results for Marsh Wrens for 1997 are presented here.

Results

Avian community structure was highly dominated by Red-winged Blackbirds in all plant communities. Marsh Wrens occurred roughly equally in *P. australis* and cattails but were absent or nearly absent in salt marsh and woods. Saltmarsh Sharp-tailed Sparrows were far more abundant in salt marsh, while Virginia Rails were most abundant in cattails, and less so in *P. australis* or salt marsh.

Correlation Studies – Bird Abundance

The Spearman rank correlation coefficients indicate a strong and positive association between *P. australis* and Red-winged Blackbirds and no association with the abundances of Song Sparrows, Saltmarsh Sharp-tailed Sparrows, Marsh Wrens, Common Yellowthroats, or Virginia Rails (Table 2). Using maxima rather than means produced the same results. Shorebird species, while among the most abundant species encountered (Table 1), were dependent on the presence of salt marsh pannes, and not on the percent cover of any plant community within the circle. As the amount of *P. australis* present was uniformly low for all locations where shorebirds were encountered, the percent of variation in shorebird abundance explained by the percent cover of *P. australis* was also low. The correlation of shorebirds with percent *P. australis* was therefore poor. This conclusion is an artifact of using univariate correlations. (Multivariate analyses are in preparation.)

Correlation Studies – Bird Species Richness

Species richness of birds was not significantly affected by the amount of *P. australis* or cattails within counting circles (Table 3). Bird species richness was slightly reduced as the percent of salt marsh increased. In contrast, species richness was dramatically increased as the percent of forest within a counting circle increased. When each counting circle was assigned to a single "site plant community", forests remained the only plant community that had a significant effect on species richness (Figure 1). This remained true whether > 50%, or >75% was used to determine dominance.

Electivity Indices

Preference for, or avoidance of, a plant community by Marsh Wrens as measured by the weighted Ivlev deviation, is illustrated using 1997 data

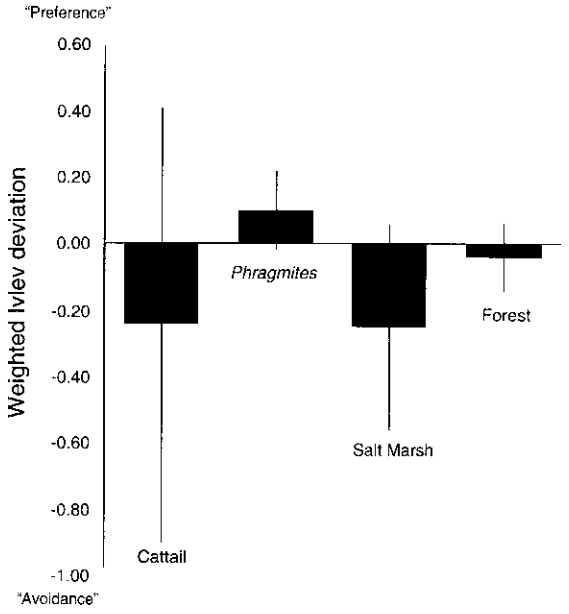

Figure 2. Marsh Wren preferences as detected by weighted Ivlev deviations (error bars=±2 SE).

(Figure 2). All error bars enclose "0", indicating that no significant plant community preferences were detected for this species.

Behavioral Observations

Behavioral observations on Red-winged Blackbirds revealed a preference for *P. australis*. In most classes of behavior except foraging, frequencies of occurrence were far greater in *P. australis* than in most other plant communities (Table 4).

Discussion

The goal of this paper is to quantify the effect of *P. australis* on the avian ecology of the coastal zone in northern Massachusetts. Using playback techniques in point count circles and bivariate correlations, we conclude that changes in percent cover of *P. australis* has a positive effect on the abundance of Redwinged Blackbirds and no effect on Song Sparrows, Saltmarsh Sharp-tailed Sparrows, Marsh Wrens, Common Yellowthroats or Virginia Rails. No significant negative effects on abundance were detected. Only the percent cover of forests in the counting circle had a significant effect on species richness. Benoit and Askins (1999), on the other hand, report that in coastal Connecticut the bird communities encountered in *P. australis* marshes were distinctly different from those found in short-grass salt marsh meadows (*i.e.*, salt marsh exclusive of tall-form *S. alterniflora*). *P. australis* marshes had significantly fewer Virginia Rails than were encountered in cattail or brackish marshes, and significantly fewer Saltmarsh Sharp-tailed Sparrows than were encountered in short-grass salt marsh. All Willets were found in salt marsh. In Connecticut, Seaside Sparrows, Snowy Egrets, Green Herons, Least and Semipalmated Sandpipers, Mallards, and American Black Ducks were entirely absent from *P. australis* marshes. Species found in *P. australis* marshes were more often habitat generalists, while species absent from *P. australis* marshes were dependent on salt marsh. Species richness, especially of those species listed by the state of Connecticut as being of management concern, was also significantly lower in *P. australis* marshes.

In north coastal Massachusetts, Saltmarsh Sharp-tailed Sparrow abundance does not appear to be significantly affected by percent cover of *P. australis*, but the number of individuals encountered was low. Only four Willets were encountered in our study area. Breeding populations of Seaside Sparrows are rare in north coastal Massachusetts (Marshall and Reinert 1990): only two individuals were encountered. Egrets and Herons may be underrepresented in both studies as the methodology used in both studies

Table 4. Relationship of Red-Winged Blackbird behaviors to plant species dominant in counting circles.

A. evidence of pairing (N=34) plant community observed in	frequency
Cattail	4
Phragmites	18
Salt Marsh	7
Forest	5
B. food or nesting material in bill (N=25) plant community observed in	**frequency**
Cattail	3
Phragmites	11
Salt Marsh	10
Forest	1
C. aggressive behaviors* (N=68) plant community observed in	**frequency**
Cattail	9
Phragmites	36
Salt Marsh	13
Forest	10

* displace, chase, sexual chase, song spread (Orians and Christman 1968), attacks on observer

may have seriously biased estimates of Heron and Egret abundance (Benoit and Askins 1999, and below). Even when our circles contained shallow pannes, the preferred feeding area for these birds (Brush *et al.* 1986), nearly all Herons and Egrets were observed considerably outside of our study circles. These birds appear more sensitive than passerines to disturbance by the observer. Censusing in full view, within 50-m circles, while broadcasting bird song appears to seriously underestimate the abundance of these birds. Least and Semipalmated Sandpipers, Mallards, and American Black Ducks depend on the presence of shallow pannes or deep pools. Benoit and Askins (1999) report significantly higher species richness of state-listed species at sites in Connecticut that have pools. We did not encounter pannes or pools in *P. australis* marshes with sufficient frequency to test their effect. The effect of *P. australis* on the abundance of pannes and pools, and on their usefulness to panne- and pool-dependent species, remains to be studied.

Pribil (1998) argues that the correlation of abundance data with environmental features such as percent cover, without regard to the availability of that feature in the habitat, can lead to false conclusions of "no effect" when these features are not limiting. We have used a weighted Ivlev deviation to test the effect of resource availability on habitat selection. For Marsh Wrens, the only species tested to date, our conclusions of "no effect" of *P. australis* on abundance remains unchanged.

Van Horne (1983) argues that abundance alone is a poor indicator of habitat quality. Vickery *et al.* (1992b) suggest the use of stereotypical behaviors associated with breeding, when direct measures of breeding success are not available. We attempted to do this, but were successful only in collecting sufficient data for Red-winged Blackbirds. Among the plant communities surveyed, the behavior data indicated a preference of Red-winged Blackbirds for *P. australis* habitats for breeding. This is in agreement with the abundance data.

Our point count circles were not selected randomly; however, given the diversity of treatments and analytic techniques described, we are confident of the robustness of our results. The 68 sites surveyed contained a wide range of percent cover of each of the plant communities studied, and were situated in a variety of matrices. *P. australis* sites were located in *P. australis* stands of various sizes and at various distances from the habitat edge. Most sites of all plant types were in moderate-density suburban communities, but several were within high-density urban areas. Agreement between different methods of calculating correlations between species abundance and the percent of a plant community present also demonstrate the robustness of these conclusions. Whether birds were counted anywhere in the circle, as in the correlation studies, or, as with the electivity index, associated only with the plant community in which they were observed produced the same results. Likewise, our results were unaffected by whether species richness was calculated as a continuous or a categorical variable, or whether >50 % or >75% cover was used to characterize study circles.

Conclusions of "no effect" are always hard to demonstrate. When the number (N) is low, "no effect" can easily be due to the low power of the test. Using maxima rather than means dramatically increases N, appearing to increase the power of the test. However, given the long period of bird observations in our study, from April to September, we did not think that the use of maxima was appropriate. Low N may explain why two marsh species, Saltmarsh Sharp-tailed Sparrow and Virginia Rail, demonstrated no significant negative relationship to the amount of forest present. Failure of bird abundances to respond to changes in the percent cover of a plant community can also be due to the scale of measurement. Fifty-meter study circles enclose only about two acres. The robustness of our conclusions is supported, however, by the number of strong patterns detected. Despite low sample sizes, Virginia Rails had a strong, positive, and highly significant correlation with cattails. Common Yellowthroats, also detected in low numbers, had a highly significant Spearman rank correlation coefficient with percent forest. It would be inconsistent to accept findings where there was strong association and reject those findings where there was none.

Management

The impact of *P. australis* on birds has management implications. Ongoing salt marsh restoration projects in New England will likely result in the loss of significant areas of *P. australis* marshes. Benoit and Askins (1999) found no bird species of management concern significantly associated with *P. australis* habitats and therefore found no impediments to its removal. They did indicate that its removal would

benefit several species of management concern in Connecticut. While we found no reason *for* its removal, we found no critical use by species of management concern that would present impediments *to* its removal in north coastal Massachusetts. Both our study and the study of Benoit and Askins, however, only censused within the breeding season or immediately before or after it. Unpublished data indicate that American Bitterns are making regular use of *P. australis* stands in both Massachusetts and New Hampshire during November and December, sometimes in urban environments (R. Donovan, R. Kleiman, and T. Diers, pers. comm.). The American Bittern is a state-listed bird in Massachusetts. We need to know if American Bittern population size is limited by non-breeding habitat loss, and what alternatives exist in the region. Salt marsh restoration is going on in a changed, and often urbanized, context. Before we remove degraded salt marsh habitats, we need to know that alternatives to the habitats we remove still exist in the landscape. We have no quantitative information on this at this time.

Future Work

Several topics for future work remain. First, *P. australis* invasions may lead to increased elevation and drying of the marsh surface through peat and sediment accumulation, and possibly lead to the filling-in of pannes or ponds. Such marshes are likely less suitable habitat for waders, shorebirds, and waterfowl. Second, dense, tall *P. australis* stands may also make ponds and pannes less accessible to those species that depend on them. Quantitative work on these issues is needed. Third, while we sited study circles in a variety of matrices, an explicitly landscape-level study is still needed. The effect of differences in size, shape, and isolation of *P. australis* stands is unknown. Likewise, the effect of the landscape context is also unknown. Buchsbaum (1994), for example, suggested that *P. australis* marshes surrounded by urban development may function differently from those in other contexts, providing local refugia, or acting as buffers from human disturbance. Fourth, long-range research is needed on how *P. australis* invasions affect marsh processes and bird populations over longer time scales than the two years of the present study. Fifth, *P. australis* in the Massachusetts coastal zone may be playing an important stabilizing role in Red-winged Blackbird populations during the breeding season. We observed flocks of several thousand non-breeding Red-winged Blackbird males in *P. australis* during September 1997 and April 1998. When lost to predation, Red-winged Blackbird territory-holding males are quickly replaced from such flocks (Yasukawa 1981; Yasukawa *et al.* 1982; Beletsky and Orians 1993). The role of *P. australis* in stabilizing these populations needs further investigation. Finally, and most critically however, work is needed on the role of *P. australis* in non-breeding avian ecology. Large numbers of Tree Swallows use *P. australis* for pre-migration roosts, and American Bitterns may have come to depend on it during fall migration. The effect of changes in the availability and location of *P. australis* stands on the conservation of American Bitterns is especially important.

Acknowledgments

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Microsatellite DNA Analysis of Native and Invading Populations of European Green Crabs

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Key words: *Carcinus*, population bottleneck, population genetics, microsatellite DNA

INTRODUCTION

The analysis of molecular genetic variation can potentially provide important insights into the invasion process, including identification of source populations, estimation of the number of founding individuals contributing to an invasion, and assessment of population dynamics following the initial introduction. To date, this potential has not been adequately explored as the majority of genetic studies have utilized molecular marker systems with relatively low levels of polymorphism (*e.g.*, allozymes) or single locus systems with large associated sampling error (*e.g.*, mitochondrial DNA). More recently developed molecular markers such as microsatellite DNA appear to be better suited for analysis of invading populations. Microsatellite DNA tends to be hypervariable, thus even introduced populations that undergo severe bottlenecks are expected to be polymorphic. In addition, microsatellite DNA is ubiquitous in eukaryotic genomes, so many independent markers can be assessed.

The worldwide invasions of green crabs in the genus *Carcinus* provide an excellent model system to evaluate the utility of a molecular genetic approach for the study of marine invasions. Green crabs native to Europe have invaded six regions in the last two centuries: Australia, Tasmania, eastern North America, western North America, South Africa, and Japan. Recent assessments of mitochondrial DNA variation for green crabs (Geller *et al.* 1997; McElligott and Geller, this volume) indicated the presence of two sibling species in Europe, *Carcinus maenas* on the Atlantic coast and *C. aestuarii* in the Mediterranean Sea. Interestingly, introduced populations in Japan and South Africa possessed mitochondrial haplotypes characteristic of both *C. maenas* and *C. aestuarii*, suggesting that these sites had been multiply invaded.

RESULTS AND DISCUSSION

We developed five microsatellite DNA markers for green crabs to complement and extend the mitochondrial DNA analyses. Our objectives for the microsatellite analysis were several fold: (1) to evaluate the hypothesis of cryptic *Carcinus* invasions posed by Geller *et al.* (1997) using an independent set of molecular markers; (2) to determine whether successful invasions were accompanied by a significant loss of genetic variability, and (3) to refine estimates of the geographic sources of each invasion.

Despite large genetic variability in native crabs (Figure 1), we were unable to detect significant genetic differentiation between sites on the Atlantic coast of Europe (western Spain and Netherlands) nor between sites in the Mediterranean Sea (southern France and Italy). Large allelic frequency differences were observed between native populations in the Atlantic Ocean and the Mediterranean Sea for most loci, and nearly fixed differences in the sizes of microsatellite alleles were observed at locus CM9 (Table 1). These microsatellite results supported earlier conclusions based on mitochondrial DNA analysis that Mediterranean and Atlantic populations repre-

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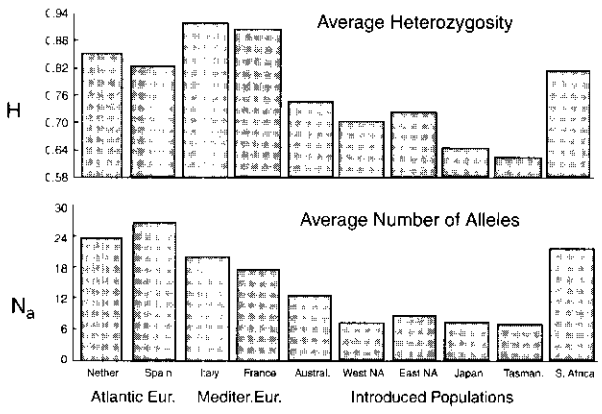

Figure 1. Measures of genetic diversity in native (Netherlands, Spain, Italy, France) and introduced green crab (*Carcinus maenas* and *C. aesturarii*) populations based on analysis of five microsatellite DNA loci.Top: Average expected heterozygosity per locus (H); bottom: average number of alleles per locus (N_a) for each population sample.

sent sibling species, but did not provide further resolution of the native source regions for invading populations.

Invasions were accompanied by large reductions in average heterozygosity and the average number of segregating alleles per locus (Figure 1). Average heterozygosity for introduced populations was 7 to 31% less than for native populations, indicating that most introduced populations underwent relatively severe genetic bottlenecks. The population in South Africa was exceptional in that it retained much more diversity than other introduced populations (Figure 1). The number of segregating alleles in introduced populations was drastically reduced, and similarities in the distributions of remaining alleles indicated that western North America and Tasmania, the two regions that were most recently invaded, had been invaded by green crabs originating from introduced populations in eastern North America and Australia, respectively.

Introduced populations in Australia, Tasmania, eastern North America, and western North America possessed allelic distributions that were characteristic of *C. maenas*, the Atlantic form of green crab. Allelic distributions for the highly diagnostic locus CM9 revealed no evidence of *C. maenas* influence in the Japanese samples; however, 14% of the alleles were of a unique size class not observed previously in either form of native crab. Since Japanese crabs also demonstrated very little genetic variability relative to native populations (Figure 1), it appears unlikely that Japan was multiply invaded by both *C. maenas* and *C. aestuarii*, as suggested by Geller *et al.* (1997). The presence of mitochondrial haplotypes characteristic of both *C. maenas* and *C. aestuarii* in the Japanese population was likely a consequence of invasion from a single source population possessing both mitochondrial haplotypes. The South African population, which was also found to possess mitochondrial haplotypes of both *Carcinus* species (Geller *et al.* 1997), possessed microsatellite alleles that were characteristic of both sibling species (Table 1). In addition, the South African population was much more genetically diverse than other introduced populations (Figure 1), which is consistent with an hypothesis of multiple invasions from distinct sources. Thus, the microsatellite data appear to support previous mitochondrial data in suggesting that South Africa has been invaded by green crabs more than once. There was no evidence for cosegregation of microsatellite alleles and mitochondrial haplotypes in South Africa, suggesting that the two forms have interbred.

These results demonstrate the power of microsatellite DNA analysis for studying marine bioinvasions. Our data indicate that green crabs were able to successfully invade several regions despite losing large amounts of genetic variation and suggest that a new trend is emerging in which prior introductions have become stepping stones for new invasions.

Table 1. Frequencies of microsatellite alleles at locus CM9 for green crabs (*Carcinus maenas* and *C. aestuarii*) in Atlantic Europe (Netherlands, Spain), Mediterranean Europe (France, Italy) and six invaded regions. Green crabs from Atlantic Europe and most invaded regions possess alleles that are predominantly 272 base pairs or smaller. Green crabs in Mediterranean Europe and Japan possess alleles that are predominantly 300 base pairs in size or larger. Two alleles identified in Japan were intermediate in size to alleles observed in native crabs.

	Allele Size Range (base pairs)		
Locale	198-272	276-280	300-360
Netherlands	0.99		0.01
Western Spain	1.00		
Southern France	0.06		0.94
Italy	0.07		0.93
Australia	1.00		
Tasmania	1.00		
Eastern North America	1.00		
Western North America	1.00		
Japan		0.14	0.86
South Africa	0.93		0.07

Genetic characterization of native and introduced populations of additional marine bioinvading species is likely to be a fruitful research approach and will allow assessment of the generality of our results.

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The Use of Molecular Genetics to Investigate the Geographic Origin and Vector of an Invasive Red Alga

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ABSTRACT: **In 1996, a large red alga, *Grateloupia doryphora*, was recorded for the first time in Narragansett Bay, Rhode Island. Since its arrival, it has continued to spread and will likely have an effect on native biota. In an effort to identify the geographic origin and vector of the Rhode Island *G. doryphora* population, RAPD (randomly amplified polymorphic DNA) analyses and sequences of the nuclear ribosomal DNA internal transcribed spacer (ITS) region were used to compare *G. doryphora* individuals from Rhode Island with specimens from locations around the world. The RAPD and ITS data sets were highly congruent. These genetic markers revealed low levels of genetic variation within the Rhode Island *G. doryphora* population as well as within the populations located in Brittany, France, and Galicia, Spain. However, there was considerable genetic variation partitioned among populations. *G. doryphora* individuals in the Rhode Island population were genetically similar to specimens from Brittany, France; Portsmouth, England; and to some specimens from the Mediterranean, suggesting that one of these locations could be the origin of the Rhode Island population. Individuals in the Rhode Island population were genetically distinct from the Galicia, Spain; Oregon, USA; and other of the Mediterranean specimens. Additional specimens from other geographic locations are currently being screened.**

Keywords: *Grateloupia doryphora*, red alga, origin of invasive species, ITS sequences, RAPDs

INTRODUCTION

Until recently, marine biological invasions by algal species have received little attention (*e.g.*, Walker and Kendrick 1998; Baskin 1996; Ribera and Boudouresque 1995; Farnham 1980). Algal invasions are of great concern because introduced species can have serious ecological consequences by reducing or replacing native macroalgae, thus leading to changes in community structure and food webs (Walker and Kendrick 1998). Efforts to stop or reduce marine bioinvasions are facilitated by a knowledge of the origin and vectors responsible for the introduction. Although these have often been difficult to identify by traditional methods (Farnham 1980), molecular techniques that use DNA characters as genetic markers may make it easier to identify and trace the origin of invasive species.

In 1996, *Grateloupia doryphora*, a large red alga (Figure 1), was found attached to rocks, pebbles, and shells in the southern portion of Narragansett Bay, Rhode Island (Villalard-Bohnsack and Harlin 1997).

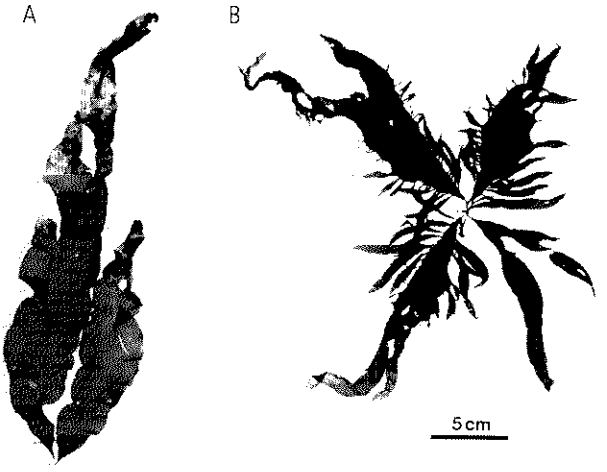

Figure 1. *Grateloupia doryphora* herbarium specimens. (A) Thalli showing foliose, undivided habit. (B) Single specimen showing basal division and marginal proliferations.

This was the first time this species had been recorded on the northeast coast of North America. Since 1996, the population has spread to new locations within Narragansett Bay and to several sites along the open ocean (Villalard-Bohnsack and Harlin submitted). The present distribution of *G. doryphora* includes the Pacific Ocean, Mediterranean Sea, and Atlantic

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Table 1. Sources of *Grateloupia doryphora* specimens used in the present study.

Collection site	Code	Collector (Date)	# Individuals Screened RAPDs	ITS
Narragansett Bay, Rhode Island, USA	R	M. Villalard-Bohnsack, M. Marston (1996-99)	10	1
Brittany, France	F	J. Cabioch (1997-98)	5	3
Portsmouth, England	E	W. Farnham (1998)	2	2
Galicia, Spain	S	J. Pérez Cirera López-Niño (1997)	3	2
Seal Rock, Oregon, USA	O	G. Hansen (1998)	1	1
Basin of Thau, (Mediterranean) France	M	M. Verlaque (1998)	4	3

Ocean from the English Channel to Angola and from Florida to Uruguay (Farnham and Irvine 1973; Dawson *et al.* 1964; Ardré and Gayral 1961). This species has repeatedly been reported to be invasive. In 1969, specimens were observed in Portsmouth, England (Farnham and Irvine 1973); in 1982 it was recorded in the Mediterranean Sea (Ribera and Boudouresque 1995; Maiz *et al.* 1986); and, more recently, the species has been reported on the coast of Brittany (Cabioch *et al.* 1997) and in the Netherlands (Stegenga and Otten 1997). It is unclear how long *G. doryphora* may have been present in the other locations along the coasts of Europe and Africa.

Considerable morphological variation in blade shape and size exists within and among populations of *G. doryphora*. Blades, growing singly or in clumps, can range from 2 to 40 cm across and up to 2 m in length. Few morphological or anatomical diagnostic characteristics are available to distinguish specimens from different geographical locations. The origin of the Rhode Island population, therefore, would be almost impossible to determine based solely on morphology and anatomy. Nevertheless, morphologically indistinguishable populations may be genetically very divergent (van Oppen *et al.* 1996a; van Oppen *et al.* 1995a). Molecular genetic techniques such as microsatellites, randomly amplified polymorphic DNA (RAPDs), and DNA sequence analysis are making it easier to identify biogeographic populations and to track introduced populations. These genetic tools have been widely applied in the study of algal phylogeography (*e.g.*, Pillmann *et al.* 1997; Vis and Sheath 1997; Lindstrom *et al.* 1996; Bakker *et al.* 1995), migrations (*e.g.*, van Oppen *et al.* 1995b), and recently to the study of the origin of introduced species (*e.g.*, Jousson *et al.* 1998; Olsen *et al.* 1998). In this study, we examine whether molecular genetic techniques could be used to identify the geographic origin of the recently established *G. doryphora* population in Rhode Island. A knowledge of the geographic origin may allow us to identify the vector responsible for the introduction and aid efforts to prevent future introductions.

Material and Methods

Specimens and DNA Isolation

Sources of material used in our analyses are summarized in Table 1. DNA was extracted from 3-cm^2 sections of thalli. Tissue was carefully examined for the presence of epiphytes prior to use. DNA was extracted from fresh tissue, herbarium specimens, or silica-gel dried material using the CTAB method (Doyle and Doyle 1987). Tissue was ground to a powder in liquid nitrogen using a mortar and pestle and then mixed with 6 mls of hot (65°C) CTAB isolation buffer [50 mM Tris-HCL (pH 8.0), 700 mM NaCl, 10 mM EDTA, 3% (w/v) CTAB (SIGMA)]. After an hour incubation at 65°C, samples were cooled at room temperature for 10 min and then extracted with 5 ml of chloroform-isoamyl alcohol (24:1; v:v) for 10 min and centrifuged in a table-top clinical centrifuge to separate phases. After centrifugation, the aqueous phase was removed with a wide-bore pipet and DNA was precipitated by adding 2/3 volume of cold isopropanol. To assist in precipitation, samples were sometimes placed in a -20°C freezer for up to 2 hr. DNA was collected via centrifugation. The DNA pellet was washed twice with 76% ethanol containing 10 mM ammonium acetate to remove the CTAB. After air drying, the pellets were resuspended in either ddH_2O or 10mM Tris-HCL (pH 8.0), 1 mM EDTA. DNA was analyzed on a 1% agarose gel.

RAPDs

Oligonucleotide primers of 10 bases were used to prime PCR reactions. Amplifications were performed in 50-µl volumes containing *Taq* reaction buffer (BRL), 1.9 mM MgCl, 100 µM dNTPs (BRL), 0.2 µM primer, 1.25 units *Taq* DNA polymerase (BRL), and 100-200 ng of template DNA. Mineral oil was added to each reaction tube prior to amplification. Reactions were carried out in a Thermolyne Temp•Tronic thermocycler with an initial denature

Table 2. Average within- and between-population RAPD genetic similarities based on Nei & Li's similarity coefficient. Bold numerals represent within-in population averages. A dash represents that only a single individual was examined.

Population	Rhode Island	France	England	Mediterranean[a] (M4)	(M1,M2,M3)	Spain
Rhode Island	**0.98**					
France	0.89	**1.00**				
England	0.67	0.74	**0.75**			
Mediterranean[a]						
(M4)	0.78	0.93	0.67	—		
(M1, M2, M3)	0.23	0.26	0.23	**0.27**	**1.00**	
Spain	0.24	0.27	0.16	0.29	0.28	**0.98**

[a] Two distinct banding patterns were observed in the Mediterranean population. Specimen M4 was genetically very distinct from specimens M1, M2, and M3.

step of 10 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 35°C, 3 min at 72°C and a final extension for 7 min at 72°C. All RAPD reactions were repeated at least once for each template/primer combination. Reaction mixes without DNA template were run as negative controls to check for contamination. After amplification, reaction products were separated by electrophoresis through a 1.0 or 1.2% TBE agarose gel stained with ethidium bromide and visualized and photographed under UV light. Presence or absence of bands was scored for all major bands. Minor or weak bands can be variable in expression, perhaps because they are products of nonspecific binding (Patwary *et al.* 1993); thus, they were excluded from the analyses. A data matrix containing the presence or absence of bands was generated and used to calculate pairwise RAPD similarities between each of the specimens using the Nei and Li's similarity coefficient: $S = 2N_{ab}/(N_a + N_b)$ where N_a is the number of amplified bands from specimen a, N_b is the number of amplified bands from specimen b, and N_{ab} is the number of matched bands between the two specimens.

ITS sequences

The primer pair TW81 (5'GGGATCCTTTCCGTAGGTGAACCTGC3') and AB28 (5'GGGATCCATATGCTTAAGTTCAGCGGGT3') was used to amplify the ITS1, 5.8S ribosomal DNA (rDNA), and ITS2 region (Goff *et al.* 1994). TW81 anneals to the 3' end of the 18S rDNA , while AB28 anneals to the 5' end of the 25S rDNA. Amplifications were carried out in 50-µl volumes as described in Goff and Moon (1993). The reaction mix contained *Taq* reaction buffer (BRL), 1.5 mM MgCl, 100 µM dNTPs (BRL), 0.2 µM of the TW81 primer, 0.2 µM of the AB28 primer, 1.25 units *Taq* DNA polymerase (BRL), and 50 - 250 ng of template DNA. Each reaction contained DNA from one individual. For each set of reactions, a control sample containing all reagents but lacking template DNA was included. The following cycling parameters were used: denaturation for 10 minutes at 97° C, 37 cycles of 95°C for 1.25 min, 68°C for 2 min, 72°C for 4 min, and a final 10-min extension at 72°C.

Products of all PCR reactions were visualized on a 1% agarose gel and the products from three different PCR reactions all containing the same primer/template combination were pooled prior to cloning. Products were cloned using a TOPO TA cloning kit (Invitrogen) following the vendor's instructions. Plasmid DNA was isolated using Qiagen-tip 20 or GFX *Micro* Plasmid Prep (Pharmacia Biotech). For each individual, two to three clones were sequenced using a ABI Prism 377 automated sequencer. Sequences were aligned using ClustalW version 1.7 (Thompson *et al.* 1994). Using the aligned sequences, nucleotide distances were calculated following the Kimura two-parameter model (Kimura 1980) and parsimony analysis was performed using the PAUP software package (Version 4.0b4a; Swofford 1991).

Results

RAPDs

Thus far, four arbitrary primers have been used to compare banding patterns among *G. doryphora* individuals from Rhode Island, Spain, France, England, and the Mediterranean. The major banding pattern for a particular combination of primer and template DNA was reproducible in repeated amplifications. The four primers amplified a total of 38 loci, 36 of which were polymorphic. Bands ranged in size from 500 to 2000 bp.

The RAPD banding patterns of individuals from the same population were very similar to one another, with similarity coefficients ranging from 0.75 to 1.00, where 1.00 indicates that the patterns are identical and 0 indicates that there are no bands in common (Figure 2, Table 2). The one exception was the Mediterranean population where two distinct banding patterns with a similarity coefficient of 0.27 were observed (Figure 2).

Even though there was little genetic variability

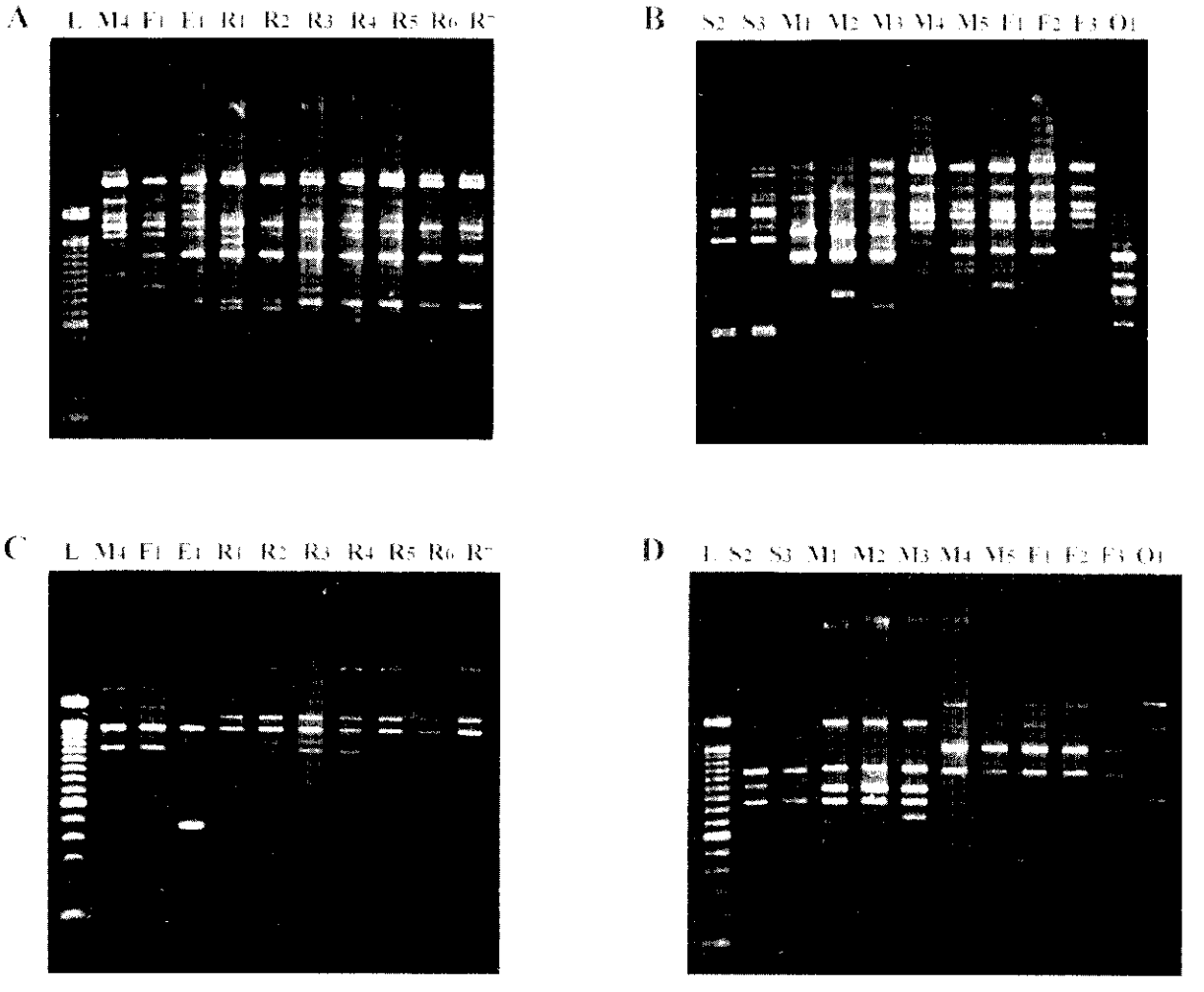

Figure 2. RAPD patterns of *Grateloupia doryphora* individuals from different geographic locations. L, 100 bp molecular weight marker; M, specimens from Mediterranean; F, Brittany, France; E, Portsmouth, England; R, Rhode Island, USA; O, Oregon, USA. Reactions shown in (A) and (B) were performed with primer (CACAGGCGGA), those in (C) and (D) with (CTGATGCTAC).

within most of the populations, RAPD markers did reveal that there is substantial genetic diversity partitioned among the populations, with the specimens falling into three distinct groupings (Table 2). The first group includes individuals in the Rhode Island, USA; Brittany, France; and Portsmouth, England, populations and one of the specimens from the Mediterranean population (M4). These individuals are genetically similar to one another, with pairwise similarity coefficients ranging from 0.67 to 0.93 (Table 2). This first group is genetically distinct from the second group, which consists of individuals collected in Spain, and from the third group, which includes Mediterranean specimens M1, M2 and M3. The average similarity coefficients between groups ranges from 0.16 to 0.29 (Table 2). We are continuing to test different primers as well as more individuals from each of these populations and additional geographic locations.

ITS SEQUENCES

The ITS2 region from selected individuals in each population was sequenced (Marston and Villalard-Bohnsack, in prep.). This region ranges from 338 to 373 nucleotides for these *G. doryphora* individuals. All of the sequences were easily aligned. Parsimony analysis produced a single most parsimonious tree of 140 steps with a consistency index (CI) of 0.97 and a retention index (RI) of 0.97. Individuals from the Rhode Island, French, and English populations, as

Table 3. Pairwise distance comparison of sequence variation in *Grateloupia doryphora* individuals from aligned ITS2 sequences.

Specimen	M1	M3	E2	F3	F2	R6	E1	F1	M4	O1	S3	S2
M3	0.000	—										
E2	0.216	0.216	—									
F3	0.216	0.216	0.000	—								
F2	0.216	0.216	0.000	0.000	—							
R6	0.216	0.216	0.000	0.000	0.000	—						
E1	0.216	0.216	0.000	0.000	0.000	0.000	—					
F1	0.216	0.216	0.000	0.000	0.000	0.000	0.000	—				
M4	0.216	0.216	0.000	0.000	0.000	0.000	0.000	0.000	—			
O1	0.244	0.244	0.082	0.082	0.082	0.082	0.082	0.082	0.082	—		
S3	0.316	0.316	0.184	0.184	0.184	0.184	0.184	0.184	0.184	0.215	—	
S2	0.316	0.316	0.184	0.184	0.184	0.184	0.184	0.184	0.184	0.215	0.000	—

well as one individual from the Mediterranean (M4), have exactly the same ITS2 sequence and thus form a distinct clade on the tree (Figure 3). The specimen from Oregon is closely related to this group (Table 3; Figure 3). However, the two individuals from Spain form another distant clade, as do two of the individuals from the Mediterranean (M1 and M3) (Figure 3). Currently, we are analyzing sequences obtained from *G. doryphora* individuals collected in additional locations.

Discussion

In this study, we are using RAPD analyses and ITS sequences both to examine the genetic variation within *Grateloupia* populations and to help identify the geographic origin of the newly established *G. doryphora* population in Rhode Island. To this end, we have thus far examined specimens from Rhode Island, USA; Portsmouth, England; Brittany, France; Galicia, Spain; and the Mediterranean. Although herbarium specimens from these locations are morphologically indistinguishable, substantial genetic variation is revealed by RAPD and ITS sequence analyses, and these two data sets are highly congruent.

RAPD analysis is based on the polyermase chain reaction (PCR). It uses short, single oligonucleotide primers of arbitrary sequence to amplify regions of genomic DNA, although the identity of the amplification products usually is not known (Williams *et al.* 1990). These products are separated by gel electrophoresis and then scored based on the presence or absence of bands. The analysis of RAPD data is not always straightforward, due to either technical limitations (*e.g.*, reproducibility of banding patterns) or assumptions made during the analysis (*e.g.*, bands at the same position on the gel are homologous) (see van Oppen *et al.* 1996b; Weising *et al.* 1995). Nevertheless, while results based on RAPDs should be interpreted with caution, they can provide a useful estimate of genetic variation among closely related taxa.

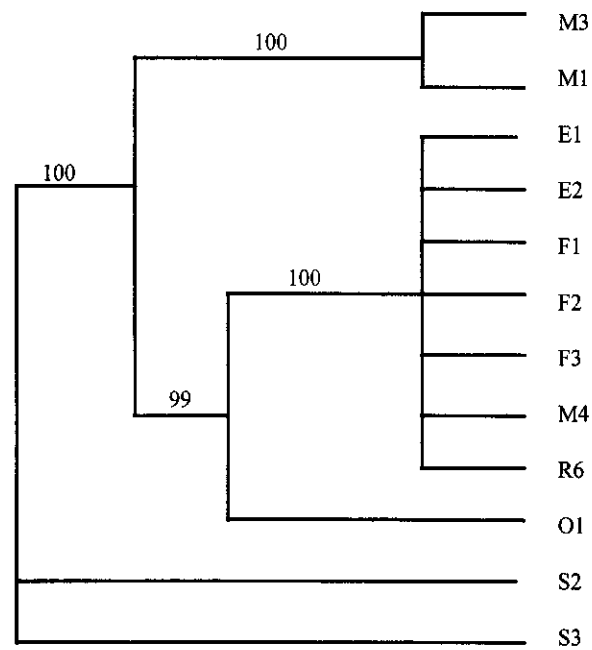

Figure 3. The single most parsimonious tree of ITS2 sequences constructed using the heuristic search option in PAUP with 100 replications of random addition sequences. Numbers above the branches are bootstrap values (500 reps). M, specimens from the Mediterranean; F, Brittany, France; E, Portsmouth, England; R, Rhode Island, USA; O, Oregon, USA.

Our RAPD analyses indicate that there is very little genetic diversity present within the Rhode Island *G. doryphora* population. Although we are limited by the small number of individuals available to us from the other populations, low levels of variation were also observed within the French, English, and Spanish populations. Our preliminary data suggest that there is more genetic variation partitioned among populations than within a population or geographic area. The one exception is the Mediterranean population where two genetically distinct groups were detected.

Both ITS and RAPD data sets reveal the same relationships among the populations we have sampled. Individuals in the Rhode Island population are genetically similar to specimens collected in Brittany, France; Portsmouth, England; and to some specimens from the Mediterranean, suggesting that one of these locations could be the origin of the Rhode Island population. The Rhode Island population is genetically distinct from other Mediterranean samples and from specimens collected in Galicia, Spain, and Oregon, USA. These data have allowed us to eliminate possible geographic origins (*i.e.*, Spain, Oregon), but cannot be used to positively identify the origin. Apparently, there has been too little time since the separation of some of these populations for variation to arise in the ITS region. It is possible that by using more RAPD primers and a larger sample size or another genetic marker (*e.g.*, microsatellites), we may be able to distinguish these genetically similar populations. This research is ongoing and we are beginning to screen *G. doryphora* individuals from other geographic locations (*e.g.*, California, Alaska, and Japan).

At this point, the vector responsible for the introduction is not known. However, based on the pattern of ship traffic in Narragansett Bay, the direction of water movement in the Bay, and the locations of the first *G. doryphora* populations, it seems likely that either hulls of ships or ballast water dumping was responsible for the introduction. Using RAPD markers and ITS sequences, we are establishing a genetic baseline of the distribution and level of genetic variation present in different geographic populations. This information can be used to monitor not only the Rhode Island *G. doryphora* population but any future introductions of this species.

Acknowledgments

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Determining the Pathways of Marine Bioinvasion: Genetical and Statistical Approaches

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ABSTRACT: **Advances in genetic technology have enabled biologists to reconstruct the history of populations, their evolutionary relationships, and geographical origins. Such information is essential in understanding the biology of invasions and in designing successful management responses. Unfortunately, the wholesale transfer of "traditional" population genetic methodology to identify the origins of marine bioinvasions is inappropriate. By definition, invading populations are characterized by rapid and recent range expansion.This has two important genetic consequences: (1) genetic diversity is often very low due to the bottlenecks in population size associated with the founding of new populations, and (2) evolutionary relationships among genes may bear no relation to the history of populations. These characteristics of invading populations limit our ability to reconstruct their geographic history. The situation is further complicated by the fact that many bioinvasions occur as a dynamic series of sequential or overlapping invasion events, the totality of which can be termed a metainvasion. Here, we evaluate the genetic markers and statistical methods currently being used to determine invasion pathways. Analyses of molecular genetic data fall into two categories: those based on phylogenies, and those based on frequency differences of genetic markers. We describe these two approaches and outline the conditions under which they are appropriate and useful in marine bioinvasions. We also outline recent technical and analytical developments that may assist in the study of marine bioinvasions.**

Key words: multilocus, genetic, DNA, population, origin of invasive species

INTRODUCTION

Following the discovery of an alien species, nearly all attempts at eradication or control (including sterile male release, biological control, and the use of transgenic plants) have a higher probability of success when source regions and the demographic parameters of invading and source populations are known. For example, very different management measures are required when invasions involve a species with panmictic population structure, compared to those that involve a species with sharply defined and genetically isolated populations (Carey 1991). Here we review genetic methods of determining the source of a bioinvasion. We begin with a brief description of the problem and evaluate the various genetic tools that are available for reconstructing invasion pathways. Next, we review the statistical developments that are needed to make sense of the new genetic data. Finally, we propose some future developments that might help to elucidate the invasion process.

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MAPPING A METAINVASION

When an invading species is recognized in a particular location, assigning the invading individuals to a source population is an important task (Davies *et al.* 1999a; Roderick *et al.* 1998). Unfortunately, many bioinvasions are hierarchical, consisting of several sequential or overlapping invasion events, which together constitute a "metainvasion" (Davies *et al.* 1999b). In many cases metainvasions are global in nature and any single invasion will often have multiple, genetically similar potential sources, many of which are likely to have been recently established themselves. Species that spread in association with human activity, especially invasive species, are likely to be characterized by newly established populations

and these represent a severe obstacle for genetic analysis. First, new populations are often genetically impoverished due to the population bottleneck associated with colonization (Nei *et al.* 1975), making it hard to find sufficiently variable markers. Second, what little variation is present tends to be ancestral, rendering phylogenetic methods less appropriate or meaningless. These are the principal challenges faced by those seeking to identify the source of marine bioinvasions and to reconstruct their invasion pathways.

Allozyme electrophoresis is a useful technique for rapidly assessing the similarity of various populations; however, only a small amount of the underlying genetic variation at any given locus can be discerned using protein electrophoresis. Consequently, allozymes have provided only very limited resolution in invading populations of species such as the medfly, *Ceratitis capitata* (Huettel *et al.* 1980; Roderick 1996a). A number of workers have identified heritable markers that reveal genetic variation at the DNA sequence level for reviews see (Geller 1996; Palumbi and Baker 1996; Roderick 1996b), with mitochondrial DNA being the most commonly used genetic marker in population studies (Avise 1994). Unfortunately, the reduction in diversity associated with colonization bottlenecks is exacerbated for mitochondrial genes because they have only a quarter of the effective population size (*Ne*) of nuclear genes (Moore 1995). In the medfly, *C. capitata*, for example, most New World populations are less than 100 years old and have a single high frequency and a single low frequency haplotype. By contrast, ancestral *C. capitata* populations in Africa display up to six haplotypes (Gasparich *et al.* 1997). Fortunately, new markers, such as microsatellites (Queller *et al.* 1993; Weber and May 1989), introns (Palumbi and Baker 1994), randomly amplified polymorphic DNA (RAPDs) (Welsh and McClelland 1990; Williams *et al.* 1990), and restriction length polymorphisms (RFLPs) (Aquadro *et al.* 1992) assay nuclear DNA variation. These markers have revealed high levels of diversity in ancestral and invading populations, even when mtDNA and allozymes are relatively impoverished (Baruffi *et al.* 1995; Villablanca *et al.* 1998). The greater variability of these markers can reveal population structure over a much finer scale. For example, genetic analysis of DNA sequence variation at four intron loci revealed significant population structure among previously indistinguishable (Gasparich *et al.* 1997) *C. capitata* populations in California, Central America and eastern South America (Davies *et al.* 1999b).

Statistical Developments in the Analysis of Bioinvasions

As the practical difficulties of finding suitable genetic markers have been overcome, severe theoretical problems became apparent. First, new populations violate the assumptions of equilibrium integral to most population genetic theory (*e.g.*, methods of estimating gene flow (Slatkin and Barton 1989). Second, phylogenetic methods that reconstruct the historical biogeography of relatively well established populations (Avise 1994; Roderick 1996b) seem inappropriate in the latter phases of a metainvasion where events are all very recent. For example, in the case of a secondary invasion event (a new invasion originating from another invading population), the likely sources are populations that were themselves only recently established as part of the primary invasion. Phylogeographic structure (Avise 1989; Avise 1994; Roderick and Gillespie 1998; Roderick and Villablanca 1996) is not expected in such recently founded populations because there has been little time for mutations to occur, and the relationships among alleles mostly reflect evolutionary events in the ancestral range of the species rather than their history in newly occupied areas (Davies *et al.* 1999a; Villablanca *et al.* 1998). For example, McGuigan *et al.* (1998) reported significant differences in haplotype frequencies over a fine geographic scale among Australian frog, *Litoria pearsoniana*, populations; however, a smaller and insignificant F-statistic was obtained when the genetic distance among alleles was considered.

With the traditional approaches being of only limited use for invasion biologists, it is fortunate that a new generation of statistical analyses have been developed based on multilocus genotype data. One of the first applications was in fisheries management, where mixed stock analysis (MSA) was developed to determine fish catch composition, mainly using allozyme data. In MSA, maximum likelihood is used to estimate the combination of potential source populations that best explain the observed allele frequencies in a catch (see Utter and Ryman 1993). Methods, such as MSA, that focus on populations are limited by the need to define those populations *a priori* and in doing so they risk missing individuals that have an unusual origin. An alternative approach focuses on

the most likely origin of an individual, or rather its multilocus genotype, and is known as an assignment test (Paetkau *et al.* 1995).

Assignment tests use maximum likelihood to assign individual genotypes to potential sources based on the allele frequencies of the source populations. These tests are particularly useful for determining the origin of an individual when there are multiple, genetically similar candidate sources. For example, chinook salmon, *Oncorhynchus tshawytscha*, occur as different temporal populations (runs) that spawn in the same river but at different times of the year. Runs are genetically very similar but have such distinct life histories that some are considered separate (and endangered) species. Microsatellites have been used to assign chinook salmon, *O. tsawytscha*, to particular runs (Banks *et al.* 1996).

There are two major sources of error associated with the population level data used in an assignment test. First, observed allele frequencies are estimates, so sampling error must be considered. Second, differences in genetic diversity among potential source populations can cause a bias because the likelihood of drawing any genotype is inversely proportional to the diversity of the population from which it is drawn. Rannala and Mountain's (1997) assignment test takes into account the sampling error associated with estimating allele frequencies and the differences in diversity among two potential sources (Davies *et al.* 1999a). Although Rannala and Mountain's (1997) test applied much needed statistical rigor to source estimation, further modifications of the test are still needed. First, laboratory scoring mistakes must be taken into account. For example, scoring errors occur in allozyme studies with a frequency of about 1% (Lathrop *et al.* 1983). One can attempt to correct for scoring mistakes prior to analysis, as we did for the errors associated with sequencing cloned polymerase chain reaction (PCR) products. Alternatively, an error rate factor may be incorporated into the analysis; such an approach was used by Marshall *et al.* (1998) in their multilocus paternity test. A second source of error that should be considered is the implications of not sampling all the potential sources. Again with a focus on paternity testing, Marshall *et al.* (1998) presented a simulation method to assess the likelihood that a more probable source remains unsampled. Finally, assignment tests focus on the origin of single multilocus genotypes, although bioinvasions usually consist of multiple invading individuals, each of which will have their own associated likelihood of being from one source or another. With such multiple assignments, one will be able to plot a distribution of likelihood statistics for the invading population as a whole, adding a new level of complexity to source estimation. For example, one interpretation of a bimodal distribution is that the invading population had two sources, but how can one assess the significance of such a conclusion, and how should one correct for multiple comparisons? Such issues are currently being examined in our laboratory using computer simulation (Bohonak *et al.*, in prep.).

Multilocus genotyping is a powerful technique and many different markers, including allozymes, mtDNA, and microsatellites can be analyzed simultaneously. In most cases additional markers will increase the power of these tests; however, some markers may be incompatible with this approach. RAPDs, for example, arevery useful in providing high levels of genetic variation and their main advantage over introns and microsatellites is that they can be applied with very little prior genetic knowledge of a species (Williams *et al.* 1990). Unfortunately, it is not clear how RAPD data can be incorporated into the same statistical framework as introns and other markers, where genotypes can be identified at each locus.

Although we have stated that phylogenetic approaches are less likely to be useful in many bioinvasions (Davies *et al.* 1999a; Roderick *et al.* 1998; Villablanca *et al.* 1998), recent data have suggested that phylogenetic data can still be used to help determine sources (Davies *et al.* 1999b). Somewhat to our surprise, we found that including an estimate of the genetic distance among alleles revealed a higher level of population structure among American *C. capitata* than estimates based solely on allele frequencies (Davies *et al.* 1999b). This was surprising because *C. capitata* only colonized the Americas this century, providing little time for the evolutionary divergence of alleles among American populations. It is possible that phylogeographic structure might reflect multiple colonizations—one of several possibilities we are exploring using computer simulations (Bohonak *et al.*, in prep.)

It is possible, therefore, that phylogenetic information might turn out to be useful even in the later stages of a metainvasion. This is especially likely if genetic markers are found with very high evolutionary rates. We have proposed (Davies *et al.* 1999a) that extragenomic markers (EGMs), such as viruses, be

used to reconstruct invasion pathways. The RNA genomes of many viruses evolve extremely quickly (Holland *et al.* 1982) and can be used to reconstruct the geographic history of their hosts (Ho *et al.* 1993) (Yanagihara 1994). Another possible set of markers that provide phylogenetic information and evolve rapidly are transposable element polymorphisms (TEPs). We are currently working with groups in Greece and Italy to explore the utility of TEPs for the *C. capitata* metainvasion. Transposable elements occur widely in nature (Li 1997) and could be applied to marine invasive species should they prove successful in model terrestrial systems such as the medfly.

If EGMs or TEPs do become widely used, approaches that utilize phylogenetic data to detect gene flow (Slatkin 1994) might be applicable. Assignment tests might be modified to consider the relationships among alleles in addition to their frequencies. Currently, assignment tests do not consider the relatedness of alleles because most multilocus markers such as microsatellites (Glenn 1998) do not easily permit the phylogenetic analysis of alleles. However, sequence data is much more phylogenetically informative, and with introns, EGMs, and TEPs becoming more widely used, restricting analysis to mere allele frequencies may waste useful information. A new test should assess the multilocus likelihood of sampling a given set of alleles from a potential source population based on the distance between alleles as well as their frequencies.

To conclude, the new genetic markers and statistical methods briefly described here will reveal much about the spread of invasive species. Bioinvasion genetics will not provide all the answers, but combined with ecological data, we might eventually be able to determine the common characteristics of invasive species and to identify the pathways that allow them to spread around the globe. Ultimately, invasion biology needs to become a predictive science, identifying species that are likely to invade, and those that will become established should they reach a new area. Such information would finally enable managers to adopt truly proactive policies.

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Atlantic Salmon *(Salmon salar)* in British Columbia

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ABSTRACT: **Farmed salmon is British Columbia's largest agri-food export product. Approximately 80% of production is Atlantic salmon (*Salmo salar*), an exotic species on the Pacific coast. Large scale escapes and small scale "leakage" of adults from marine net pens are not uncommon and lead to over 2600 marine and 150 freshwater reports of Atlantic salmon in B.C. waters in 1997 alone. The continuous addition of free ranging adult Atlantic salmon into the coastal environment combined with the weak state of many native Pacific salmon (*Oncorhynchus* spp.) stocks has been suggested to enhance the likelihood of colonization. We present preliminary results of our work conducted to delineate what, if any, ecological or genetic impacts are associated with aquaculture escapee Atlantic salmon on native Pacific salmon species. We also present evidence that suggests colonization may be occurring on a Vancouver Island river, an event which would mark the first anadromous expansion of the species beyond its native range.**

Keywords: Atlantic salmon, aquaculture, Pacific Northwest, niche competition, steelhead

INTRODUCTION

Atlantic salmon (*Salmo salar*) account for approximately 80% of finfish aquaculture production in British Columbia (B.C.) and is the largest agri-food export of the province. The increasing frequency of *S. salar* observed in coastal marine waters and rivers has generated a lively debate regarding the eventual fate of this species along the Pacific coast of North America. At present, empirical data regarding potential genetic and ecological impacts of escaped Atlantic salmon in this region are nonexistent. Thus, any current predictions regarding the persistence of free-ranging *S. salar* and what effects may result are not robust.

S. salar is endemic to most countries with drainage into the North Atlantic Ocean and Baltic Sea (Mills 1989). This encompasses an area roughly from Portugal to the Arctic Circle and from Iceland to southern Greenland to Labrador south to the Connecticut River. Introductions of *S. salar* have been documented in every continent save Antarctica; the vast majority of these introductions have ended in failure (MacCrimmon and Gots 1979; Alverson and Ruggerone 1997; McKinnel *et al.* 1997). Indeed, a self-supporting anadromous population of *S. salar* has never been established outside the species' native range.

Only two notable successful introductions outside the native range have been recorded–one in Argentine Patagonia, the other in New Zealand–both are nonanadromous (MacCrimmon and Gots 1979). In North America, success of *S. salar* introductions has followed a similar pattern and has been limited to a number of small oligotrophic lakes in eastern Canada and northeastern United States (MacCrimmon and Gots 1979).

The first transfer of *S. salar* to western Canada occurred in 1905 when 90,000 Atlantic salmon eggs from New Brunswick were transferred to the Fraser River Hatchery and the Cowichan Hatchery on Vancouver Island (Prince 1905 reviewed by MacCrimmon and Gots 1979; Carl and Guiguet 1958). The fry were released into three lower mainland rivers and six Vancouver Island systems (Carl and Guiguet 1958). Importation of eggs from New Brunswick and later Scotland continued until 1933. Estimates of total number stocked (mostly eyed eggs and alevins) are not consistent, having been reported as 5.5 million (Castledine 1991), 6 million (Needham 1995),

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>7.5 million (Burt *et al.* 1992 in Alverson and Ruggerone 1997), and 13.2 million (McKinnell *et al.* 1997). Although some returning adults were recovered in the Cowichan River system (MacCrimmon and Gots 1979), these introductions failed to establish a permanent population.

The seeming inability of Atlantic salmon to establish viable populations when deliberately planted suggests that the potential to do so via farm escapees is remote. However, failure of these early attempts is likely attributable to causes that would have undermined the potential success of any species' introduction. Historical introductions of *S. salar* were made into mature, stable environments that were saturated with predators and niche-equivalent competitors (*i.e.*, Pacific salmonids) and as a result tended to be more resistant to a biotic invasion (reviewed in Pimm 1991). Predation and interspecific resource competition would have been much more severe than is likely today, when, because of severely depressed native stocks, progeny of present-day *S. salar* grow in underutilized habitat, which is more accessible to colonization. Historically, Atlantic salmon were released at early life history stages (egg and fry) that experience naturally high mortality. Large, healthy, immunized adults are the norm today. The two scenarios, historical and present-day, are clearly different, and using the outcome of one to predict the fate of the other is not valid.

Aquaculture escapes

Escapes of *S. salar* from marine net-pens in the eastern Pacific often occur as large-scale escapes, typically due to weather events, human error, and predators (Alverson and Ruggerone 1997). Unaccounted-for losses (leakage, predation, unrecovered mortalities, etc.) have been estimated to be between 10 and 30% of the cage population (Moring 1989). From 1991 to 1997, 28 escape events involving 162,453 salmon were reported at B.C. marine net-pens (Thomson and Candy 1998). Reporting of escape events is a mandatory condition of a farm license; however, there are no mechanisms to evaluate compliance and therefore reported escape numbers should be considered as minimum values.

In British Columbia, the first free-ranging *S. salar* was caught in 1987 (one year before the first reported escape) (McKinnell *et al.* 1997). Reported marine captures in B.C. waters peaked in 1993 at 4,543 fish (Thomson and McKinnel 1994). Capture data are compiled opportunistically from various management databases and voluntary reports by commercial and sports fishers. As such, these data, like escape data, are considered to be minimum values only and likely do not reflect the actual number of captures. *S. salar* often go unreported: commercial crews and to a lesser extent sport fishers no longer consider the capture of a *S. salar* noteworthy and often do not go through the trouble of reporting it to authorities (Volpe, pers. obs.). *S. salar* landed commercially may be disposed of through unofficial channels or are frozen and used for halibut bait (Volpe, unpublished data).

Evidence of Natural Reproduction of Escapees

In 1997, Atlantic salmon were reported in 40 freshwater systems in British Columbia (34 on Vancouver Island). Again, because most of these data are collected opportunistically, this figure should be considered as a minimum only. To date, three river systems, all on the northeast coast of Vancouver Island, have been identified as supporting juvenile Atlantic salmon: the Tsitika River (Volpe *et al.*, 2000), Amor de Cosmos Creek (Volpe, pers. obs.), and the Adam River (Volpe, pers. obs.). At least two age-classes are present in both the Tsitika River and Amor de Cosmos Creek. Parr estimated to be one-year old have been observed in the Adam River. To date, scales and otoliths from only the Tsitika River fish have been examined and were consistent with these fish being wild reared (Volpe *et al.*, 2000). Further, there are no commercial aquaculture activities of any kind on or around any of these systems, therefore, the only logical explanation for the presence of these fish is as products of natural spawning events of aquaculture escapees. The presence of multiple age- and size-classes raises the possibility of previous year-classes having successfully reared and smolted undetected. At present, there is no way to discriminate between wild-reared adult *S. salar* and escaped adult *S. salar* in the field. Therefore, there is no way to easily discern if feral progeny are successfully completing their life cycle.

Ecological Concerns

Domesticated *S. salar* may not be behaviorally adapted for interspecific competition and other challenges of a natural environment (Olla *et al.* 1994). Dickson and MacCrimmon (1982) noted differences

in behavioral patterns between hatchery and wild-reared salmon, which they suggested may account for poor survival of planted *S. salar*. Many studies have reported similar results (reviewed by Hindar 1994). Due to intense artificial selection to repress wild traits (in contrast to supplemental hatchery programs), it is possible that behavioral deficiencies may be even more pronounced in aquaculture escapee salmon (Gausen and Moen 1991; Olla *et al.* 1994). However, each farm generation undergoes selection for traits appropriate to the B.C. coastal environment. As long as *S. salar* are reared in open net-pens, selection for a "Pacific strain" of Atlantic salmon will continue. Further, typical aquaculture production fish are already proven capable of spawning in a controlled west coast stream channel (Volpe; unpublished data) and in the wild (Volpe *et al.* 2000, Volpe, per. obs.). If spawning events continue to occur, adaptation will be hastened by natural selection altering the behavioral or phenotypic profile of the feral population. Released from old constraints and under a radically different selection regime, adaptive changes can occur rapidly in invading populations (Carrol and Dingle 1996). For instance, American shad (*Alosa sapidissima*), when introduced from the Atlantic to the Pacific, evolved novel, locally adapted life histories in less than a century (Shoubridge 1977, reviewed by Dingle 1980).

Steelhead (*Oncorhynchus mykiss*) and Atlantic salmon share similar environmental requirements, habitat preferences, growth rates, and life histories (Bley and Moring 1988). Therefore, we predict that any negative effects resulting from the presence of *S. salar* in B.C. will manifest first among sympatric *O. mykiss*. We are aware of only three studies that directly compare competitive ability of juvenile *S. salar* and *O. mykiss* (Gibson 1981; Hearn and Kynard 1986; Jones and Stanfield 1993). All three concluded that *O. mykiss* were more aggressive than Atlantic salmon. However, these results are confounded by their experimental design. All three investigations did not quantify intra- versus interspecific competition, therefore, defining unambiguous interaction terms is not possible (Underwood 1986; Fausch 1998). Our data, generated in the laboratory and the field, suggest that *O. mykiss* individuals are indeed more aggressive than *S. salar* individuals. However, *O. mykiss* individuals are much more likely to attack a conspecific than an Atlantic salmon (Volpe, unpublished data). Thus, while *O. mykiss* individuals are aggressive, agonism is unlikely to result in *S. salar* being competitively excluded.

Genetic Concerns

Potential genetic concerns of *S. salar* on the west coast center on the possibility of *S. salar* x *Oncorhynchus* spp. hybridization events. Laboratory trials of all possible crosses involving *S. salar* and *Oncorhynchus* spp. in coastal British Columbia demonstrated that the most successful combinations in terms of the survival of F_1 to hatching would involve steelhead females (6.07%) and pink salmon (*O. gorbuscha*) males (0.36%) (reviewed in Alverson and Ruggerone 1997). Therefore, although hybrid progeny can be produced in a laboratory environment, survivorship is poor in all cases and reproductive viability of the few F_1s produced remains unknown. It should be noted, however, that these data were extracted from a small, unpublished pilot study and this issue awaits rigorous examination.

Conclusions

Current methodologies used to estimate *S. salar* abundance in the wild provide minimum values only. The actual numbers of individuals remain undefined. Invasion potential of escaped *S. salar* cannot be assessed until reliable estimates of escapes and captures are generated or until colonization occurs, at which point the issue is moot.

Historical anecdotes cannot be used in a predictive context. The failure of sporadic attempts to introduce fry and eggs over half a century ago bears no relationship to the present continuous introduction of robust adults.

When a species colonizes novel territory, adaptive modification of the phenotype to a more appropriate state is possible. Selection pressure and thus rate of change is likely to be greatest during the initial generations of the colonization. Predicting the fate of *S. salar* in the Pacific based on static models (derived from analyses of domestic fish only) is likely to be decreasingly valid as colonization continues

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Biomonitoring of an Aquacultured Introduced Seaweed, *Porphyra yezoensis* (Rhodophyta, Bangiophycidae) in Cobscook Bay, Maine, USA

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ABSTRACT: **The intentional introduction of organisms for the purpose of mariculture requires a balance between minimizing ecological impact and maximizing economic gain. Phycogen Inc. (formerly Coastal Plantations International) has commercially farmed an introduced species of nori, *Porphyra yezoensis*, in Cobscook Bay, Maine, for the past eight years. Permits were granted based on the presumed inability of this seaweed to sexually reproduce under Gulf of Maine temperature regimes. *P. yezoensis*, a cultivar from Japan, has been grown by Phycogen at both a nursery and a grow-out farm site since 1991. A monitoring program was begun in 1996 to examine the potential dispersal and establishment of *P. yezoensis* around the sites. This report describes our monitoring program's results for the nursery farm site at Huckins Ledge from 1997 and 1999. *Porphyra* samples were collected from 10-m intertidal transects and artificial substrates constructed of Japanese netting. *Porphyra* species were identified using morphological characteristics and isoenzyme electrophoretic markers. Collections from transects and artificial substrates at this site suggested that *P. yezoensis* only recruited ephemerally during Phycogen's summer/autumn growing season and did not overwinter. The overwintering potential of *P. yezoensis* was further examined by deploying established blades attached to Japanese netting in the field from December until March. *P. yezoensis* was not observed on the netting the following spring. Based upon the results at Huckins Ledge, *P. yezoensis* does not appear able to establish a permanent population. However, data from Phycogen's grow-out site at Mathews Island is still being examined and will be reported in a future publication.**

Key words: *Porphyra*, monitoring, Maine, aquaculture

INTRODUCTION

Porphyra is the second most widely cultivated seaweed in the world. Currently, over 900,000 mt worth over $1.5 billion dollars are cultivated in Japan, China and Korea annually (Hanisak 1998). *Porphyra* is grown for the production of "nori", which is eaten as dried sheets in soups and sushi. The first attempt to farm a nonindigenous *Porphyra* species in North America occurred in Washington state in the 1980s. Although cultivation attempts were successful, this early effort ultimately failed due to permitting difficulties (Mumford 1990). In 1990, Phycogen Inc., of Portland, Maine (formerly Coastal Plantations International), received state, federal, and international (International Council for the Exploration of the Sea) permits to introduce and farm *P. yezoensis*, a species native to Japan, in the Cobscook Bay region of Maine (Levine 1998).

Permits were granted for the aquaculture of *P. yezoensis* in the Gulf of Maine based on temperature restrictions in its reproductive cycle. *P. yezoensis* demonstrates a heteromorphic alternation of generations typical of the genus, with a haploid foliose blade phase alternating with a diploid filamentous conchocelis phase. The diploid conchocelis phase requires temperatures exceeding 28°C to mature sexually and release conchospores, which give rise to the

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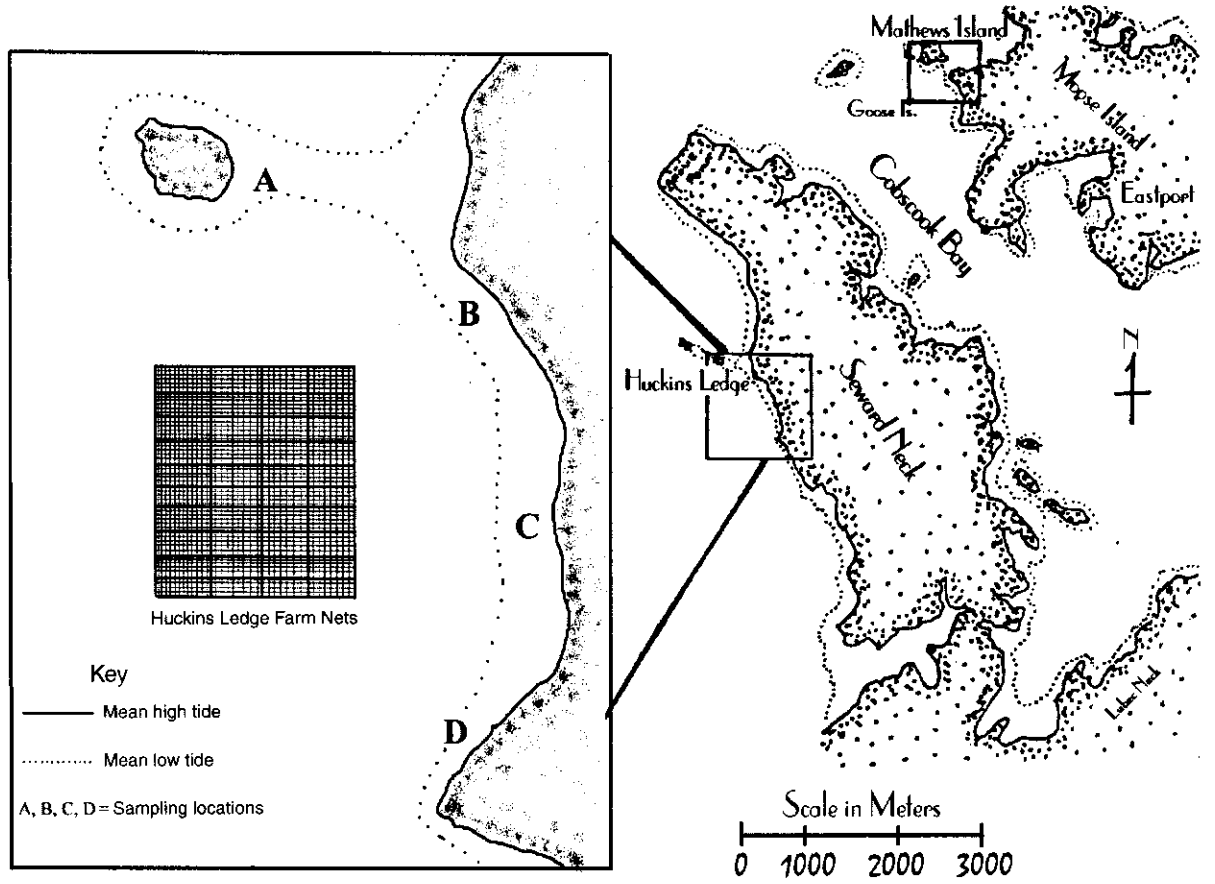

Figure 1. Phycogen farm sites in Cobscook Bay near Eastport, Maine, USA. Inset shows Huckins Ledge sampling locations.

haploid blade phase (Melvin *et al.* 1986). *P. yezoensis* was thought to be incapable of sexual reproduction in the Gulf of Maine due to this temperature requirement. Haploid blades, however, can also reproduce asexually by releasing monospores during the Gulf of Maine's summer temperatures (12-16°C), which coincide with Phycogen's summer/fall growing season. This raised concerns regarding the potential establishment of this seaweed in the local intertidal via asexual reproduction, especially in the vicinity of Phycogen's nursery farm site where juvenile blades would be producing the greatest numbers of monospores. The present study was undertaken to monitor the establishment of *P. yezoensis* near Phycogen's farming sites and is one of the few studies to examine an aquacultured introduced seaweed.

Phycogen initially utilized two sites for its nori farming operation: a grow-out site at Mathews Island, northwest of Eastport, Maine, and a nursery site at Huckins Ledge, southwest of Eastport (Figure 1). Farming was conducted at Mathews Island from 1991 to 1997, until the grow-out nets were moved and combined with nursery nets at the Huckins Ledge site in August 1997. Farming at Huckins Ledge was continuous until May 1998. *P. yezoensis* was aquacultured seasonally during favorable conditions, usually in the late spring/early summer and in the autumn/early winter. A monitoring study was initiated at Phycogen's sites in August 1996. The ability of *P. yezoensis* to recruit at the nursery farm site was evaluated by sampling *Porphyra* blades from intertidal transects and artificial substrata we constructed of Japanese netting strung between poles. *Porphyra* blades have simple morphologies and species can be difficult to discern. In order to identify and distinguish *P. yezoensis* from the six species described for the North Atlantic we used both morphological traits (Bird and McLachlan 1992) and isoenzyme electrophoretic markers. Isozyme markers have proven useful for distinguishing *Porphyra* species in previous studies (Lindstrom and Cole 1990, 1992a,b). Data from our monitoring program at the Huckins Ledge nursery farm site from August 1997 until July 1999 will be addressed in this paper.

Figure 2. Artificial substrata (Japanese netting between poles) used to collect *Porphyra* spores at Huckins Ledge, shown in the high intertidal zone.

Materials and Methods

Monitoring of *P. yezoensis* at the Huckins Ledge nursery site was conducted using two methods: sampling of intertidal transects and artificial substrata. Randomly placed 10-m transect lines were run parallel to the shoreline in the vicinity of the artificial substrata at high- and low-tide marks. Eight transects were run during each sampling event. All *Porphyra* plants encountered along the 10-m transect line were collected and analyzed.

The artificial substrata were constructed of specialized netting from Japan suspended between two poles. Similar artificial substrata have been reported in the past for growing seaweeds (Sylvester and Waaland 1983). The netting was considered an ideal substrate for collecting *P. yezoensis* spores and was developed by the Japanese to improve monospore settlement during nori farming. The netting is constructed of synthetic fibers to which *Porphyra* spores readily attach (Mumford and Miura 1988). Netting pieces (2 m x 1 m) were suspended vertically between 3-m metal posts, approximately 0.5 m off the ground (Figure 2). Eight artificial substrata were placed at low- and high-tide marks at four locations (A, B, C, and D) in the vicinity of the farm site (Figure 1). Artificial substrata and transect samples were collected seasonally from August, 1997 to July 1999.

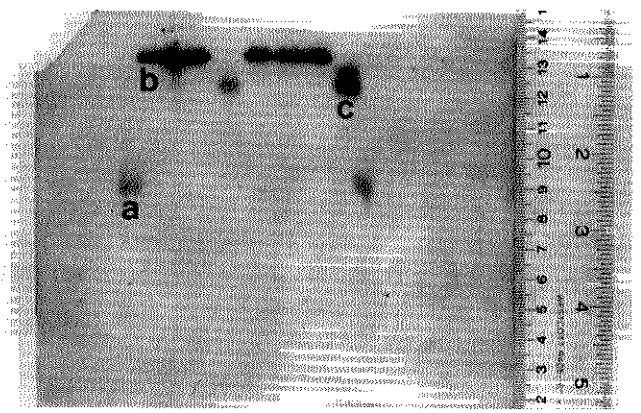

Figure 3. Sample polyacrylamide-starch gel stained for phosphoglucose isomerase (PGI). A = *P. yezoensis* control, B = *P. purpurea* control, and C = *P. umbilicalis* control. Remaining bands are *Porphyra* samples taken from netting at Huckins Ledge showing matches to controls.

Porphyra species identification was first attempted using classical features (thallus shape, size, cell height, number of cell layers and patterns of reproductive cells when available) described in the key of Bird and McLachlan (1992). After initial examination, isozyme electrophoresis was used to identify a set of random samples and any questionable samples to support visual identification. A modified starch-polyacrylamide isoenzyme electrophoresis gel system was used with a Tris-citrate buffer (Cheney and Babbel 1978; Cheney 1985). Although several enzymes were intitially tested, including malate dehydrogenase (MDH) and phosphoglucose mutase (PGM), we found that phosphoglucose isomerase (PGI) gave reproducible banding patterns that could distinguish *P. yezoensis* from the local *Porphyra* species in question. PGI has been used to distinguish Pacific coast *Porphyra* species (Lindstrom and Cole 1990, 1992a, b; Brostoff and Gordon 1997) and is currently being used in phylogenetic studies of Atlantic coast species (C. Neefus, pers. comm.). *P. yezoensis* farmed by Phycogen is from a single conchocelis stock culture and as expected showed the same allele for the PGI locus throughout this study. *P. yezoensis* PGI migrates more slowly (*i.e.*, has lower bands) than that of the local species (Figure 3) and electrophoresed in a consistent pattern. Samples from our monitoring study were always run against stock laboratory cultures of *P. yezoensis* and the two most frequently encountered local species, *P. umbilicalis* and *P. purpurea*, as controls on each gel (Figure 3). *P. umbilicalis* and *P. purpurea* samples occasionally showed polymorphism at the PGI locus, but samples always corresponded to at least one of the control bands when screened.

The ability of *P. yezoensis* blades to overwinter in the field was tested by subjecting blades to winter conditions *in situ*. Individual strands of netting seeded with established *P. yezoensis* blades were affixed by cable ties to the middle of new artificial substrata at

high- and low-intertidal locations at the Huckins Ledge site at the end of Phycogen's growing season, in the autumns of 1997 and 1998. These strands were grown and supplied by Phycogen, and had blades ranging from 1 mm to 10 cm long, in densities >100 blades/strand. Both seeded strands and nets were analyzed in the spring of 1998 and 1999 for survival of the original blades and for potential recruitment and establishment on the surrounding netting. Strands recollected in the spring were cultured under laboratory conditions (aerated, sterile, enriched seawater media, 15°C, 12:12 light-dark cycle) to permit regeneration of any *P. yezoensis* blades from spores that might be present but not visible. Similarly seeded strands were deployed in May 1999 and collected in July 1999 to test the ability of *P. yezoensis* to persist in the intertidal under favorable conditions. Recollected strands were cultured as above and *Porphyra* recruits on the surrounding artificial substrata were screened electrophoretically.

Results

Monitoring Study

The abundance of *Porphyra* species from transects at Huckins Ledge varied with location and season. Greater numbers of individual blades were found epilithically on small cobble in muddy substrata near sampling location A and sparser populations were found on sandy cobble substratum near sampling locations B, C, and D along the rocky shoreline (Figure 1). The performance of the artificial substrata exceeded expectations and recruited *Porphyra* plants in large numbers. A total of 653 individual *Porphyra* blades were collected from artificial substrata at Huckins Ledge during the course of this study and 334 individual blades were collected during transect sampling. Approximately 26% of the *Porphyra* collected on artificial substrata and 48% collected in transects were analyzed electrophoretically. The most common *Porphyra* species identified from both artificial substrate and transect collections taken throughout the year was *P. purpurea*. The second most commonly encountered species was *P. umbilicalis*. The seasonal and spatial distribution of these species between the two years sampled was consistent.

None of the *Porphyra* blades collected in transect surveys between August 1997 and July 1999 were identified as *P. yezoensis*, of a total of 163 individuals tested. However, five blades from the artificial substrata collected in November 1997 were identified as *P. yezoensis*. *P. yezoensis* was not identified in subsequent sampling from March 1998 to July 1999 in either transect or artificial substrate surveys (Figure 4).

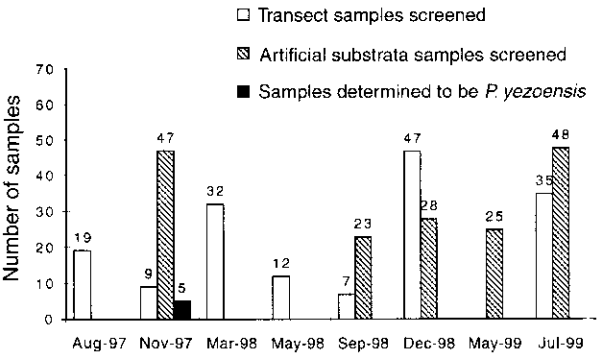

Figure 4. Electrophoretic screening results for transect and artificial substrata *Porphyra* samples collected at Huckins Ledge from 1997 to 1999.

Overwintering Study

Six *P. yezoensis*-seeded strands of netting used in the overwintering study at Huckins Ledge in December 1997, were collected and analyzed in March 1998. *P. yezoensis* was not found on the original pieces or on the surrounding netting. The netting pieces were overgrown with *Enteromorpha* spp. and *P. yezoensis* blades did not regenerate after a month in laboratory culture. This study was repeated in December 1998, with 11 seeded strands. Of the 11, 9 strands were recovered in May 1999. One strand had two *Porphyra* blades that were identified morphologically and electrophoretically as *P. umbilicalis*. All recovered strands were cultured in the lab for in excess of 1 mo to allow for any spores that might be present to grow into blades; however, no blades were observed. Strands deployed in May 1999 and recovered in July 1999 were also bare of *P. yezoensis* blades and did not regenerate after lab culture. However, the netting surrounding the strands was well recruited with *P. purpurea* blades.

Discussion

Monitoring of the nursery farm site at Huckins Ledge was initiated in 1997 because this is where we believed monospores would have been released in the greatest numbers and was therefore the most likely area for *P. yezoensis* to recruit and establish a population. During the two years of this study, only limited and ephemeral recruitment of *P. yezoensis* was

observed at Huckins Ledge. This recruitment was observed only on artificial substrata, immediately following a farming season and in small numbers compared to the presence of local *Porphyra* species. The *P. yezoensis* blades collected from artificial substrata in November 1997 represented only 8% of the total *Porphyra* blades collected during that sampling event and *P. yezoensis* was not found in either transect or artificial substrate collections taken the following spring. Thus, it appears that *P. yezoensis* was able to release asexual monospores during Phycogen's farming season, but new blades survived only ephemerally in the intertidal surrounding the farm site. Overwintering experiments conducted in 1998 and 1999 at Huckins Ledge also suggest that *P. yezoensis* is not capable of overwintering in the field. The local species of *Porphyra* produce blades in the spring/summer, generally die off over the winter, and then return again in the spring when conchospores are released by subtidal conchocelis populations. However, *P. yezoensis* was not identified in any spring transect or artificial substrate sampling when the local species blades had reappeared, most likely due to the temperature restriction in its life cycle upon which permitting was granted. The inability of *P. yezoensis* to overwinter is further supported by the fact that an intertidal population of *P. yezoensis* was never discovered during our transect sampling or that of another researcher (A. Mathieson, pers. comm.), at the Huckins Ledge site.

It must be acknowledged that this paper reports only results for the Huckins Ledge nursery farm site and that differences in site locations may play a vital role in the ability of *P. yezoensis* to recruit and survive in Cobscook Bay. Data from Phycogen's former grow-out site (Mathews Island), currently under investigation, will be described in a forthcoming publication. Together, our monitoring studies at Huckins Ledge and Mathews Island should be able to assess whether *P. yezoensis* can become established in Cobscook Bay and demonstrates the usefulness of monitoring species introduced for aquaculture.

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The "Silver Lining"—The Economic Impact of Red Sea Species in the Mediterranean

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Key words: economic impact, invasion, Red Sea, Mediterranean, fishery, prawn, jellyfish

INTRODUCTION

Biological invasions threaten natural ecosystems with impacts at genetic, population, and ecosystem levels (Ruiz *et al.* 1997). The Mediterranean, with its particular geological history and nearly landlocked geography, has been exceptionally susceptible to biological invasions. Ecologically, environmentally, and economically, the invasions into the Mediterranean Sea have had serious consequences (Boudouresque 1994). The major pathway of anthropogenic introduction into the Mediterranean Sea is the Suez Canal (Zibrowius 1994). Despite physical and hydrological impediments, hundreds of Red Sea species traversed the Suez Canal and settled in the Mediterranean, forming thriving populations along the Levant coasts, some invaders spreading as far west as Malta, Sicily, and Tunis (Galil 1994). Some abundant invaders constitute a nuisance or an economic burden, others outcompete local species, and yet others are exploited commercially.

DISCUSSION

Each summer since the mid-1980s huge swarms of the invading jellyfish, *Rhopilema nomadica* appear along the southern Levantine coast. In 1995 the jellyfish was recorded off the southeastern coast of Turkey (Kideys and Gücü 1995), and in 1998 a specimen was collected near Izmir (A. Karatas, pers. comm.). These massive swarms of voracious planktotrophs must play havoc with the meagre resources of this oligotrophic sea, and when the shoals draw nearer shore, they impact fisheries, coastal installations and tourism. Local municipalities report a decrease in holiday makers frequenting the beaches because of the public's concern over the painful stings inflicted by the jellyfish. Coastal trawling and purse-seine fishing is disrupted for the duration of the swarming due to net clogging and inability to sort yield. Jellyfish-blocked water intake pipes pose a threat to cooling systems of port-bound vessels and coastal power plants: in the summer of 1996 Israel Electric removed 25 tons of jellyfish daily from its seawater intake pipes at the Hadera power plant and has since installed a "jellyfish barrier" at the entrance to the cooling pond. Yet, that same jellyfish, *R. nomadica*, known to shelter among its tentacles the juveniles of a Red Sea carangid fish, *Alepes djedaba*, (Galil *et al.* 1990), may have precipitated the sudden population increase of this commercially valuable fish (Grofit 1987).

A native penaeid prawn, *Melicertus kerathurus*, was "very commonly caught by trawlers on Israel coastal shelf especially on sandy or sandy mud bottoms" (Holthuis and Gottlieb 1958) and supported a commercial fishery throughout the 1950s. It has since nearly disappeared and its habitat overrun by the Red Sea penaeid prawns. Geldiay and Kocatas (1972) reported that *Marsupenaeus japonicus* has also replaced *M. kerathurus* off the southern coast of Turkey and the rapid advent of another Red Sea prawn, *Metapenaeus monoceros*, into the Gulf of Gabes, Tunisia, has raised concerns over the fate of *M. kerathurus* fisheries there (Chaouachi *et al.* 1998).

Red Sea fish constitute nearly half of the trawl catches along the Israeli coast (Golani and Ben Tuvia 1995). In the late 1940s the invading goldband goatfish, *Upeneus moluccensis*, made up 10-15% of the total mullid catches off the Israeli coast. Following the exceptionally warm winter of 1954-55, its percentages

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increased to 83% of the catch, then dropped to 30% of the catch (Ben Tuvia 1973). In the 1990s, both invading mullids, *U. moluccensis* and *U. pori*, formed 87% of the mullid catch off the coast of Israel at depths of 20 m, and 50% at 55 m, whereas the native mullids are more abundant at greater depths (Golani and Ben Tuvia 1995). Following the winter of 1954-55, the brushtooth lizardfish, *Saurida undosquamis*, has become a commercially important fish and its share in trawl fisheries catches rose to 25% in 1979 (Grofit 1987). The population then diminished and catches have stabilized at about 5% of the total trawl catch (Ben Yami and Glaser 1974; Snovsky and Shapiro 1999). The Red Sea obtuse barracuda, *Sphyraena chrysotaenia*, has outnumbered the native sphyraenids in inshore trawl and purse-seine catches along the Israeli coast (Grofit 1987). In addition, two of the four species of Red Sea clupeids that established populations in the Levant–*Dussumieria acuta* and *Herklotsichthys punctatus*–are of importance in the inshore-pelagic fishery.

Red Sea species make up most of the commercially valuable crustacean catch along both Egyptian and Israeli coasts (Galil 1986). An early invader, the swimming crab, *Portunus pelagicus*, was recorded from Port Said in 1898 (Calman 1927), where it soon became abundant, and by the beginning of the century was offered in the markets of Port Said, Alexandria, and Haifa (Fox 1924). Red Sea penaeid prawns are highly prized and a small fleet of Israeli coastal "mini" trawlers has specialized, since the mid 1980s, in shrimping, bringing in a quarter of the total trawl catch volume and a third of the trawl gross income (Snovsky and Shapiro 1999). *Marsupenaeus japonicus*, *Metapenaeus monoceros*, and *M. stebbingi* compose most of the prawn catch off the Mediterranean coast of Egypt and in the Nile delta lagoons (Dowidar and Ramadan 1976; Bishara 1976).

The sizable assemblage of Red Sea species that has taken up residence along the Levantine infralittoral, modifying the composition and structure of the biota, and enhancing its tropical affinities, has disrupted the biogeographic unity of the Mediterranean, and turned the Levant into a "quasi-tropical" province. The unique history of the easternmost Mediterranean that left it warm, salty, and impoverished is at the base of a singular synergy between anthropogenic and environmental factors. Though the expected outcome of invasion is reduction in diversity, we witness an invasion that increases faunal diversity, and augments the local fisheries – every cloud has a silver lining.

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Source of Unpublished Material

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How and When to Protect Native Species from Exotic Invaders: Lessons from a Predictive Model[1]

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Key words: *Batillaria*, bottom-up effect, *Cerithidea*, coexistence, control, estuary, exploitative competition, individual based models, local extinction, monitoring, mudsnail, prediction, top-down effect

INTRODUCTION

The exotic mudsnail, *Batillaria attramentaria*, was introduced to the west coast of North America in the early part of this century with aquaculture imports of the Pacific oyster, *Crassostrea gigas*, and has been displacing the native mudsnail, *Cerithidea californica* (Byers 1999). Both species produce demersal egg sacs with directly developing larvae and exhibit limited movement as adults, and thus comprise essentially closed populations within bays. Both snails also are susceptible to infection by trophically transmitted trematode parasites that typically infect *C. californica* at a higher rate than *B. attramentaria* (Sousa 1983; Lafferty 1993; McDermott 1996; Byers 2000). The trematode species substantially affect these snail species by castrating infected individuals, eliminating future reproduction. Byers (2000) demonstrated that these snail species compete for shared, limited diatom food resources and that the introduced snail, *B. attramentaria,* is superior in exploitative competition due to its higher resource conversion efficiency. Predictions of individual-level interspecific effects of each species upon the other based on consumer-resource data were highly accurate at an individual level (Byers 2000).

RESULTS AND DISCUSSION

Here we use an individual-based model to expand these predictions and project the population-level impact on the native snail, *C. californica*, caused by the nonindigenous species. By using empirical data to parameterize the model, we can project times of local extinction for the native snail, and also focus on two areas of primary importance to invasion biology not directly amenable to field manipulations: (1) the relative importance of mechanisms responsible for the exclusion of *Cerithidea* by *Batillaria*, and (2) identification of the metrics and biological measurements within a system that provide useful information about the likely course of the invasion in a given location. Identification of the metrics most sensitive to invasion impact would greatly aid resource managers in making decisions about which characteristics of a native species or system to monitor to provide earliest detection of problematic exotic species.

Our model tracked the species, sex, age, size, and infection status of each snail species through time. We set most demographic rates and interaction coefficients with empirical data derived from populations of the snails in Bolinas Lagoon, California (Byers 2000; Byers unpublished data). In summary, the model parameters and operations allow the number, sizes, and species of snails to determine the amount of resource available. Snail growth rates vary with resource level. Both species exhibit similar per capita rates of resource consumption; however, *Batillaria* is superior to *Cerithidea* in terms of how it responds to resource level (resource conversion efficiency). Egg production also is dependent on resource level, since no reproduction occurs if the level falls below 0.5-mm^2 diatom surface area/mm^2 sediment surface area. Egg mortality is constant and equal between the species. Infected and immature snails (<14 mm) cannot reproduce; otherwise, reproductive output increases with snail size. Monthly mortality is density independent and *Cerithidea* dies at a higher rate than

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Table 1. State variables and parameters and their associated mathematical relationships used within the model. For the first three state variables, empirical relationships were determined experimentally from plots with snails of single species (*Cerithidea* or *Batillaria*) and sizes (10 mm or 20 mm); hence, corresponding values are denoted by the first initial of each species with size class in mm as a subscript. For the first three state variables, snails of intermediate sizes were calculated by linear interpolation from the 10- and 20-mm snails. Snails below this range were treated as 10 mm and snails above this range were treated as 20 mm. Parameter values appear at bottom of table. Parasitic infection rates were derived from field data; egg survival and shell erosion are informed estimates.

Model component	Relationship/Values			Comments
A. State Variables				
Diatom level†	= a * exp (b * snail density)			When snails of mixed species and sizes were present, the coefficients (a and b) were the means of their separate values.
(μm^2 diatom surface area/ μm^2 sediment surface area)	4.3	−0.0044	C_{10}	
	4.02	−0.0067	C_{20}	
	4.46	−0.0024	B_{10}	
	4.22	−0.0061	B_{20}	
Resource-dependent individual growth†	= m * diatom level + b			Growth was constrained to be positive; a small decrease due to erosion or basic metabolism was applied separately. (In the field, large *Cerithidea* at low resource levels exhibit negative tissue growth).
(mm)	0.36	1.73	C_{10}	
	0.40	−0.26	C_{20}	
	0.03	2.85	B_{10}	
	0.26	1.48	B_{20}	
Density-independent mortality*	= m * length + b			For infected snails, the calculated value was increased 10%; The maximum age for all snails = 20 years.
(probability of death/month)	0.24	−2.07	C	
	0.0093	0.23	B	
Reproduction (eggs/year)	= 0.079 * (snail length)$^{2.7}$			None if snail infected, if <14 mm, or if diatom level <0.5
B. Parameters				
Egg survival (probability of surviving/egg/year)	1/300			
Parasitic infection rate* (probability per season)	p(*Batillaria*)=0.3*p(*Cerithidea*)			Probability was applied once during spring and once during summer; Snails <14mm not susceptible to infection
Shell erosion	−0.1mm/yr			

*For one or more of the state variables and parameters that differed between the species, some runs set the value for *Batillaria* equal to the value for *Cerithidea*, to determine which differences may be most important in promoting the displacement of the latter by the former.
†Both a species' effect on the diatom resource and its growth response to diatom levels combined represent its exploitative competitive abilities. In simulations that removed *Batillaria*'s competitive advantage, both state variables were equalized to *Cerithidea*'s values. Since the species impact the diatom level similarly, the majority of the effect of equalizing competition derives from changes in the efficiency of *Batillaria*'s conversion of resources to growth.

Batillaria. Maximum longevity for both species is capped at 20 years. Finally, parasitic infection rates, which are naturally lower in *Batillaria*, reflect the probability of uninfected individuals becoming infected at a given time step within the model, with infection increasing the typical monthly mortality rate for an infected snail by 10% (Table 1). The basic flow of the model is depicted in Figure 1.

We first tested the relative importance of *Batillaria*'s demonstrated advantages in parasite resistance (top-down effect), exploitative competition (bottom-up effect), and mortality rate (demographic advantage) in driving its successful invasion and displacement of *Cerithidea*. Such an analysis pinpoints the key pathway through which the exotic species derives the majority of its success, and also suggests pathways of intervention that may more successfully control or delay the impact of the exotic species. Model results indicate that displacement and ultimate local exclusion of *Cerithidea* by *Batillaria* takes

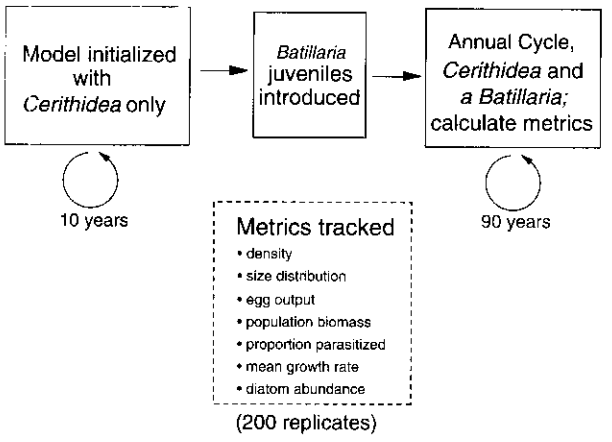

Figure 1. Flow chart of model.

between 55 and 70 years. Furthermore, exploitative competition and susceptibility to parasitic infection are relatively weak mechanisms in driving the overall success of *Batillaria*. Although these interactions provide the mechanism for *Batillaria* to exert an influence on *Cerithidea*, *Batillaria*'s lower density-independent mortality rate plays the key role governing *Batillaria*'s displacement of *Cerithidea*. Management techniques can therefore focus particular attention on alleviating species-specific differences of this mechanism, for example, through physical removal of *Batillaria*, to most effectively neutralize the invasion.

To determine the earliest point that a monitoring program could detect the impact of the invader in the native system, we tested the sensitivity of many response variables of *Cerithidea* at the population and individual level, including density, population biomass, egg production, mean size, proportion of infected individuals, and individual growth rate. We also tracked the overall level of diatom resource in the marsh. For these model simulations, we chose an initial number of *Batillaria* invaders to inoculate into *Cerithidea* populations that previous results showed would guarantee extinction of the native snail in 100% of the runs within 90 years. In this manner, we could identify which biological responses of the native snail gave the earliest signal that a detrimental invader had entered the system. Despite almost immediate detection of increasing invader populations, all metrics for *Cerithidea* were slow to exhibit evidence that this observed increase in invader density impacted the native species. Most metrics took at least 25 years from the beginning of the invasion to show significant declines. By this point, the presence of the exotic species and its effect on the native species was essentially irreversible. Native snail egg production and marsh-wide diatom abundance reflected impact from the invader the quickest of all metrics—within 15-20 years. Difficulty in finding reliable, early-warning metrics has crucial implications for how we should view and conduct monitoring programs and risk assessment analyses.

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Ballast Water

'Which technologies gain prominence is of less importance than the integrity of the overall system of coastal protection from ballast-mediated invasions.'

BALLAST WATER OVERVIEW

Ballast Water Management: Developments in Policy and Technology

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INTRODUCTION

United States policy to prevent ballast water transfers of harmful aquatic organisms and pathogens has evolved rapidly throughout the 1990s. This paper briefly outlines some of the major policy and technology developments in the United States to date, and discusses pressing policy and technology needs of the future.

A BALLAST POLICY AND TECHNOLOGY TIME LINE

The Great Lakes region, ground-zero for some of the worst infestations of harmful aquatic organisms including the zebra mussel, was the first to become politically active over the detrimental effects of non-indigenous species. Interest in the impacts of exotic aquatic organisms on the Great Lakes ecosystem increased throughout the 1980s and in 1989, with the explosion of the zebra mussel population in the lakes, the region sought Congressional action. Congress responded with the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (P.L. 101-646 - NANPCA). This legislation, as introduced, would have created a national regulatory ballast management program, along with a national program for managing impacts of aquatic nuisance species (S. 2244). But the demand for ballast management from other coastal regions was not yet adequate to overcome industry opposition and the legislation as enacted created a ballast program targeted at the Great Lakes only. In 1993, the program became the first mandatory prevention program globally, requiring ballast management on all ships entering the Great Lakes after operating outside the Exclusive Economic Zone.

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While the ballast program was limited to the Great Lakes, NANPCA did set up a national aquatic nuisance species task force and program, and planted strategic information requirements to cultivate interest in a national ballast management program. In particular, the legislation called for a study of the risk posed by invasive aquatic organisms in U.S. waters other than the Great Lakes. The resulting "Shipping Study", completed in 1993 by Carlton *et al.* and published in 1995 by the U. S. Coast Guard (USCG), showed very clearly that foreign ballast water was discharged in commercial harbors and coastal areas around the country. The report indicated that these coastal areas were already sustaining impacts of ballast-mediated transfers of exotic organisms.

Events also highlighted the interests of other regions. In 1992, the Food and Drug Administration and the Centers for Disease Control, responding to detection of *Vibrio cholerae* in shellfish beds of Mobile Bay, analyzed the ballast tanks of ships entering Mobile Bay from South America. The agencies found a strain of cholera consistent with that in the shellfish beds and in harbors in South America in several vessels (McCarthy and Khambaty 1994).

Work began in 1995 on the reauthorization of NANPCA, particularly to make it national in scope. The Northeast Midwest Institute held a National Forum on Nonindigenous Species Invasions of Marine and Fresh Waters in early 1996 to raise the awareness of Congressional offices regarding the national scope of the problem (NE-MWI 1996). Scientists from around the country came together for the first time to present their respective findings regarding impacts of invasive aquatic organisms on their regions. The Pacific Northwest, California, Hawaii, the Gulf of Mexico, the Mississippi, the Great Lakes, New England, the Mid-Atlantic, and the Southeast were represented. The Forum was

effective at communicating a national need, and the National Invasive Species Act of 1996 became law (P.L. 106-232).

With the enactment of the National Invasive Species Act (NISA) in the United States, negotiations to create an international ballast-management regulation at the International Maritime Organization (IMO) took on new urgency. Flag countries began to take the process more seriously as it became clear that unilateral mandatory action beyond that in the U.S. Great Lakes would take place even in the absence of a timely international framework. This outcome is still likely. Even though the Coast Guard published its final rule establishing the national ballast management program 18 months late (in mid-1998), the program must become regulatory by 2001 if compliance or reporting on a voluntary basis is not adequate. This time frame will precede any final IMO action, currently scheduled for the 2002-2003 biennium. Meanwhile, environmental groups petitioned the U.S. Environmental Protection Agency, (USEPA) to regulate ballast water under the Clean Water Act. The time frame for initiating regulation under the Clean Water Act may not be faster than that outlined in NISA (even given the Coast Guard delay), but the interest in it underscores the reality that ballast management requirements are coming in the United States; there are more questions about who will regulate than whether regulation will occur.

Pressure has built for technology research and development in direct response to ballast policy innovations. In 1990, while Congress considered NANPCA, concern over the limitations of the ballast exchange option for treatment led to a National Research Council (NRC) study of ballast water treatment alternatives. The NRC report, *Stemming the Tide,* completed in 1996, recommended research and development work on a number of potential treatment technologies (NRC 1996). As a result of the report, the Northeast-Midwest Institute and the Lake Carriers' Association launched the Great Lakes Ballast Technology Demonstration Project (GLBTDP) in collaboration with several federal, state and industry interests, primarily with state-level funds from the Great Lakes Protection Fund. The GLBTDP has researched the merits of filtration and will review various forms of secondary treatment during 2000. More funds for research and development of treatment technologies became available pursuant to NISA. The Fish and Wildlife Service and the National Oceanic and Atmospheric Administration (NOAA)'s National Sea Grant College Program began to solicit proposals for ballast treatment research pursuant to this program in 1998. Table 1 provides a summary of policy and technology developments in ballast management.

Future Directions

Developments in the state-of-the-art of ballast treatment have tended to follow from policy advancements. The reason for this effect is that as demand for action increased, focus on the insufficiencies of the suite of currently available ballast treatments also increased. The current set of options and some of their limitations are summarized below:

1. High-Seas Ballast Water Exchange

This technique is fairly effective but has several limitations associated with it. Probably the greatest limitation of the technique is that it is effective only on high-seas voyages. The technique involves replacement of near-coastal ballast water and organisms with open-ocean ballast water and organisms. The method owes its efficacy to the fact that open-ocean organisms are not likely to survive in near-coastal ports of call. Thus, in instances of coastal voyages, the efficacy of ballast exchange is greatly compromised if not eliminated because the ship would be replacing near-coastal ballast water with similar water, with organisms adapted to near-coastal areas. A second limitation is that ships may be fully loaded in their transoceanic voyage. In this case, a ballast exchange would overload the vessel. Yet unpumpable sludge in the bottom of the tank can later be resuspended in subsequent ballast operations and discharged into harbors. In addition, there are sea conditions that are considered unsafe for ballast exchange operations. Finally, ballast exchange is difficult to verify, which complicates accurate compliance monitoring.

2. Ballast Water Exchange in Alternate Exchange Zones

The effectiveness of ballast water exchange as a treatment option in coastal voyages and in stormy conditions could be enhanced by the designation and use of alternative exchange zones in near-coastal areas. To be effective, such zones must be located where prevailing currents are seaward. The Ballast Exchange Study, required by NANPCA 1990 and released in draft by Becton *et al.* in 1998, surveyed U.S. coastal waters for possible alternate exchange

Table 1. Time line for ballast management policy in the United States

1980s	Attention to U.S. exotic species problems grows primarily in the Great Lakes region due to species impacts; spurs demand for ballast management requirements.
1989	Zebra mussel infestation creates political will in Great Lakes region to accept ballast management restrictions. Primarily at urging of Australia, International Maritime Organization (IMO) Marine Environment Protection Committee convenes a Ballast Working Group.
1990	Congress enacts the Nonindigenous Aquatic Nuisance Prevention and Control Act (NANPCA) creating a regional ballast management program for the Great Lakes and initiating studies to document need for national program.
1992	Great Lakes ballast management program becomes mandatory pursuant to NANPCA. Shellfish beds in Mobile Bay close temporarily due to contamination by *Vibrio cholerae* possibly transported in ships' ballast tanks. Ballast management recognized as public health issue. U.S. initiates National Research Council Study to explore ballast management alternatives.
1995	U.S. Coast Guard (USCG) Shipping Study carried out pursuant to NANPCA is released detailing national need for ballast management.
1996	National Forum on Nonindigenous Species Invasions in US Fresh and Marine Waters presents evidence of national scope of the problem to Congressional offices. Congress enacts the National Invasive Species Act creating a national ballast management program. The National Research Council completes its review of possible ballast technologies. The Great Lakes Protection Fund awards grant to initiate the Great Lakes Ballast Technology Demonstration Project.
1997	The IMO's Marine Environment Protection Committee names ballast management a top priority and approves Assembly Resolution 868 requesting all member nations to implement ballast management guidelines. Hundreds of scientists sign a letter to Vice President Al Gore requesting a national program to address exotic species of all kinds.
1998	USCG publishes draft national ballast management program pursuant to NISA. IMO begins crafting treaty language for an international regulation Fish and Wildlife Service and the National Oceanic and Atmospheric Administration award first ballast technology development grants.
1999	Environmental groups petition U.S. Environmental Protection Agency (USEPA) to regulate ballast discharges under the Clean Water Act. Clinton Administration issues an Executive Order calling for a more coordinated federal response to the exotic species problem. USCG issues final rule for U.S. national ballast management program. Great Lakes Ballast Technology Demonstration Project filtration experiment completed. Fish and Wildlife Service and the National Oceanic and Atmospheric Administration award second round of ballast technology development grants.
2000	Great Lakes Ballast Technology Demonstration Project secondary treatment trials to be completed.
2001	Earliest date the U.S. national ballast management program could become mandatory.

sites. The study concluded that few, if any, near-coastal areas meet statutory requirements for alternate exchange zones. The only such zone that has been designated is located in the Gulf of St. Lawrence in Canadian waters. Canadian scientists are currently investigating possible detrimental effects of ballast exchange at this site on the Gulf ecosystem.

3. Retention of Ballast Water on Board

Ships can retain ballast water on board and not threaten the environment. However, only rarely is this option consistent with cargo loading and unloading operations, although new container ship design may make retention on board increasingly possible. Despite its limited applicability, this method should be listed as an option because, when it is applied, no other treatment is needed.

4. Best Management Practices

Ships can undertake a variety of best management practices, termed precautionary practices in the IMO guidelines, which can provide marginal protection against species transfers, even on coastal voyages. These practices are not considered substitutes for ballast water exchange, and cannot on their own adequately reduce the risk of ballast-mediated

transfers of organisms. However, they are important to an overall prevention strategy. They include such methods as timely cleaning of ballast tanks, avoidance of ballast uptake in known areas of harmful species outbreaks (such as red tide zones), and discharge of only the minimum amount of ballast water needed to carry out cargo operations.

While better than nothing, the current set of options presents serious gaps in resource protection (especially related to coastal voyages and treatment of ballast residuals), and problems for ship operators (relating to safety, monitoring difficulty, and crew time). On-board and shore-side treatment are the best prospects for improving the practicability and effectiveness of ballast water treatment. These approaches could be used alone or together to achieve adequate treatment. The technologies of greatest interest are heat, ultraviolet radiation, filtration, cyclonic separation, ozonation, and biocides.

The next generation of ballast management in the United States will likely incorporate some or all of these options. Which technologies gain prominence is of less importance than the integrity of the overall system of coastal protection from ballast-mediated invasions. That integrity will depend upon whether the ballast management system is:

- Comprehensive–Future ballast management should address coastal as well as transoceanic voyages, and loaded as well as unloaded vessels. This requirement almost mandates that treatment technology replace to a large extent ballast exchange as the primary approach to ballast management.
- Flexible–The system should be spatially flexible, i.e., able to accommodate fluctuations and changes in both the nature and patterns of shipping and the nature and patterns of invasions. The system also should include a diverse enough set of treatment alternatives that operators can maximize efficiencies given the particulars of their ships and voyage patterns. Some treatments, such as forms of heat treatment, may be effective only on ships engaged in lengthy and tropical voyage patterns; for these ships the method may prove the least costly, yet the same method would be insufficient for ships on other types of voyages.
- Safe and Practicable–The system should include methods that protect the safety of crew and ship, present minimum maintenance and operational difficulties, and do not create undue delay for ships. These characteristics will prevent the system from failing due to non-use.
- Effective–The system should include methods that are better and more consistent than ballast exchange at removing or killing organisms. It should also incorporate a level of redundancy, providing backup for instances in which the primary system of treatment fails or becomes unavailable (for example, shore-side treatment for ships should be available for those ships for which on-board treatment malfunctions or ballast exchange is unsafe).
- Environmentally Sound–Given today's level of sophistication in pollution prevention technology, the system should be expected to actually solve environmental problems rather than just replace one with another.
- Efficient–The system should provide this protection for coastal resources at a minimal cost to society.
- Accountable–The system will need to be one in which effective participation can be monitored actively and accurately.
- Globally Applicable–The U. S. system should be supportive of a credible global system of ballast management. A U.S.-only system will never be enough to protect U.S. waters because a proliferation of source areas abroad would raise the odds that transfers to U.S. waters will take place passively or through the cracks in our own prevention system. The next system must be compatible with the needs and realities of developing countries as well as with those of relatively wealthy nations.

Shore-side treatment, ship-board treatment, and ballast water exchange are all likely elements of the next generation of ballast management. One scenario that meets the above criteria and that we are on the way to achieving includes (1) continuing to allow high-seas ballast exchange for transoceanic voyages, but over time combining it with an obligatory back-up system of alternate exchange zones or shore-side treatment to address instances in which ballast exchange is not an option (*e.g.*, coastal voyages and stormy conditions); and (2) continuing to provide ships with the option of installing and using ship-board treatment to relieve themselves of the obligation to conduct ballast exchange/back-up treatment. Retaining the treatment obligation on board the vessel as much as possible will help make this system flexible and globally applicable. However, adding the

backup requirements will help improve the comprehensiveness and effectiveness of the system and create incentive to industry to move from ballast exchange to more reliable technological alternatives.

A great deal of thought and experimentation should precede any conclusions about which particular technologies and treatment alternatives should or should not be part of the next generation of ballast management. Hopefully, more rather than fewer such options will prove consistent with a long-term and effective ballast management system. If the next generation of ballast management is to become a reality in the foreseeable future, government, industry, and resource protection interests should actively undertake new research that reflects a partnership between resource management and industry interests, is scientifically rigorous, and meets environmental and safety requirements.

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Changes In Ballast Water Biota During Intracoastal and Transoceanic Voyages

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Key words: ballast water, survivorship, intraoceanic, transoceanic

INTRODUCTION

To develop predictive models of marine invasions, information is needed concerning which inoculants fail, as well as which succeed and why (Carlton 1996). The movement of ballast water by commercial ships is recognized as a primary mechanism for the global transfer of marine organisms (Carlton 1985; Carlton and Geller 1993; Smith *et al.* 1999), yet surprisingly little is known about patterns of survivorship during transit. To date, most ballast water studies have provided a checklist of taxa present in ballast water at the *end* of voyages (*e.g.*, Williams *et al.* 1988; Carlton and Geller 1993). Quantitative counts of organisms are needed at the start and finish of voyages to determine which taxa comprise an inoculant pool and which are more likely to survive the passage.

In separate long-term studies, we examined the intracoastal and transoceanic transport of organisms in the ballast water of coal ships. Our objectives were to:

- characterize the initial planktonic assemblages in the ballast water,
- assess the type and number of ballasted organisms remaining over voyages of both short and long duration, and
- test for inter-voyage differences or taxonomic biases in survivorship.

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MATERIALS AND METHODS

To understand patterns of survival in ballast water assemblages, we sampled plankton at the start and finish of voyages that differed in length and point of origin (Figure 1). We report findings from two of these routes. Between 1994 and 1997, we sampled plankton from the cargo hold of a coal carrier transporting ballast water from Israel to Chesapeake Bay, USA (n = 13 voyages) (Smith *et al.* unpublished data). Between 1996 and 1997, we sampled plankton from the topside deck tanks of a domestic coal carrier transporting ballast water from Somerset,

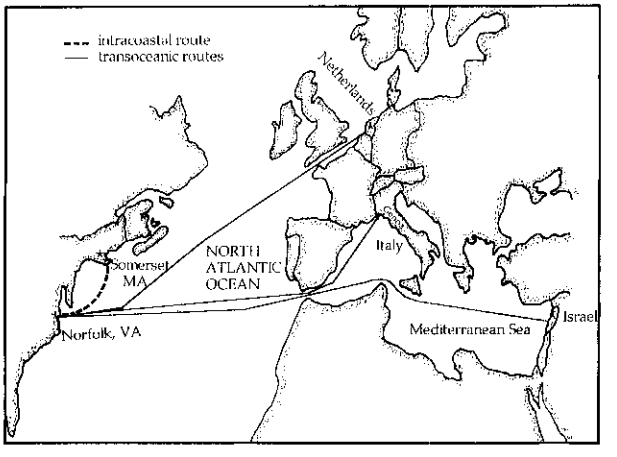

Figure 1. Routes of intracoastal and transoceanic voyages sampled between 1994 and 1997. Intracoastal voyages transported ballast water from Somerset, Massachusetts (41° 42′N, 71° 11′W) to Norfolk, Virginia (36° 51′N, 76° 19′W) in Chesapeake Bay. Transoceanic voyages transferred ballast water from Israel, Italy, and the Netherlands to Chesapeake Bay. Of the transoceanic voyages, only the Israeli component is reported here.

Massachusetts to Chesapeake Bay (n = 7 voyages) (Lavoie *et al.* 1999). Initial plankton samples were taken immediately after ballasting and final samples just prior to deballasting in the respective ports. In all instances, we sampled plankton in the ballast water to a depth of 2 m using replicate tows of a plankton net (0.15-m diam. opening, 80-μm mesh). Temperature and salinity of the ballast water were also taken at the start and finish using a salinity-conductivity-temperature meter.

Plankton samples were processed at the Smithsonian Environmental Research Center in Edgewater, Maryland (transoceanic voyages) and at the Northeastern University Marine Science Center in Nahant, Massachusetts (intracoastal voyages). After initial observations for living organisms, all samples were preserved in 10% formalin. Organisms were later identified to the lowest taxonomic group possible and enumerated using a dissecting microscope (see Lavoie *et al.* 1999 for details). We counted preserved organisms only if they showed evidence of internal tissue (or pigment) and no gross signs of deterioration (*e.g.*, disarticulation of copepod exoskeletons). We feel our data provide a good approximation of survivorship. Any bias in our calculations would likely lead to overestimation of transit success, but unless such biases were large, they would not significantly affect interpretation of our results.

Replicate tows were averaged to obtain mean initial and final abundances for each taxon per voyage. These valves were then used in repeated measures multivariate analysis of variance (MANOVA) models to test if there was an overall effect of time on abundance.

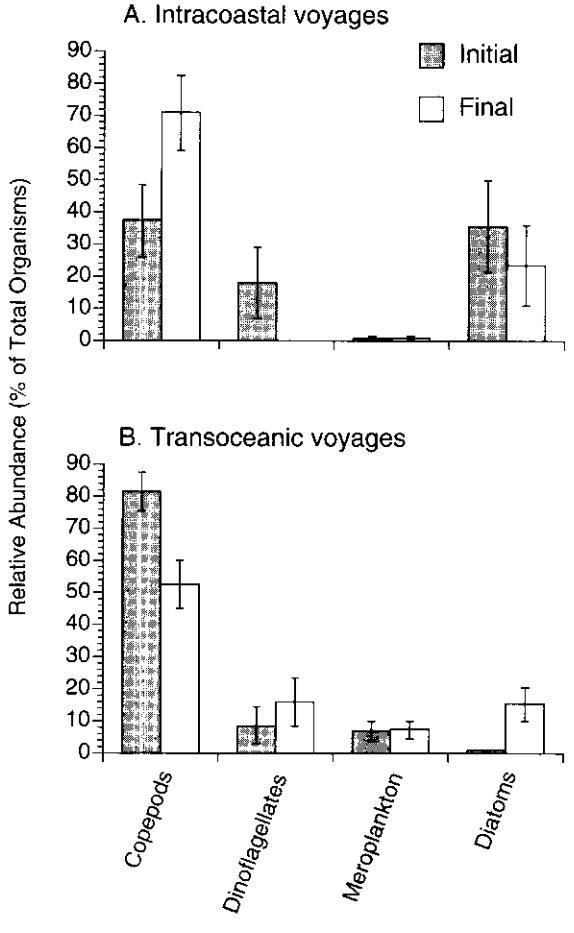

Figure 2. Relative numerical abundance (% of total organisms) of major groups at the start and finish of (A) intracoastal and (B) transoceanic voyages. Error bars are ± 1 S.E.M.

Results

A diverse assemblage of organisms was transported in the ballast water during both intracoastal and transoceanic voyages. At least 40 distinct taxa of net phytoplankton and zooplankton were identified from

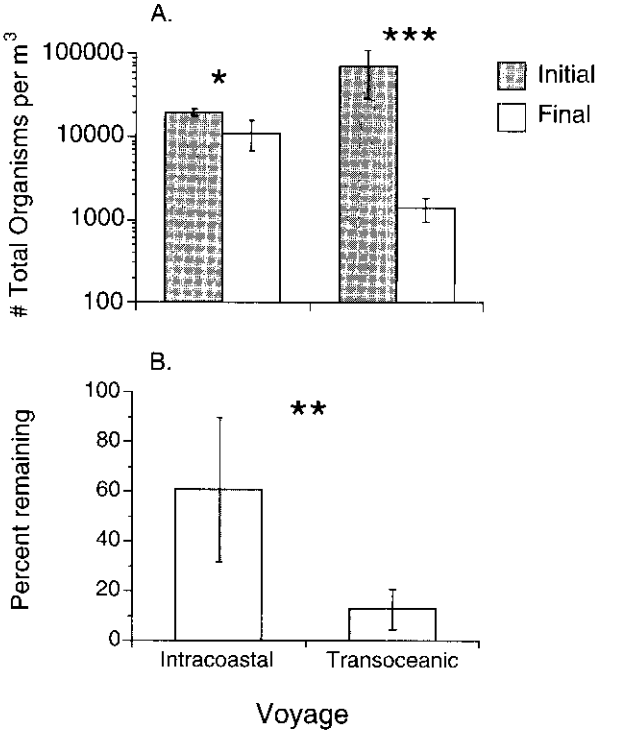

Figure 3. (A) Comparison of the total number of organisms m^{-3} at the start and finish of intracoastal and transoceanic voyages on a $\log_{10}$ scale. (B) Comparison of the percent of all organisms remaining at the end of intracoastal and transoceanic voyages. Error bars, ± 1 S.E.M. Kruskal-Wallis tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

combined intracoastal voyages (Lavoie *et al.* 1999). Over 70 taxa were collected from transoceanic voyages (Smith *et al.* unpublished data). Initially, intracoastal samples were dominated by copepods, dinoflagellates, and diatoms (Figure 2A). Copepods also dominated initial transoceanic samples (>80% of total organisms), with dinoflagellates and meroplankton (*e.g.*, larvae of benthic polychaetes, crustaceans) making up most of the remainder (Figure 2B). The relative abundance of the major groups changed over the course of both intracoastal and transoceanic voyages. For example, the relative abundance of copepods increased during the course of intracoastal voyages, while that of dinoflagellates decreased (Figure 2A). In contrast, the relative abundance of copepods decreased during transoceanic voyages, while that of dinoflagellates and diatoms increased (Figure 2B).

Overall, the total number of organisms declined significantly for both intracoastal and transoceanic voyages (Figure 3A). The decline in numbers, however, was greater for transoceanic voyages (87%) than for intracoastal (39%) voyages (Figure 3B) and may reflect the substantial difference in average transit time (19 d vs. 1.5 d respectively).

Despite a general pattern of declining abundance, survivorship varied greatly among taxonomic groups for both intracoastal and transoceanic voyages (Figure 4A, B). Dinoflagellates and diatoms suffered particularly sharp declines during intracoastal voyages, while copepod abundances showed smaller losses (Figure 4A). In contrast, dinoflagellates and diatoms fared relatively better than copepods during transoceanic voyages (Figure 4B). Survivorship of combined taxa varied significantly among voyages for intracoastal (repeated measures MANOVA, $p = 0.036$) and transoceanic ($p = 0.032$) routes (data not shown). There were, however, no seasonal differences in survivorship during transoceanic voyages (repeated measures MANOVA, $p = 0.62$). Too few intracoastal voyages were surveyed to test for seasonal differences.

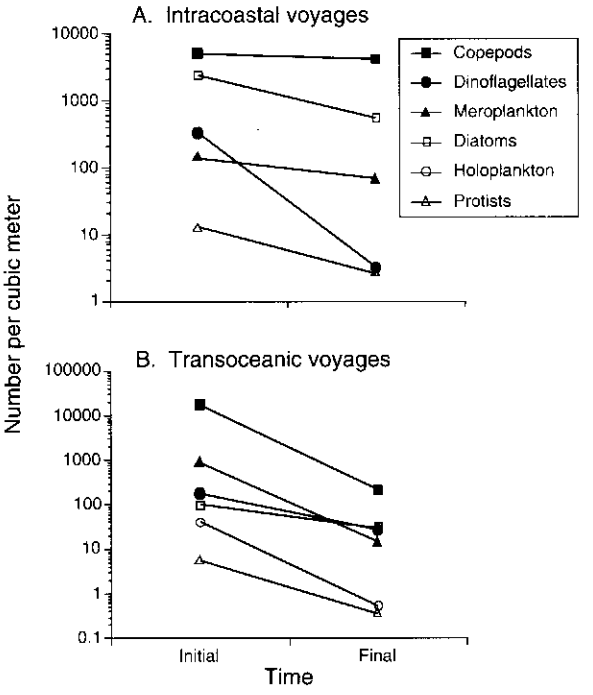

Figure 4. Mean abundance (no. organisms m^{-3}) of major taxonomic groups in the ballast water at the start (initial) and finish (final) for combined (A) intracoastal ($n = 7$) and (B) transoceanic ($n = 13$) voyages. Meroplankton consisted of planktonic larval forms of benthic invertebrates. Holoplankton included multicelled organisms that spend their entire life cycle in the plankton (other than copepods). Protists included all single-celled planktonic organisms other than dinoflagellates and diatoms. Taxon responses were not uniform over time for either intracoastal (repeated measures MANOVA, $F = 6.06$, $df = 4, 30$, $P = 0.0011$) or transoceanic ($F = 5.17$, $df = 5, 72$, $P < 0.001$) voyages. Mean abundances were back-transformed and presented on a $\log_{10}$ scale.

Discussion

We attribute the decreases in net plankton abundance during both intracoastal and transoceanic voyages to mortality. While the possibility exists that some species of dinoflagellates and diatoms may have encysted and settled out of the water column, analysis of ballast water sediments showed few organisms in the bottoms of ballast tanks (Lavoie *et al.* 1999). The potential causes of mortality are numerous and include physiological stress due to changing temperature or salinity, starvation, predation, or toxic substances in the tanks (Lavoie *et al.* 1999). We discount the first option, because our data showed little change in either temperature or salinity of the ballast water during most voyages (Lavoie *et al.* 1999; Smith *et al.* unpublished data). Oxygen concentration and pH of the ballast water also remained constant during one transoceanic voyage (Smith *et al.* unpublished data).

A successful ballast-mediated invasion is a multistep process in which organisms must survive uptake, transit, and release before establishing (Carlton 1985; Lavoie *et al.* 1999; Smith *et al.* 1999). Our data suggest that the transit stage is a significant selective event, both numerically and taxonomically, for ballasted organisms. Thus, the ballast water biota at the end of a voyage represents only a subset of the original

inoculant pool, even for voyages of short duration. Mortality, however, was significantly greater for the longer route in our studies. Our data indicate that certain taxonomic groups (*e.g.*, copepods) dominated the ballast water assemblage at both the beginning and end of voyages. Regional surveys designed to assess numbers and types of invasive species would do well to devote special attention to these more abundant taxonomic groups.

Our studies need to be repeated for different routes and for voyages of varying duration to separate out potential regional differences in survivorship from true temporal effects associated with the voyage. Such information will be vital in assessing the risk of ballast-mediated invasions for different vessel routes.

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Transport of Phytoplankton Via Ship's Ballast into Ports Around England and Wales

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ABSTRACT: **Ballast water was sampled from vessels arriving at 21 English and Welsh ports. The majority of the ballast water originated from Northern Europe. All but one of the water samples contained phytoplankton with some evidence of a seasonal pattern of temperate spring and autumn blooms. A number of toxic and/or nuisance species were found, *e.g., Pseudonitzschia* spp., *Dinophysis* spp., *Ceratium* spp., *Dictyocha* spp., and *Phaeocystis* spp. Dinoflagellate cysts were recorded in 84% of sediment samples and included *Scrippsiella hangoei, Pentapharsodinium tyrrhenicum* and *Gymnodinium catenatum*, species not previously recorded in British waters. In addition, some potentially toxic species, of which *Alexandrium tamarense/catanella* was the most common, were also found. This project confirms previous studies in that viable organisms are being transported into English and Welsh ports and may subsequently become established within British waters. Thirty of the fifty non-native species in British waters are thought to have been introduced via shipping, either in ballast water or hull fouling. The International Maritime Organization regulations may reduce the transfer of non-native species between continents. However, much of the shipping that discharges ballast in British waters will not be subject to these regulations and there is therefore still a risk of secondary introduction of species from established populations at other European ports.**

Key words: ballast water, nonindigenous species, introduced species, dinoflagellate, toxic alga, phytoplankton

INTRODUCTION

Vessels have carried marine organisms to areas beyond their ability to reach by natural range expansion (Carlton 1985). Organisms can travel attached to the hull or be carried aboard with the ballast water and associated sediments. There has been a steady increase in numbers of introductions of marine species worldwide and a small number of these species have caused major ecological, economic and public health problems (Carlton 1985). International attention has focused on ballast water as a primary means of introduction on a regional, national, and international level. Species that have commanded particular attention include the zebra mussel, *Dreissena polymorpha,* introduced to Lakes St. Clair and Erie in the late 1980s (Herbert *et al.* 1989), the ctenophore, *Mnemiopsis leidyi,* introduced from America into the Black Sea in the 1980s (Shushkina and Musayev 1990) and the potentially toxic dinoflagellate, *Gymnodinium catenatum,* introduced from Japan to Australian waters (Hallegraeff *et al.* 1988).

Ballast water introductions are an international problem and this has been recognized by the International Maritime Organization's (IMO) Marine Environment Protection Committee, which is in the process of introducing legislation to control the introductions of marine organisms and pathogens via ship's ballast. Presently, their guidelines recommend to member states measures that include:

- avoidance of ballasting in known bloom areas or areas with a heavy sediment load
- retaining ballast water where possible and not discharging it in port
- exchanging ballast water in mid-ocean.

Fifty-one non-native marine species are known to be established in Britain, half of which are thought to have been introduced by shipping either via hull fouling or in ballast water (Eno *et al.* 1997). The earliest of these records was the Southeast Asian diatom, *Odontella sinensis,* which appeared in 1906 (Ostenfeld, 1908) and more recently the American crab, *Rithropanopeus harrisii,* found in 1996 (Eno *et al.*

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1997). Non-native species in Britain have generally originated from similar latitudes, in particular from the east coast of the United States and the western Pacific, although secondary introduction from mainland Europe is also a common route of entry (Eno *et al.* 1997). To date, the introductions into British waters have not caused major problems–the main effect has been the displacement of native species (Eno *et al.* 1997). There have also been some localized effects such as fouling by *Undaria pinnatifida* (Fletcher and Manfredi 1995) and damage to fishing nets caused by the scum formed as blooms of *Coscinodiscus wailesii* break down (Boalch and Harbour, 1977). However, with continued ballast water transport this may not necessarily be the case in the future.

Macdonald and Davidson (1997) studied organisms in ballast water aboard vessels arriving at Scottish ports. Potentially toxic dinoflagellate cysts were recorded, including two species (*Alexandrium minutum* and *Gymnodinium catenatum*) not currently described from UK waters. Potentially toxic diatoms and dinoflagellates were also found, *i.e., Pseudonitzschia* spp., *Dinophysis* spp., and *Alexandrium* spp. The zooplankton examined contained a great diversity of taxa including two non-indigenous species of calanoid copepods and five other species (four copepods and one polychaete) only rarely seen in Scottish waters.

In England and Wales, the Ministry of Agriculture, Fisheries and Food (MAFF) is responsible for the marine environment and has funded a three-year research project designed to investigate the transport of marine organisms in ships' ballast water. The results will enable the development of government policy on the discharge of ballast water and sediments. The project is being carried out at the School of Ocean Sciences at the University of Wales, Bangor. It is closely associated with the project carried out in Scotland (Macdonald and Davidson 1997) and will share a common database of results. The project has also been associated with a European Union Concerted Action on Testing Monitoring Systems for Risk Assessment of Harmful Introductions by Ships to European Waters and has been involved in developing ballast water sampling systems.

The main objectives of this project are to establish a sampling strategy for collection of ballast water and associated sediment from ships docking at ports around England and Wales and to investigate the range and numbers of organisms present in the ballast tanks. The project aims to examine as wide a range of ports and vessels as possible.

This paper describes the collection of both phytoplankton and zooplankton from ships' ballast but provides preliminary results for phytoplankon work only as the zooplankton analysis is still underway.

Materials and Methods

A questionnaire survey carried out by Laing (1995) identified ports most likely to have received foreign ballast, and these ports were selected for sampling. The sampling methods were based on those developed at the Marine Laboratory in Aberdeen (Macdonald and Davidson 1997). Safety restrictions prevented use of electrical and battery-operated equipment on certain classes of vessel such as those carrying inflammable or explosive products. Consequently, all sampling methods avoided powered sampling apparatus.

Sampling was via a deck hatch. However, vessel design varied and it was not always possible to use the same method, and so several methods for sampling were employed.

Water Samples

A vertically integrated sample was taken by lowering a weighted, reinforced hose (25-mm internal diam.) through the hatch opening to the bottom to the tank. A valve at the top of the hose was closed off and the hose was quickly pulled up. The contents of the hose were emptied into a bucket in order to collect sub-samples. Samples were collected for phytoplankton analysis and preserved immediately with Lugol's iodine. A clear glass bottle (500-ml) was filled for future salinity analysis; this sample required no preservation. The temperature was measured and a pH reading was taken as soon as possible after sampling.

Sediment Samples

The weighted hose was lowered to the bottom of the tank, connected to a hand driven Mono pump (Model GH25) and a sediment slurry pumped up and collected in two 10-l carboys. The carboys containing the sediment slurry were placed in a dark cold room (4°C) on arrival back at the laboratory and left for a week to allow the sediment to settle. The water overlying the sediment was then removed

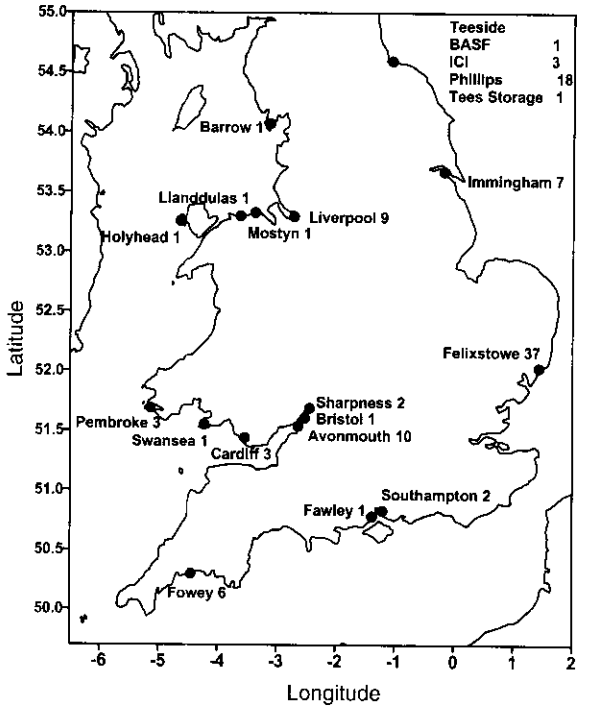

Figure 1. The location of the ports sampled and the number of ships sampled at each.

by siphoning. The sediment was then transferred to a smaller container for centrifuging at 3000 rpm for 10 min. The supernatant was poured off and the remaining sediment stored at 4°C until dinoflagellate-cyst analysis.

Some samples were only possible using a sounding pipe. A narrow reinforced hose (13-mm internal diam.) was pushed down the sounding pipe, connected to the hand pump, and any sediment slurry was pumped up and collected in carboys. This sample was collected first, as the hose cannot be moved to try to obtain sediment from other areas of the tank. The hose was then pulled up slightly and water was pumped into a bucket in order to collect subsamples as described above. Integrated samples could not be taken from sounding pipes, as it was not possible to pull the hose up before it emptied.

On some occasions it was not possible to sample using either of the above methods. In this case a variety of methods was used to collect samples. Samples were collected from an air pipe on deck if the ballast water was "pressed up" until it overflowed from the air intake pipe. All samples were collected by placing the buckets, carboys, and net under the flow of water. If the vessel was deballasting, samples were collected by holding a bucket under the outflow. Samples were also collected with a bucket if the level in the ballast tank was too low for the pump to work efficiently but there was room to lower a bucket. Some samples were taken from the ballast pump system: the pumps were turned on and samples taken via a bleed valve.

Sample Examination and Microscopy

Phytoplankton

Phytoplankton were settled by measuring 1, 5, 10, or 50 ml into settling chambers and leaving for 24 hr. The settled sample was then examined using a Nikon Diaphot inverted microscope using brightfield and phase-contrast illumination. The whole bottom plate was scanned at a low magnification (150x) in order to record large diatoms and dinoflagellates. Two transects were scanned at a higher magnification (300x) to count and identify smaller organisms, including small flagellates. Sometimes fine sediment made examination of the sample very difficult: these samples were sieved using 10-µm mesh before settling.

Dinoflagellate cysts

Sub-samples of sediment (or the whole sample if there was very little sediment) were placed in an ultrasonic bath for 2 min to remove detritus from cyst walls and to break down any aggregations. The sonicated sediment was then washed with 0.2-µm filtered sea water and fractionated through 106-µm and 20-µm sieves. The material retained on the 20-µm mesh was washed thoroughly with filtered sea water into a beaker and diluted to a known volume; density gradient centrifugation was then used to concentrate the cysts (Bolch 1997). A subsample of this suspension was then examined at 300x magnification under a Nikon Diaphot inverted microscope using brightfield and phase contrast illumination. Any full cysts found were counted and identified as far as possible.

Results

To date, 112 vessels have been sampled at 21 ports. Figure 1 shows the locations of the ports and how many times each were visited. Eight types of vessel were sampled, the majority being container vessels and bulk carriers (Table 1). The range of ballast water origins are shown in Figure 2, with northern Europe and near continent being the most common last port of call. In some cases it could not be

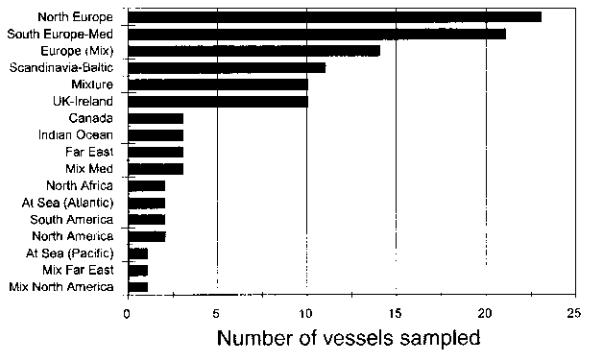

Figure 2. All recorded origins of the ballast water and how many times a vessel was sampled carrying ballast from each origin

ascertained exactly where the ballast water had been taken on board- some were of unknown origin and some were a mix from two or more sources. A total of 106 water samples, 88 net samples, and 102 sediment samples were collected. Of these, 44 were sampled via a deck hatch, 41 through sounding pipes, 6 from the ballast pump system, 5 using a bucket and 16 by some other means as described in the methods (*e.g.*, air pipe overflow, bleed valve).

Of the 100 ballast water samples analysed to date, only one did not contain any phytoplankton. The samples contained marine and freshwater organisms, in numbers ranging from 1,000 cells l^{-1} to 49 x 10^6 cells l^{-1} and their abundance reflected a seasonal pattern of temperate spring and autumn blooms (Figure 3). Table 2 shows the taxonomic diversity of the phytoplankton found in the water samples. The most common group was diatoms, represented by 42 genera, compared to 19 genera for dinoflagellates. Although it was possible to identify some diatoms to species level using the light microscope, it would only be possible to identify others to species using the scanning electron microscope. Of the nine diatom genera occurring >30% of the samples, all were cosmopolitan species and comprised both planktonic and benthic species. The most common diatoms were small centrics (10-20-µm diam.), tentatively identified as *Thalassiosira* spp. Other diatoms, *Nitzschia* spp., *Chaetoceros* spp., *Navicula* spp., *Paralia sulcata, Gyrosigma/Pleurosigma* spp., *Skeletonema costatum, Nitzschia longissima,* and *Thalassionema nitzschoides,* were present in >30% of the samples. Representatives of the genus *Pseudonitzschia* were recorded in 26% of the samples.

The most common dinoflagellates from water samples were *Gymnodinium* spp. and unidentified thecate and naked dinoflagellates, which were present in >20% of the samples. *Prorocentrum* spp. and *Prorocentrum micans* were present in 17% and 16% of the samples, respectively. Other dinoflagellates included eight species of *Protoperidinium* and seven species of *Ceratium. Dinophysis* spp. was recorded in 4% of samples. Some of the water samples also contained dinoflagellate cysts, including *Protoperidinium* spp. (13 samples) and an *Alexandrium tamarense*-type cyst (2 samples).

The flagellates were difficult to identify as they are easily damaged during sampling and preservation methods distort their shape. Potentially toxic or nuisance species included *Dictyocha speculum* and *Phaeocystis* spp. Among the common freshwater genera was the diatom genus *Fragilaria* spp. (31% of the samples), chlorophytes such as *Pediastrum boryanum, Scenedesmus quadricauda,* and *Ankistrodesmus* spp., and a cyanobacterium, *Microcystis* spp., some strains of which can be toxic.

Of the 60 sediment samples analyzed 84% contained dinoflagellate cysts. A total of 33 species representing 15 genera were identified; the most com-

Table 1. The different types of vessels sampled and the number of occasions each type was sampled during the course of the project.

Type of vessel	Number of samples
Container	39
Bulk Carrier	21
Gas Tanker	20
Oil Tanker	13
Car Transporter	9
General Cargo	5
Tanker	3
RollOn-RollOff	2

Table 2. Taxonomic diversity of phytoplankton samples.

Environment	Group	Water Samples		Sediment Samples	
		Species	Genera	Species	Genera
Marine	Diatoms	109	42		
	Dinoflagellates	35	19	33	15
	Flagellates	N/A	7		
Freshwater	Diatoms	7	8		
	Chlorophytes	12	14		
	Cyanobacteria	5	5		

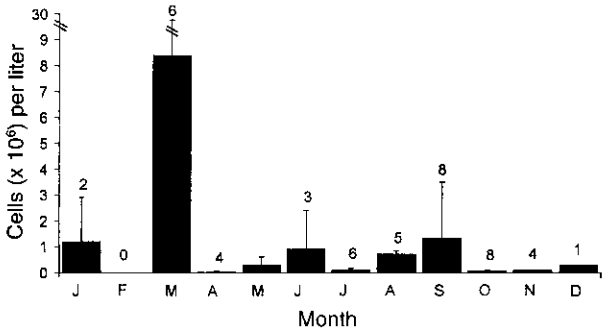

Figure 3. Monthly mean phytoplankton cell counts using ballast water samples originating from European ports. Number of samples analysed for each month indicated by number above error bar.

mon cysts were *Protoperidinium* spp. and *Scrippsiella* spp. The concentration of cysts varied from 1 to 8125 cysts ml^{-1} of wet sediment, with the majority of the samples containing a total of <500 cysts ml^{-1} of wet sediment. Four samples contained >1000 cysts ml^{-1} of wet sediment. Three species, *Scrippsiella hangoei, Pentapharsodinium tyrrhenicum,* and *Gymnodinium catenatum,* not previously recorded in British waters were identified. *Scrippsiella hangoei* was present in two samples with a maximum density of 8125 cysts ml^{-1}. Cysts of potentially toxic species were common and included *Alexandrium tamarense/catenella* (the most common), *A. minutum* and a *Gymnodinium catenatum*-type cyst (present in 13%, 6%, and 0.6% of samples, respectively).

Discussion

The water samples examined contained a wide range of organisms from both marine and freshwater environments, reflecting the variety of ballast water origins. As reported in previous studies, diatoms were the most numerous phytoplankters found in water samples (Carlton and Geller 1993; Subba Rao *et al.* 1994; Gollasch 1995; Macdonald and Davidson 1997). This is not unexpected as the flora of coastal regions is dominated by diatoms for most of the year and vessels would generally load ballast in coastal ports. In many cases organisms such as dinoflagellates and diatoms were found to be viable. This is consistent with Macdonald and Davidson (1997), who found 51% of cysts that were incubated from sediment samples hatched into motile dinoflagellates and with Hallegraeff and Bolch (1992), who successfully germinated 20 out of 53 cyst species from ballast tank sediments. All of these studies indicate that a wide range of organisms survives the journeys in ballast tanks and that there is a risk of introducing unwanted species into English and Welsh ports.

Diatoms from the potentially toxic genus *Pseudonitzchia* were common; these may be *P.* cf. *delicatissima* and *P.* cf. *seriata* and require SEM work to provide a definite identification. Diatoms from this genus were also found in 38% of the ballast water samples analysed by Macdonald and Davidson (1997). *Pseudo-nitzschia multiseries* has been linked to an outbreak of Amnesic Shellfish Poisoning (ASP) in Canada (Bates *et al.* 1989) and several other species within this genus are known to be toxic. There is a wide distribution of *P. pseudodelicatissima, P. delicatissima* and *P. seriata* in North Atlantic waters but *P. multiseries* is not common in European coastal waters (Hasle *et al.* 1996). *Pseudo-nitzschia* spp. were included in the Scottish toxic algal monitoring programme in 1996 and the first recorded occurrence of ASP in mussels was reported in the same year (Kelly and Macdonald 1997). The presence of ASP in Scottish waters has led to closures of fisheries in 1998 and 1999 (M. Kelly, pers. comm.).

Potentially toxic or nuisance flagellates were also present. Nuisance species may cause blooms that discolor the water, deoxygenate the water column, or clog fishing nets with the scum formed when the bloom breaks down. Two species found in this study, *Phaeocystis* spp. and *Ceratium* spp., fall into this category. Potentially toxic species included *Dinophysis* spp., known to cause Diarrhetic Shellfish Poisoning (DSP),which can affect humans and other mammals (Larsen and Moestrup 1989). The silicoflagellate *Dictyocha speculum* was also present in some water samples; this species has been implicated in fish kills in southwestern Denmark in 1983 (Larsen and Moestrup 1989). Although these species are native, ballast water transport is one way in which nuisance or toxic species could be transported to areas where they may not have previously caused a problem.

Dinoflagellate cysts have been recorded in 84% of the sediment samples, a greater frequency than has been recorded in previous studies by Hallegraeff and Bolch (1992), who found cysts in 50% of sediment samples and Macdonald and Davidson (1997), who found cysts in 64% of sediment samples. One of the cysts found in this study closely resembled *Gymnodinium catenatum,* a toxic species that has not been previously recorded in British waters but was also found in the study carried out by Macdonald

and Davidson (1997). The potential introduction of this species is of concern as it is a known Paralytic Shellfish Poison (PSP) producer. However, the taxonomic position of this species is unclear and there are indications that there is a non-toxic European species. The cysts found may therefore be those of the European species, *G. nolleri*, rather than the non-native *G. catenatum* (Ellegaard 1998).

Cysts of the toxic dinoflagellates *A. catenella/tamarense* and *A. minutum* were found in 13% and 6% of the samples, respectively. Cyst densities ranged from 21-1400 cysts ml^{-1} of wet sediment for *A. catenella/tamarense* and from 1-900 cysts ml^{-1} of wet sediment for *A. minutum*. These are densities greater than those found by Macdonald and Davidson (1997), who recorded *Alexandrium*-type cysts in 17% of sediment samples with maximum abundances of 94 cysts ml^{-1} for *A. catenella/tamarense* and 125 cysts ml^{-1} for *A. minutum*. Both this study and the Scottish study (Macdonald and Davidson 1997) had cyst densities much lower than the 22,500 *A. tamarense* cysts cm^{-3} reported by Hallegraeff and Bolch (1992) from a sample taken from a ballast tank containing water loaded during a bloom. Several *Alexandrium* species are known to cause PSP but *Alexandrium* type cysts are difficult to identify without germination studies. *A. tamarense* is present in U.K. waters and has been implicated in PSP events (Macdonald and Davidson 1997). Dense blooms of *A. minutum* along the Brittany coast have caused shellfish contamination by PSP (Erard-Le Denn *et al.* 1993), but this species is not common in British waters and has not been known to cause any problems.

The vessels were sampled from a range of specialized ports dealing with containers, oil and gas, grain, general cargo and cars. The majority of studies in the past have focused on one type of vessel such as cargo vessels (Hallegraeff and Bolch 1991; Carlton and Geller 1993) or sampled a relatively small number of ports (Gollasch *et al.* 1995) as these were considered to represent the greatest risk in terms of ballast water introductions. The majority of the ballast water (70%) sampled in this study originated from within Europe and the vessels carrying the ballast generally had short journeys of up to a few days between ports. Laing (1995) reported that of the estimated 16.8 million mt of ballast water discharged into English and Welsh ports only 11% was estimated to have originated from outside Europe; this percentage is reflected in our sampling data.

All vessels may pose a potential risk of introducing non-native species. However, our results indicate that, owing to the large the number of factors involved, generalizations regarding the number of species present within the tanks are difficult. The variety and density of the organisms present in the ballast tanks will be influenced not only by the port of origin and the season but also by factors such as sampling methods used, ballast management, and ship type. The large number of vessels visiting from ports in Europe with correspondingly short journey times to ports in England and Wales suggests that there would be a greater risk of secondary introduction of non-native species from established populations at other European ports than from primary introductions from areas outside Europe. There is also a risk of translocating toxic or nuisance native marine organisms to areas where environmental conditions may mean bloom or toxin formation may be more likely.

It is apparent that the proposed IMO regulations will have limited effect with respect to preventing the introduction of non-native species into UK waters. The regulations generally deal with ballast carried on a deep-sea voyage, *i.e.*, in waters 500 m deep and 200 mi offshore for more than 48 hr. A vessel on such a voyage would then be required to carryout some form of ballast management such as mid-ocean exchange. As the majority of the ballast arriving in UK waters originates in Europe, ballast exchange would not be required. There will therefore be a risk of continued introductions of non-native species from ports within Europe.

Acknowledgments

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Patterns of Marine Bioinvasion in New Zealand and Mechanisms for Internal Quarantine

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ABSTRACT: **About 130 marine species have been introduced to New Zealand by human-mediated pathways. Trends in these invasions are described in relation to changing trade patterns. Since the 1960s when emphasis on trade shifted from the United Kingdom to Asia, there has been an associated increase in the proportion of Asian species among recently discovered invaders. Notable species include the Pacific oyster *(Crassostrea gigas)*, the Asian date mussel (*Musculista senhousia)*, and the Asian kelp *(Undaria pinnatifida)*. Trends in overseas trade highlight the importance of updating donor regions in marine biosecurity risk assessments for border control agencies. Our studies suggest it is only a matter of time before further well-known invaders such as the northern Pacific seastar *(Asterias amurensis)* and the European shore crab *(Carcinus maenas)* arrive in our ports—if they are not already established. While it appears generally accepted that ballast water is the primary mechanism of trans-oceanic dispersal, the role of other mechanisms should not be ignored. Of particular concern are the periodic events when ships laid up for long periods in foreign harbors arrive in New Zealand waters with heavily fouled hulls. At a national level, the problems of internal quarantine to prevent the spread of unwanted species from "infected" harbors and ports are considered. Results from recent shipboard trials suggest that the flow-through dilution method of ballast exchange may be ineffective at ridding ballast tanks of unwanted organisms.**

Key words: marine invasion, trade pattern, ballast water, fouling, quarantine, *Crassostrea gigas*, *Musculista senhousia*, *Undaria pinnatifida*.

INTRODUCTION

At least since the arrival in New Zealand of Captain Cook's sailing ship the *Endeavour*, New Zealand's harbor and coastal ecosystems have been modified by numerous fouling organisms and other marine flora and fauna introduced from foreign shores. Although New Zealanders are very familiar with the considerable damage inflicted on their terrestrial native flora and fauna by small mammals deliberately or accidentally introduced from Australia (opossums) and Europe (rabbits, goats, pigs and deer), marine exotic species are also a problem despite the lack of public awareness. As a consequence, New Zealand's marine biosecurity is low compared to the terrestrial scene.

Several of New Zealand's prominent marine bioinvaders are controversial. For example, the Pacific oyster, *Crassostrea gigas*, was introduced in the 1970s and now supports a marine farming industry worth several millions of dollars to New Zealand each year. But, in some areas such as city beaches, *C. gigas* is a pest owing to this species' reef-building habit, which results in a displacement of sand and fouling of inshore structures and facilities such as boat ramps. Similarly, the Japanese kelp, *Undaria pinnatifida*, arrived in the 1980s and has export potential as a farmed sea vegetable (wakame) but there also are concerns that *U. pinnatifida* is seriously affecting New Zealand's coastal ecosystems (Battershill *et al.* 1998; Hay 1998).

Global trends in marine bioinvasion, patterns of overseas trade, and shipping statistics indicate that other potentially more harmful marine species such as the northern Pacific seastar, *Asterias amurensis*, now established in Tasmania and Port Phillip Bay (Furlani 1996), and the European shore crab, *Carcinus maenas*, will invade New Zealand, primarily as a result of ballast water discharges. Ballast water is undoubtedly a key player in the trans-location of marine species around the globe, and at the close of the twentieth century, important transport routes exist for the

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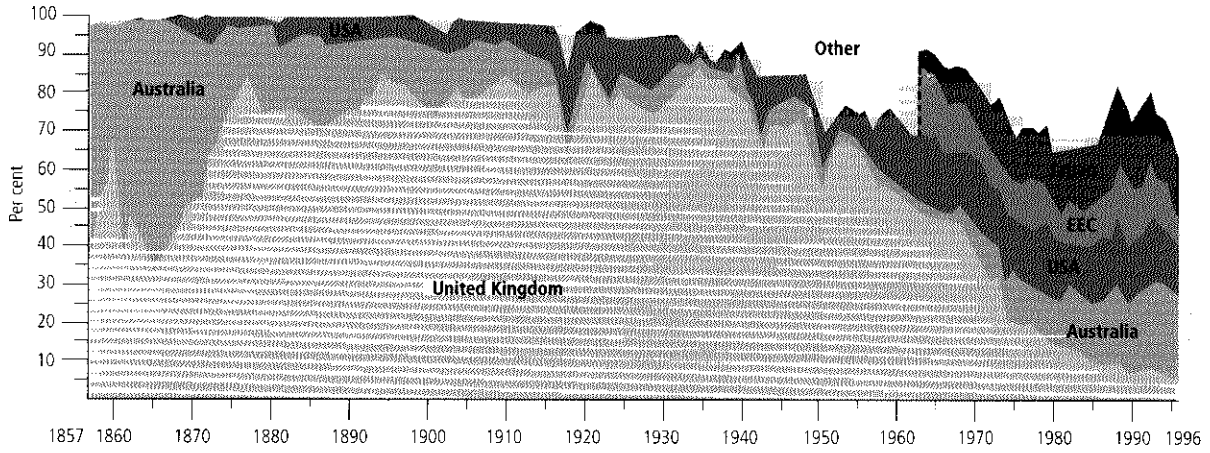

Figure 1. History of New Zealand's overseas trading patterns showing the major diversification in trade during the 1960s (data from Statistics New Zealand 1998).

dispersal of nonindigenous marine species into and within the Pacific Ocean (Carlton 1985; Carlton 1987; Cohen *et al.* 1995).

Patterns of Overseas Trade

The New Zealand economy is particularly reliant on exports of agricultural products (dairy, wool, and other edible products of animal origin), forestry products, and raw materials (aluminium and mineral fuels). More than 99 percent of these exports, totalling about 20.5 million mt, are loaded at New Zealand's international sea ports (Statistics New Zealand 1998).

Although New Zealand's trade with Australia was significant during the late 1800s, up until the 1960s New Zealand's most important trading partner was the United Kingdom (Figure 1). Since the early 1960s there has been considerable diversification in New Zealand's trade with other European countries (EEC), Japan, and other parts of Asia and various countries belonging to the Asian Pacific Economic Co-operation (APEC) group including the USA, Canada, and Chile. Today, more than half of New Zealand's overseas trade is with Australia and Asia (Statistics New Zealand 1998).

Patterns of Introduction

Associated with changes in New Zealand's trading patterns since the 1960s has been a notable increase in the proportion of foreign marine species established in New Zealand waters that are native to Japan and other parts of the northern Pacific and Asia. Prior to 1960, less than 2 percent of New Zealand's foreign marine species were thought to have arrived from Japan and other northern Pacific countries, whereas after 1960, about 38 percent of new introductions originate from this region (Figure 2). The majority of species introduced from European countries and the Atlantic Coast of the USA are fouling organisms such as bryozoans and ascidians, many of which are thought to have been transported around the world on the outside of ships' hulls during the early part of this century.

About 130 species recorded as established in New Zealand waters are thought to be introduced (Cranfield *et al.* 1998). Sixty-nine percent arrived as fouling organisms on the outsides of vessels' hulls and floating structures, while only about 3 percent most likely arrived in ballast water (Figure 3). It is unknown, however, whether 21 percent were introduced as a result of transport on the outsides

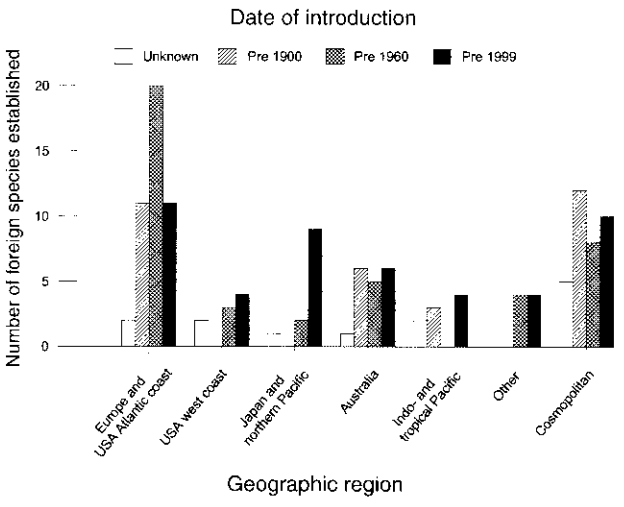

Figure 2. Native ranges of New Zealand's nonindigenous marine species (data from Cranfield *et al.* 1998).

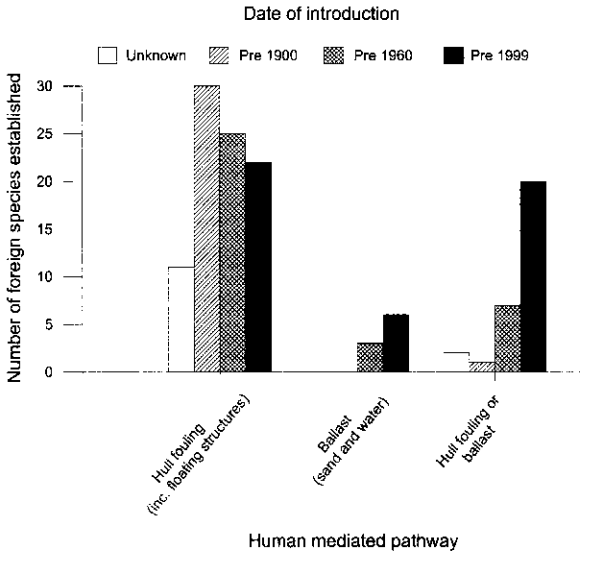

Figure 3. Pathways of introduction of New Zealand's nonindigenous marine species (data from Cranfield *et al.* 1998).

of ships' hulls or on floating structures, or whether they were discharged in ships' ballast. Some nonindigenous species probably remained unidentified for several decades (Forrest *et al.* 1997). A high level of taxonomic skill is required to differentiate between native and nonindigenous marine flora and fauna and new exotic species usually go undetected until they are well established (MacKenzie 1996).

Although many of New Zealand's conspicuous fouling organisms were introduced by shipping in the early part of this century, today most modern ships visiting New Zealand—including container vessels, bulk carriers, and gas and oil tankers—have clean hulls with very little external fouling. All modern ships carry seawater or freshwater ballast, however, generally in segregated tanks. Significantly, the last few decades have also seen the evolution of the relatively fast modern bulk carrier and today ships enroute to New Zealand from temperate northern hemisphere countries spend only a few days in the tropics, compared to several weeks during the early days of shipping.

The importance of trends in New Zealand's trading patterns and ballast water in the trans-location of new marine species to New Zealand is substantiated by the recent arrival of several molluscs that are native to northern parts of Asia such as the bivalve, *Theora lubrica*, the Asian clam, *Musculista senhousia*, and algal species including *U. pinnatifida*. The early life-history stages of these species appear especially suited to uptake, survivorship, and discharge in ship's ballast water (Cranfield *et al.* 1998; Willan 1987; Hay and Luckens 1987).

The likelihood of delivering an inoculum of an unwanted marine species via ballast water is related to the number of ship visits as well as to the total volume of ballast water discharged. New Zealand currently receives 2,500 to 3,000 foreign ship visits per year and this results in an annual total discharge of four to six million mt of ballast water (Hayden 1995; Hay *et al.* 1997).

The risks to New Zealand's marine biosecurity from hull fouling as well as fouling of floating structures such as barges and oil and gas drilling platforms cannot be ignored (Nelson 1993). Oil rig platforms and clip-on side structures floated in from Asia during the 1970s for Auckland's Harbour Bridge, are responsible for introducing some of New Zealand's conspicuous fouling organisms (Foster and Willan 1979; Nelson 1993). Since the 1970s a significant proportion of New Zealand-owned and -operated fishing companies have chartered foreign fishing vessels. These vessels are often tied up for long periods in foreign ports before being commissioned by these companies to fish intensively for deep-sea fish stocks (squid, southern blue whiting, and hoki). Fishing is usually seasonal and maintenance and cleaning operations are often carried out in New Zealand harbors.

An event that happened to result in a positive outcome in terms of improving New Zealand's level of marine biosecurity was the arrival in 1994 of a heavily fouled Russian super trawler. Prior to coming to New Zealand to fish for hoki on the west coast of the South Island, the trawler had been laid up for 18 months at Port Novorossiysk in the Black Sea, a port known to have relatively high numbers of laid-up fishing vessels. Unfortunately, at no time prior to it having its hull cleaned while in dry dock in New Zealand was the vessel observed by a marine biologist; however, after hearing about the state of the vessel's hull a serious concern was raised by Cawthron Institute staff (Hay and Dodgshun 1997).

As a consequence, the New Zealand Fishing Industry Association recognized that some overseas fishing vessels operating in New Zealand waters are a significant biosecurity hazard and adopted an industry-wide Code of Practice. This Code of Practice stresses that the charter company obtain a guarantee from the vessel's owner that the hull of the vessel is free from plant and animal growth and provides the company with rights for having the vessel

inspected at the owner's cost and, if necessary, cleaned on arrival in New Zealand and the fouling material disposed at a designated land-based refuse site (Pfahlert 1997).

New Zealand's marine biosecurity remains at risk from these chartered foreign vessels, however, and in recent years New Zealand's rapidly expanding domestic foreign fishing fleet poses additional risks when these vessels return to fish in New Zealand waters after fishing in the territorial seas of other countries such as the west coast of South America (Potter 1998).

Patterns of the Future

As overseas trade and shipping traffic increases, we can be almost certain that the new millennium will see more nonindigenous marine species reaching New Zealand shores, especially via ballast water from countries in relatively close proximity such as Australia and Asia as well as from other parts of the Pacific. Because of the global spread in the past few decades of the European shore crab, *C. maenas* (Cohen *et al.* 1995) and the relatively recent arrival of the northern Pacific seastar, *A. amurensis,* in Tasmania and Port Phillip Bay as mentioned above, it seems only a matter of time until these potentially harmful marine species are introduced into New Zealand waters–if they are not already established.

Major considerations for New Zealand's marine biosecurity in the new millennium are the geography of international trade routes and the level of international as well as domestic shipping activity. Future overseas trading patterns, therefore, will be important in determining which exotic species will invade New Zealand waters and when these invasions might occur.

In addition to the major increase in trade between New Zealand and Australia and Asian countries over the last three to four decades, there has been a steady increase in trade with several new trading partners, especially South American countries such as Chile. It can be expected, therefore, that in the next few years far greater quantities of ballast water will be discharged in New Zealand ports and harbors by the increased number of ships plying transport routes originating from South American ports. New Zealand spans the same southern latitudinal range as the southern coast of Chile, and the climate and marine flora and fauna and ecosystem structure in parts of this region show some striking similarities to New Zealand's marine ecosystems. For example, the circum-subantarctic open-coast bull kelp, *Durvillea antarctica*, is native to New Zealand as well as to Chile.

New Zealand's coastal marine ecosystems are likely to be suitable environments for the establishment of numerous South American marine species, some of which may be indigenous to that region and currently have very little or no invasion history. As demonstrated in the last few decades by Japan, countries with growing economies are the important donor regions of exotic species in the future (Ricciardi *et al.* 1998) and South America is the most rapidly growing region in the world (Statistics New Zealand 1998).

Moreover, isolated oceanic islands such as New Zealand are predisposed to human-related bioinvasions. This is largely because of their long isolation from many of the selective forces that have played a role in determining the species composition of continental biological communities (Loope and Mueller-Dombois 1989). New Zealand's terrestrial ecosystems are characterized by a long list of invasive species which were not considered a pest in their native region, but became one when introduced into a new environment (*e.g.*, feral European mammals, opossums, and brown trout), a pattern which is at least partly attributable to New Zealand's extreme geographic isolation (Townsend 1996).

This pattern of ecosystem vulnerability apparent in New Zealand's terrestrial ecosystems may also apply to marine ecosystems. For example, *U. pinnatifida* is a highly opportunistic annual; an invasive life-history characteristic attributable to this species' rapidly growing, reproductively active sporophyte stage. Prior to *U. pinnatifida* becoming established, however, all large New Zealand kelps and large fucalean seaweeds were perennials that are far less successful at pre-empting available space. Although searches for invasion patterns based on the biogeography of the Pacific Ocean are limited by our knowledge of systematics and species distributions (Carlton 1987), the application of principles and predictions from island biogeography may prove very useful in future risk assessments aimed at improving New Zealand's level of marine biosecurity.

Mechanisms for Internal Quarantine

If it is inevitable that new foreign marine species will invade New Zealand waters, there currently are

no marine biosecurity management strategies in place to limit the spread of unwanted species such as the European shore crab, *C. maenas*, or the northern Pacific seastar, *A. amurensis*. The newly formed Ministry of Fisheries is currently in the process of improving surveillance procedures for newly introduced marine organisms (Cox 1998). However, there are no current standards for regulating hull fouling or for the discharge of ballast that was loaded in one New Zealand port into the waters of another port.

New Zealand has 16 domestic shipping ports which are distributed throughout its North and South Islands. On arrival in these ports, international as well as domestic ships (mainly container vessels) and fishing vessels frequently discharge ballast water that was uplifted from their previous New Zealand port of call. In the case of new introductions in source ports, the only reliable management strategy may be quarantine of the infected area by preventing or strictly limiting all local shipping activity. However, strict quarantine of the major ports is clearly not a viable option.

Limiting shipping traffic is also unlikely to be a viable management option, however, owing to the national and regional demand for domestic transport to and from New Zealand's main centers. Even if quarantine were an option, it would be impossible to prevent the spread of high-risk species suited to travelling large distances (100s–1000s km) in coastal currents to suitable habitats outside the region of initial infestation. For example, the northern Pacific seastar, *A. amurensis*, has a planktonic larval life of 50–120 days (cited in Bruce *et al.* 1995) and would be capable of natural dispersal over these scales.

In coastal waters, the only management strategy currently applied to limit the regional spread of marine species in ships' ballast is mid-ocean ballast exchange, either by complete reballasting, or by flow-through dilution. These measures are recommended by the International Maritime Organisation (IMO) to minimize the global spread of nonindigenous marine species. Unfortunately, however, these methods are no panacea for the ballast water problem, domestically or internationally (see below).

Although some studies have demonstrated that the chances of trans-locating marine organisms from source to recipient ports are considerably reduced after carrying out a mid-ocean exchange (Williams *et al.* 1988), other studies have demonstrated that mid-ocean exchange is only partially effective in removing species that have settled in the bottom sediments of ballast tanks, such as dinoflagellate cysts (Hallegraeff and Bolch 1991; 1992). During 1990, Locke *et al.* (1991) sampled the ballast water of 59 foreign ships bound for the Great Lakes, USA and calculated the effectiveness of mid-ocean exchange at eliminating live freshwater zooplankton to be 67 percent.

During 1995-97, the Cawthron Institute sampled the ballast water from 161 ballast tanks of 50 foreign ships and found that about 80 percent of the tanks contained live phytoplankton and zooplankton. The proportion of tanks containing marine organisms belonging to each of the major taxonomic groups encountered in the samples was similar for tanks that were exchanged (either by complete reballasting or flow-through dilution) and tanks that were not exchanged (see Hay *et al.* this volume). Many of these taxonomic groups were characterized by coastal species (cysts, molluscs, and annelids) and most of the exchanged tanks contained a mixture of coastal species mixed with oceanic species. Thus mid-ocean exchanges were only partially effective at removing coastal phytoplankton and zooplankton from ballast tanks (Hay *et al.* 1997).

Although flow-through dilution may have the advantage over reballasting as a method of carrying out a ballast exchange when maintenance of ship stability and limits on longitudinal stresses and structural loads are at risk (because of marginal weather conditions), flow-through dilution has a number of significant disadvantages:

(1) Air ventilators, manholes, or tank lids have to be used for the overflow and these are not designed to vent water.
(2) If air ventilators are too narrow (they should be designed to cope with 125 percent of tank capacity), or are occluded or blocked, the tanks may be pressed up too far (over pressurized) during exchanges.
(3) Removing manholes or deck plates may compromise the strength of the deck, especially in heavy seas.
(4) Many vessels are not designed for flooding vast amounts of water on deck. This may impose serious safety risks on crew members.
(5) In icy conditions, overflow may cause accretion of ice.
(6) Not generally suited for double-bottom tanks because the high head of water may cause excess internal pressure.

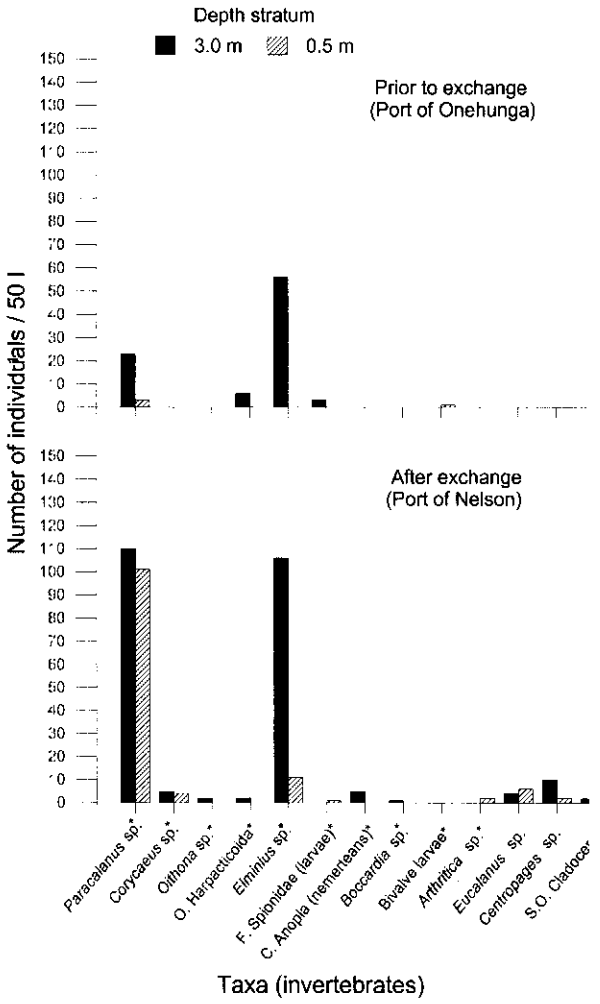

Figure 4. Abundance of invertebrate taxa at two strata in the forepeak tank of a domestic container ship prior to (Port of Onehunga) and after (Port of Nelson) an open ocean ballast exchange by flow-through dilution (*refers to invertebrate taxa that were present in the water uplifted in the donor port, but were not present in open ocean samples collected during the exchange).

(7) The length of time required to make the exchange (three times the tank volume) may be longer than the voyage, especially on domestic routes.
(8) Ballast pumps may be required for other functions.
(9) There are also a number of scientific concerns with the flow-through dilution method.

Its effectiveness at ridding ballast tanks of unwanted marine organisms may be highly variable, and therefore unreliable, as a marine biosecurity management tool for use by internal quarantine agencies. For example, a recent shipboard trial conducted by Cawthron on the forepeak tank of a domestic container vessel produced what at first appeared to be a counter-intuitive result. Certain harbor and estuarine zooplankton species being used by Cawthron as indicator species to measure the effectiveness of mid-ocean exchanges were common at the source port but were not present in plankton samples collected from the open ocean (outside the 12 mi limit) during the time the ballast exchange was carried out. However, some of these indicator species (the calanoid copepod *Paracalanus* sp. and nauplii larvae of the barnacle *Elminius modestus*[2]) occurred in higher numbers in samples taken from the water column of the tank immediately after carrying out an exchange using the flow-through dilution method (three times the original volume of water in the tank was pumped through the top of the tank via vents located on the foredeck), compared to samples taken from the tank immediately prior to departure from the source port, several hours after the tank had been filled (Figure 4). This result indicates that sampling prior to departure or during mid-ocean exchange was not sufficiently representative, or that some of the zooplankton uplifted at the source port are resuspended from the bottom of the tank during the exchange and retained in the tank throughout the exchange (authors' data).

Even if mid-ocean exchange is effective, hull fouling may be important with respect to regional and local dispersal. Fishing vessels as well as small pleasure craft commonly sail between coastal destinations around the coast. These vessels are very effective at distributing reproductively viable life-history stages of fouling species such as gametophytes and sporophytes of the kelp *U. pinnatifida*. Secondary dispersal by coastal shipping and small boats is responsible for the regional spread of *U. pinnatifida* along the east coast of New Zealand's South Island (Hay 1990).

Conclusion

It seems inevitable that in time certain high-risk invaders will reach New Zealand shores, but invasion rates can be minimized by recourse to decision-making tools such as risk assessments for ballast water discharges in relation to current and future overseas

[2] *Elminius modestus* is the most numerous barnacle species in New Zealand's sheltered waters and harbors where it attaches itself to practically any hard substrate. *Elminius* was translocated to Britain during World War II on vessels sailing from Australia and New Zealand and has subsequently spread to other parts of Europe (Gunson 1993).

trade patterns. Clearly, more knowledge is required about those marine species particularly suited to trans-location by shipping and which have invaded countries that will be New Zealand's important trading partners in the future.

At present, New Zealand requires an effective marine biosecurity surveillance program aimed at detecting new introductions. The benthos, plankton, and artificial structures, and the outsides of vessels' hulls in ports and harbors should be regularly monitored, especially in international ports. Regular surveillance will be crucial to the success of response measures such as attempts at eradicating unwanted species from highly valued coastal areas.

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Survival Rates of Species in Ballast Water during International Voyages: Results of The First Workshops of The European Union Concerted Action

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ABSTRACT: International shipping has been recognized as a major vector for the introduction of nonindigenous and harmful organisms through the transport of species in ballast water as well as fouling on hulls. In recent decades, ballast water discharges have increased throughout the world in and near most major ports, causing an increased probability of successful transfer and establishment of self-sustaining populations of exotic species. Although many data reviews and sampling programs have been carried out, little information exists on the survival rates of species or individuals during ship journeys. Detailed information on the survival rate of species would assist in evaluating the risk of unintentional introductions. During a previous study, the survival of planktonic organisms in ballast water tanks was studied by accompanying a container vessel on its 23-da voyage from Singapore to Germany. Results of that study are compared with results obtained during workshops of the recently initiated European Union Concerted Action in an attempt to intercalibrate methods of sampling ballast tanks. During both inter-oceanic and shorter voyages, most species and individual organisms decreased in number, although a species of harpacticoid copepod increased dramatically in one tank. The results of the first workshop on intercalibration of ballast water sampling techniques and the initial results of two ocean-going workshops are presented and discussed in this paper.

Key Words: ballast water, sampling method, survival rate, introduced species, *Tisbe*

INTRODUCTION

Since the late 1870s, ballast water has been used to submerge the propeller and rudder in the water and to control the trim while increasing the stability of ships that are not fully loaded. Ballast water is usually carried in segregated ballast water tanks or in emptied cargo holds. Fully loaded ships carry ballast water as well (Carlton 1985, 1987, 1994). Depending on the construction of the ballast tanks and the pipework, several metric tons of residual water can remain in maximally emptied ballast tanks.

Nonindigenous species have been transmitted with ballast water, associated sediments, and as fouling organisms attached to the hulls of ships. It has been estimated that the 70,000 major cargo vessels of the world (Stewart 1991) annually transfer about 10 billion mt of ballast water world-wide, leading to a global concern for possible effects on biodiversity. Ballast water containing high densities of many

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species maybe taken in from eutrophic coastal areas. Some of these species may survive voyages of several months, presenting a true risk of introducing viable populations into non-native environments. On average, 3,000 to 4,000 species (Carlton and Geller 1993; Gollasch 1996) are transported between continents daily by ships. Species discharged with ballast water into ports-of-call may threaten native populations, fishing industries, and public health.

The European Union Concerted Action Study

In cooperation with five European countries (Finland, Ireland, Sweden, the United Kingdom (England and Scotland), and Lithuania) and the International Maritime Organization (London), Germany coordinated a 2-yr (1998-1999) European Union Concerted Action (EUCA) effort called "Testing Monitoring Systems for Risk Assessment of Harmful Introductions by Ships to European Waters." Several experts from around the world (*e.g.*, North America, South America, Mediterranean countries, and Australia) also participated. Various monitoring methods were studied in order to evaluate, qualitatively and quantitatively, the fate of exotic species in ballast water and to compare the efficiency of various sampling methods. The main objectives of the EUCA included (1) summarizing the state-of-the-art of ballast water studies, (2) collecting case histories of introduced species in European waters, (3) assessing potential treatment measures to reduce the risks arising from ballast water releases, (4) developing material to raise public awareness, and (5) intercalibrating techniques of ship-board sampling.

This investigation of ballast water carried by ocean-going vessels represents a new contribution to our understanding of the survival potential of transported species while documenting the variety of organisms unintentionally transported. Such studies support the assessment of the ecological and economic risks involved in discharges of untreated ballast water.

Methods and Materials

During the EUCA study, the standardization of ship-board sampling techniques was undertaken during one intercalibration workshop. The recommended suite of sampling methods was tested onboard ships during their voyages in order to test the practicability of these methods.

Standardization of sampling methods

Standardization of techniques for sampling ballast water is essential not only to enable a reasonable comparison of data from scientific studies, but also to establish comparable standards for risk assessment. Some standardization experiments were carried out during a 2-da workshop attended by scientists previously involved in European ship sampling studies. The experiments were carried out by sampling a land-based plankton tank capable of holding 5.3 m^3 of water. The test tank was spiked with a known density of phyto- and zooplankton organisms. For proper mixing, aeration using compressed air supplied at the bottom of the test tank was applied for 15 min prior to employing each sampling technique. Before sampling the plankton tank, nine pre-samples were taken to document the number of species and specimens in the water body of the plankton tower. A total of 48 samples was taken from the test tank and analyzed in this experiment, prior to testing the various sampling methods onboard ships during ocean-going workshops (see below). The efficiency of each method was analyzed by comparing the number of specimens recovered to the initial number added to the tank.

The methods tested were developed during onboard studies in Germany, Norway, Scotland, and Wales. The equipment used included pumps (for sampling ballast tanks onboard ships through sounding pipes) nets, buckets, and the Ruttner sampler (a bottle-like device with a capacity of 1.5 L that can be closed at certain depths by lowering a weight) for sampling through open manholes. All methods were employed through the hatch-like opening of the test tank.

Sampling Through Sounding Pipes (Pump Samples)

A small hand pump and a 30-kg "Monopump" were tested for sampling through sounding pipes onboard ships. To sample the test tank, both pumps were deployed through the hatch at the top of the tank. A hose connected to the pump was lowered down the test tank to 3 m depth. The comparatively little and lightweight hand pump allowed sampling by one person. Practical experience in employing this method onboard vessels in the Kiel Canal has shown that the hand pump is the easiest to handle and most flexible method when sampling via the sounding pipes.

Sampling through Open Manholes (Nets, Buckets, and Pump Samples)

The mesh sizes of the four plankton nets used were 10 µm, 20-30 µm, 53 µm, and 68 µm. A plankton net with a cone-shaped opening and a mesh size of 55 µm was also used. All nets were lowered to the bottom of the tank and kept in this position for 1 min. The net was then lifted by hand at a speed of approximately 0.5 m sec^{-1}. Pump samples, bucket samples, and samples taken with the Ruttner sampler were analyzed.

Ocean-Going Sampling

Although many desk studies and onboard-ship sampling programs have been carried out in the past, there is a lack of data on survival rates of phyto- and zooplankton species during ship journeys. A total of five ocean-going workshops will be undertaken on ships in short-term and intercontinental service. This paper summarizes the results of short-term voyages from St. Petersburg, Russia, to Lisbon, Portugal, on a research vessel and from Cork, Ireland, to Sture, Norway, on an oil carrier. The results of an earlier study (carried out in 1995) in which the ballast water of a container ship was sampled daily on a trip from Singapore to Bremerhaven, Germany were used for comparison. The duration of the voyages ranged from 3 to 23 days (Table 1). Three long-term ocean-going voyages were undertaken in 1999, starting along the coasts of New Zealand, Taiwan, and the Black Sea.

St. Petersburg, Russia to Lisbon, Portugal

The first EUCA ocean-going workshop was conducted aboard a Russian research vessel, the *Sibiryakov*. The ballast water study was carried out during a cruise organized by the Russian State Hydrometeorological University (RSHMU), within the framework of the Baltic Floating University (BFU) program. The vessel left St. Petersburg for Lisbon on 22 July 1998 and returned to St. Petersburg on 28 August 1998. The ballast water was exchanged three times en-route and sampling was therefore divided into four periods: (1) ballast water taken onboard in the Gulf of Finland shortly after leaving the port of St. Petersburg (22-25 July), (2) ballast water of northern Baltic Sea origin (25 July to 4 August), (3) water pumped onboard during the passage through the Strait of Dover (4 -18 August), and (4) ballast water pumped onboard in the Zund, Denmark region (19-22 August).

Table 1. Details of the ships and ballast tanks of the vessels in this study. (DWT is dead weight metric tons.)

	St. Petersburg - Lisbon	Cork - Sture	Singapore -Bremerhaven
Name of Ship	*Sibiryakov*	*Nordic Torinita*	*DSR America*
Type of Ship	Research vessel	Oil tanker	Container vessel
Duration (days)	13	2.7	23
Tanks investigated	1	3	2
Number of Samples	95	61	41
Ship size (dwt)	3,442	108,682	45,000
Maximum volume (m^3) of ballast	268	43,818	14,860

Because it is a research vessel, the *Sibiryakov* is not equipped with ballast water tanks. A small (11 mt) tank that usually carries freshwater is located under the ship's main propeller shaft. This tank was filled with seawater, which was sampled as ballast water. Access to the tank was possible either through a manhole or a sounding pipe.

Two sampling methods were used to compare species recovery: (1) bucket (10 L), (2a) hand pump via an opened manhole, and (2b) hand pump through the sounding pipe. After sampling, the ballast water was filtered through a plankton net (mesh size 55 µm). A total of 52 zooplankton and 43 phytoplankton samples was taken.

Cork, Ireland, to Sture, Norway

During this ocean-going workshop, several sampling methods were used in order to support the standardization of European techniques to sample ballast water. The usual route of this oil tanker is between the ports of northwestern Europe, predominantly those in the North Sea. The ship transports crude oil from Norway to ports in Western Europe and on its return trips to Norway it is in ballast, *i.e.*, no cargo is transported. In 1996 the vessel was docked for routine inspection in Japan. The sediment was removed from the ballast tanks and the antifouling paint renewed. After the departure from Whitegate Terminal in Cork, Ireland, on 8 October 1998 (2130), the vessel went northward passed the Scottish Hebrides, sailed around Scotland, and crossed the North Sea. It arrived at Sture Oil Terminal, Norway, on 11 October at 2100. Average

cruising speed was 14.6 kn and the duration of the voyage was 64 hr and 30 min (2.7 da).

This vessel is equipped with 12 ballast tanks (aft peak tank, fore peak tank, and 10 wing tanks), which reach a maximum depth of 18 m. The total ballast tank capacity is 43,818 m^3.

When manholes were opened, species attracted by light were collected by hand using sampling jars. In addition, hand pump and net samples were taken from both wing tanks investigated. The forepeak tank was treated differently. To sample the forepeak tank, three hoses were positioned at depths of 5 m, 10 m, and 15 m before ballast water was taken onboard. Because of safety reasons, it was not possible to install a hose deeper than 15 m. After the tank was filled, ballast water was sampled using the hand pump at the surface and at the three depths of previously installed hoses. In addition, bucket samples were taken at the surface only.

To compare efficiency of different sampling strategies, bucket, net, and hand pump samples were taken from both wing tanks. Bucket samples were taken at the surface. Hand pump and net samples were taken at a depth of 2 m (starboard wing tank) or 2.5 m (portside wing tank) by lowering either the hose or net depending on the tank installation and water level. The mesh size of the plankton nets used were 10 µm, 20-30 µm, 53 µm, and 68 µm. Additionally, a plankton net with a cone-shaped opening and a mesh size of 55 µm was used. A Ruttner sampler was employed for phytoplankton sampling.

Singapore to Bremerhaven, Germany (Long-term Voyage)

In 1995, ballast water was sampled for phytoplankton, zooplankton, and environmental conditions (temperature, salinity and oxygen) during a 23-da journey. The vessel left Singapore for the port of Colombo, Sri Lanka, passed the Suez Canal, called at Rotterdam in the Netherlands, and terminated its journey in the German port of Bremerhaven.

Four tanks were sampled, including the aft peak tank, fore peak tank, and two of the eight side tanks (side tank number three both portside and starboard). The aft peak ballast tank was filled with water while the ship was entering the Strait of Malakka close to Singapore. The fore peak tank and both side tanks were filled with ballast water while leaving the port of Colombo, Sri Lanka. Immediately after filling the tanks, the ballast water sampling program was started.

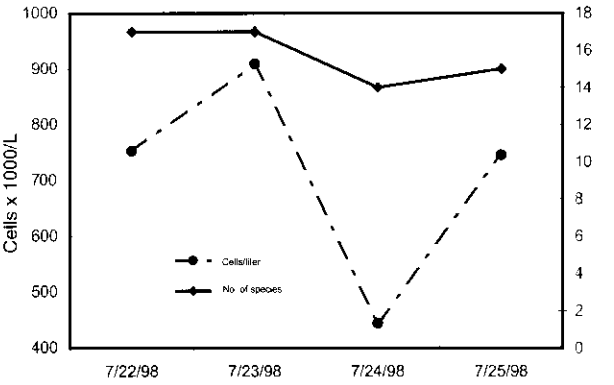

Figure 1. Number of phytoplankton cells and species in ballast water originating in the Gulf of Finland during a voyage from St. Petersburg to Lisbon. Samples were taken via an opened manhole over a period from July 22-25, 1998.

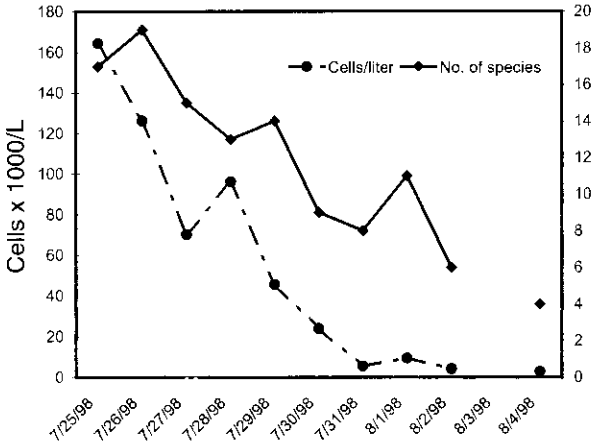

Figure 2. Number of phytoplankton cells and species in ballast water originating in the northern Baltic Sea during a voyage from St. Petersburg to Lisbon. Samples from July 27-30, 1998 were taken via an opened manhole; all other samples were taken through a sounding pipe.

All ballast tanks, accessible through open manholes, were sampled daily, between 1000 and 1100. The ballast water of the aft peak tank was sampled for phytoplankton and zooplankton using vertical hauls of a plankton net with a conical top (mesh size 10 µm for phytoplankton samples and 55 µm for zooplankton samples). The net was drawn from approximately 6 m above the bottom to the surface. The construction of the side tanks included several partition floors and support frames, preventing use of this sampling method. Therefore, the ballast water of these tanks was sampled by filling a 10 L bucket 10 times with surface water and pouring this water through the 10-µm mesh and 55-µm mesh plankton nets outside the ballast tank. Samples were preserved in 4% buffered formalin (phytoplankton) and 70%

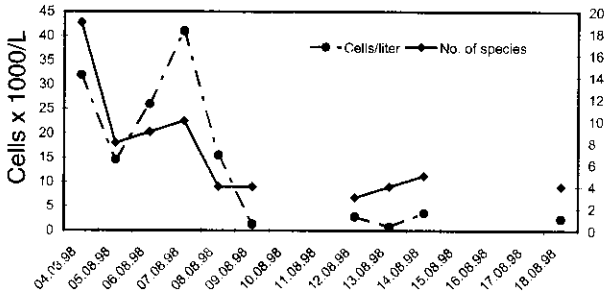

Figure 3. Number of phytoplankton cells and species in ballast water originating in the Strait of Dover during a voyage from St. Petersburg to Lisbon. Samples from August 4, 5, 7, 8, 14, and 18, 1998 were taken via an opened manhole all other samples via a sounding pipe.

ethanol (zooplankton). Specimens were identified and counted onboard using a microscope for phytoplankton and a dissecting microscope for zooplankton (Gollasch *et al.*, in press).

Results

Survival rates of the zooplankton and phytoplankton during the first EUCA ocean-going sampling studies are presented according to the route of the vessel. For comparison, results of the earlier voyage from Singapore to Bremerhaven, Germany, have been included.

St. Petersburg, Russia, to Lisbon, Portugal

During the voyage from St. Petersburg to Lisbon and back, ballast water was exchanged completely three times. The number of phytoplankton species in all four ballast water sampling trials decreased during each of the four sampling periods (Figures 1-4). The same pattern was observed for phytoplankton density, with a few exceptions on day 2 (Figure 1) and days 3-4 (Figures 2 and 3), when increases were observed. Living phytoplankton cells were at very low densities at the end of 13 da during the fourth sampling period (Figure 4).

Zooplankton analyses of the ballast water pumped onboard in the northern Baltic Sea showed a similar decrease of both species and individuals (Figures 5 and 6). The higher density of copepods on the second day of the investigation period and of total species on the second and eighth days (Figure 5) is noteworthy. Furthermore, it is noted that the number of individuals caught with the plankton net through an open manhole is about three times higher than the number sampled by pumps via sounding pipes. The density of living zooplankton specimens

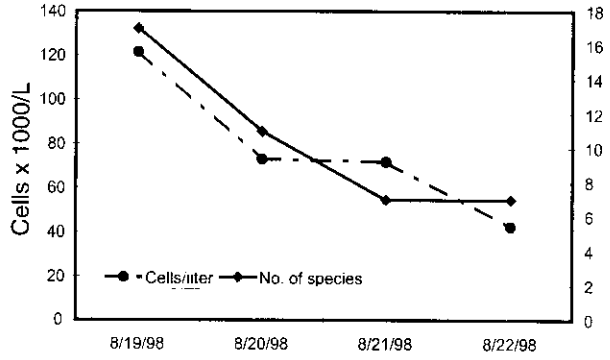

Figure 4. Number of phytoplankton cells and species in ballast water originating in Zund during a voyage from St. Petersburg to Lisbon. Samples from August 19, 21, and 22, 1998 were taken via an opened manhole all other samples via a sounding pipe.

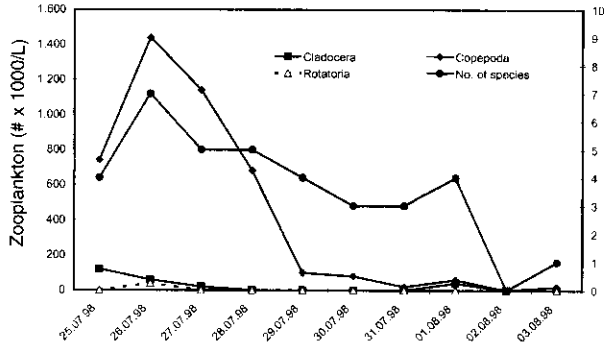

Figure 5. Origin of the ballast water investigated: Northern Baltic Sea. Number of zooplankton species and individuals in ballast water originating in the northern Baltic Sea during a voyage from St. Petersburg to Lisbon. All samples were taken using a hand pump via a sounding pipe.

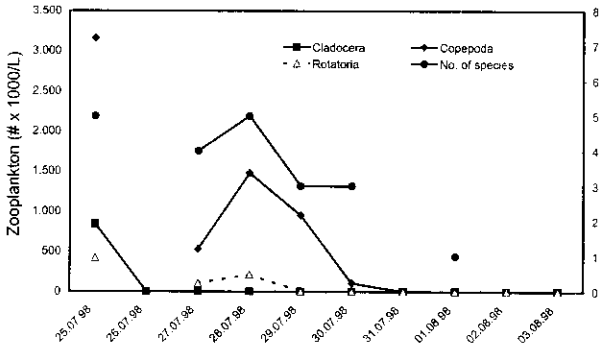

Figure 6. Number of zooplankton species and individuals in ballast water originating in the northern Baltic Sea during a voyage from St. Petersburg to Lisbon. All samples were taken using a plankton net via an opened manhole.

captured by either method at the end of 10 da was extremely low (Figures 5 and 6).

Cork, Ireland, to Sture, Norway

The number of phytoplankton cells per liter in samples taken from both side tanks on the second

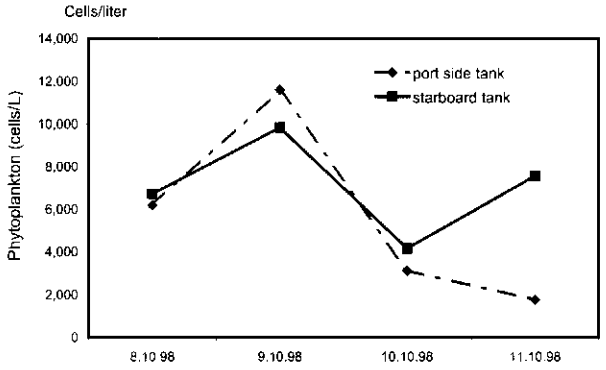

Figure 7. Number of phytoplankton cells in ballast water sampled in water originating in Cork during a voyage from Cork, Ireland, to Sture, Norway. All samples were taken from side tanks via an opened manhole.

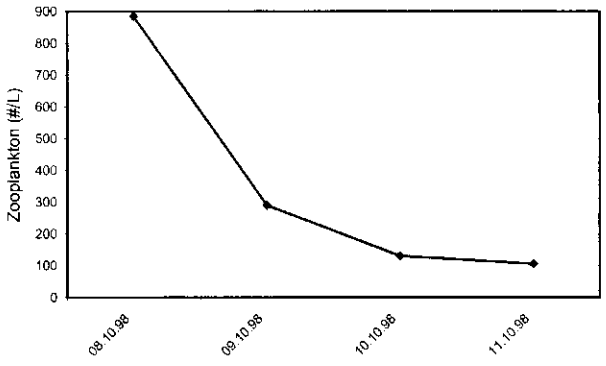

Figure 8. Number of zooplankton individuals in ballast water sampled in water originating from Cork during a voyage from Cork, Ireland, to Sture, Norway. All samples were taken from side tanks via an opened manhole.

day was higher than the number recorded on the first day (Figure 7). The increase of cells in the starboard side tank at the end of the voyage may have been caused by an upcoming gale with wind forces of 8 Beaufort (Figure 7).

The number of zooplankton individuals decreased more rapidly at the beginning of the voyage than towards the end (Figure 8). At the beginning of the voyage, the density of zooplankton in the fore peak tank was greater at the surface and 5-m depth than at the 10-m and 15-m depths (Figure 9). By the end of the voyage, zooplankton density at the surface was greatly reduced and was uniform throughout the water column, probably due to increasing water currents in the ballast tank caused by the wind-induced movements of the vessel (Figure 9).

Singapore to Bremerhaven, Germany

Results of this study have been published elsewhere (Gollasch *et al.*, in press) and are summarized

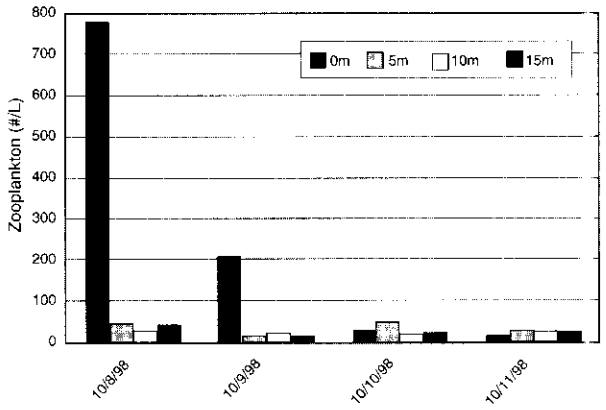

Figure 9. Number of zooplankton individuals in ballast water originating in Cork during a voyage from Cork, Ireland to Sture, Norway. All samples were taken from the fore peak tank using a pump connected to hoses with certain lengths via an opened manhole and taken at depths of 0, 5, 10, and 15 m.

here. The ballast water loaded shortly after leaving the port of Colombo contained enormous numbers of copepods. It is supposed that the copepods reduced the phytoplankton through predation. The ballast water taken onboard near Singapore showed phytoplankton species survival of 10% after the 23-da voyage, whereas the survival rate of individual cells was 0.2 %.

The survival rates of zooplankton species in the Colombo (23%) and Singapore (17%) ballast waters were comparatively high. The survival rate (2%) of zooplankton individuals in the Singapore ballast water was 10 times higher than that of phytoplankton. In the samples of the Colombo ballast water, 15% of the zooplankton specimens survived. In general, the number of zooplankton species and individuals decreased over time in the ballast tank; the increased density of the harpacticoid copepod *Tisbe graciloides* was an exception. Eleven individuals of *T. graciloides* were found per 100 L at the beginning of the experiment; at the end of the 23-da voyage, 1040 specimens/100 L were found (Gollasch *et al.*, in press).

Intercalibration of Sampling Techniques

Sounding pipe sampling

For sampling phytoplankton, the hand pump seems to be more efficient (maximum 89%) in species recovery than the Monopump (76%). Only the Ruttner sampler was more efficient (96%) in retaining phytoplankton specimens, but this device cannot be used for sampling through sounding pipes.

Zooplankton catches were higher using the Monopump (maximum 96%) compared to the hand pump (maximum 62%).

Manhole sampling

The Ruttner water sampler produced the best phytoplankton samples (96%). The 10-µm-mesh plankton net was 75% efficient, comparable to samples obtained by the Monopump operated via sounding pipes. Bucket sampling of the surface water (with an efficiency of 59%) seems to be an inappropriate method.

Three of the four plankton nets used for sampling zooplankton had comparable levels of efficiency: 69% (cone net, mesh size 55 µm), 72% (mesh size 68 µm), and 74% (mesh size 20-30 µm). The plankton net with a mesh size of 53 µm was less than 4% efficient and is inappropriate for sampling zooplankton.

Discussion

The likelihood that an introduced species will succeed in new regions and establish a self-sustaining population depends on a number of factors, related primarily to the biological characteristics of the species and the environmental conditions in the receiving area at the time of introduction. Additional important factors are the number of introduced specimens (size of founder population), number of repeated transfers, the ecological capacity of native competitors to keep numbers of the invaders down, and the availability of adequate food. Species are more likely to become established in environments that are similar to those of their native range. Therefore, environmentally similar conditions in both the last port of call and the port of discharge may lead to a high degree of successful species transfer.

Efficiency of the Sampling Techniques Tested

Studies on the efficiency of sampling techniques currently used to sample ballast water allowed comparison of the results of onboard sampling studies. It was shown that all methods had different results. Depending on the accessibility of the ballast water, different methods have to be used. Net samples showed greater efficiency compared to pumps, but can be used only through manholes. Pumps, however, may be operated via sounding pipes.

Sounding pipe sampling

Recommendations for which pump to use in different circumstances are given. The large Monopump was more efficient than the small hand pump method, but was cumbersome to use. Additional manpower is needed to transport and operate the approximately 30-kg pump and equipment onboard. The advantage of the small, lightweight hand pump is its convenient size, enabling sampling and transport by a single person. Two disadvantages of this pump are the limited pumping height of approx. 8 m and the lower efficiency compared to the Monopump. Using the Monopump, water could be pumped from a depth of at least 20 m if the ballast tank is filled completely. If the tank is not filled to the top, but the distance down to the water surface is higher than 8 m, the pump can still bring water to the operational deck. The water flow is approximately three times higher compared to the small hand pump. Using the heavy Monopump seems to be the most suitable method for sampling zooplankton in ballast water via sounding pipes. In contrast, the hand pump was more efficient for sampling phytoplankton.

Manhole sampling

The net hauls using the cone-shaped net (mesh size 55 µm), the plankton net with a mesh size of 68 µm, and the net with 20-30 µm mesh size seem to be comparable in their efficiency as demonstrated during the experimental sampling of the plankton tank. The short cone-shaped net and the net with 20-30 µm mesh are the preferred methods to sample the zooplankton in ballast water via manholes because of their short length (it is unlikely to become stuck in the tank frames or other installations), comparably small diameter, and efficient capture results. Additionally, the net opening of short nets can be lowered closer to the bottom compared to long nets. Previous onboard studies have shown that on many occasions the depth of the water column sampled was limited to less than 4 m. In these cases, shorter nets were preferred. Because the Ruttner sampler was the most efficient phytoplankton sampler, it is recommended for use wherever possible.

Comparison of net versus pump

In general, the pumps collected about a third of the specimens collected by plankton nets. Fast-swim-

ming zooplankton, such as fish larvae, amphipods, or isopods, may not be caught by the pumps due to the limited pump suction. The net method is therefore preferred over the pump method; however, nets can be used only when manholes are open. Previous onboard studies have shown that manholes could not be opened on many occasions due to overlaying cargo and lack of time or manpower (Gollasch 1996; McCollin *et al.* 1999). In such cases, sampling the ballast water by operating pumps through sounding pipes is preferable to leaving the ship without any sampling.

Species Found in Ballast Water

Previous sampling studies during ship voyages were carried out on a limited scale by *e.g.* Carlton (unpublished data), Rigby and Hallegraeff (1993, 1994), and Fukuyo *et al.* (1995). All investigations showed a rapid decline of plankton concentration during the first days after filling the tanks. As reported by Carlton (1985) and Williams *et al.* (1988), the current results show that the diversity and number of specimens decreased with increasing duration in the ballast tank. Williams *et al.* (1988) documented the greatest decrease of diversity in ballast water during the first 3 wk of a voyage. On the other hand, during the German study, living amphipods, *Corophium acherusicum*, were observed even after 116 da in a ballast tank (Gollasch 1996). Macrofaunal species were found to survive for longer time periods compared to viable phytoplankton species (excluding cysts). It is suggested that some of the macrofaunal species matured in the ballast tanks. For others, especially phytoplankton and their predators, the low numbers of surviving species and individuals may be due to the absence of light in the ballast tank. In addition, some delicate species may be adversely affected by harsh environmental conditions, such as rolling of the water in ballast tanks (Gollasch *et al.*, in press).

Never before documented is the surprising observation made during the voyage from Singapore to Germany that a zooplankton species increased considerably in density after 10 da in the ballast tank. At the start of the voyage, 11 specimens of *Tisbe graciloides* were collected in 100 L of ballast water. After 15 da, the density of this species had increased by 9,450% to 1,040 specimens, demonstrating that conditions in ballast tanks can indeed sustain certain species. It is believed that the reproduction rate did not increase, but rather the survival rate of the subadult stages was higher due to the absence of natural predators (*e.g.*, fishes) in the ballast water, low competition through decreasing diversity, and unlimited food supply. Dying zooplankton and phytoplankton cells (the last living cells were recorded on day 13 of the voyage) were an excellent food supply for harpacticoid copepods. Previous studies showed *Tisbe* sp. to be a common species in ballast water (Carlton 1985; Locke *et al.* 1993; Gollasch *et al.* 1998). The present study showed that *Tisbe graciloides* is able to survive, reproduce, and develop enormous densities inside ballast tanks. This result suggests that ballast water might be the main vector of distribution for *T. graciloides*, or even the genus *Tisbe*, and perhaps explains the zoogeography of the genus. This new dimension of species transport in ships' ballast tanks shows that ballast tanks can be incubators under special conditions and emphasizes the risk of species transport with this vector.

Vessels in these studies transported ballast water and organisms from the western Indo-Pacific and Indian Ocean, the Bay of Biscay, and the Irish Sea to the North Sea and Baltic Sea regions of Europe. Some taxa, such as the larvae of molluscs and echinoderms, were indeterminable; therefore, we do not know if these species are native to European waters or not. However, it is assumed that these vessels pose a risk of species transportation either due to primary or secondary introductions of new species with its ballast water.

EUCA Recommendations

- Researchers conducting onboard studies should consider the recommended methods standardized by the EUCA partners. Wherever possible, these methods should be compared with their own methods in order to develop correction factors to allow comparison of sampling results.
- Wherever possible, the standardized sampling techniques should be used in any future ballast water study.
- From the intercalibration exercises undertaken so far, it is clear that existing risk assessment methods (a) need to be modified to include a larger range of scenarios and (b) require further studies with intercomparison according to, *e.g.*, ship design categories.
- The EUCA welcomes any advice or comments that would support the standardization of ship sampling methods.

The presence of human disease agents (*e.g.*, cholera bacteria) in ballast water emphasizes the need for ballast water treatment (McCarthy & Khambathy 1994). Ignoring the problems related to species introductions could be analogous to an ecological roulette (Carlton and Geller 1993; Hedgpeth 1993). Because of the great number of parameters involved, we cannot estimate the probability of future species introductions and whether these might cause severe harm to the environment or the economy. Major problems may occur, impacting local aquaculture businesses or other economically important activities. In 1988, the total world aquaculture production was estimated to be at 14 million mt (FAO 1990), while in 1998 production reached 25 million mt. A large proportion of this increase took place in coastal habitats, often near shipping routes (H. Rosenthal, pers. obs.). Therefore, operational and procedural practices with ballast water are necessary to reduce the risk of unwanted impacts on the aquaculture industry (Subba Rao *et al.*1994). The treatment of ballast water is necessary in light of increasing risks involved with ballast water releases. Furthermore, it has to be taken into account that each single vessel in interregional service has the capacity to introduce an unwanted non-native species to a new habitat. Efforts to prevent or minimize introductions should be given high priority.

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Systems for Evaluation of Shipboard Ballast Water Treatment Technologies for Preventing Transfer of Unwanted Organisms

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Key words: ballast water, treatment, management, disinfection, filtration, biocide, dinoflagellate, pilot-scale study

Introduction

Environmental damages caused by transfer of unwanted organisms in ballast water are well understood relative to how to prevent and manage unwanted invasions. More focused research efforts are needed for treatment and control. The Environmental Technology Institute (ETI), Singapore, in collaboration with the Maritime and Port Authority of Singapore (MPA) and the National University of Singapore (NUS), has completed the first phase of a multifaceted research and development project on ballast water treatment, including a pilot-scale evaluation of possible shipboard treatment systems to remove unwanted organisms. This paper presents the salient features of this project and preliminary findings.

Results and Discussion

The first phase of this project consists of construction of a dockside pilot treatment facility, located at Sembawang, Singapore (Straits of Johor), that filters 50 mt/hr of ballast water. This flow rate was selected because it would allow a number of system modifications and optimization experiments in a relatively short time. The seawater turbidity at Sembawang varies between 4 and 15 NTU (Nephelometric Turbidity Unit). The mean particle size in Sembawang seawater (analyzed by Malvern laser particle size analyzer) also varies considerably–from 5 to 100 μs–with tidal levels. Hence, the test facility provides an ideal platform to study the filtration technologies under different solids-loading levels. The solids-loading levels are determined by measuring the suspended solids levels and particle oversize, at any point of time. The treatment facility includes two continuous, self-cleaning screen filters and a multimedia pressure filter with an automatic air-augmented backwash system. The facility includes automatic controls and data-logging capability to monitor the treatment system performance on a continuous basis. The studies included

(1) evaluation of treatment efficiency (filtration efficiency and biological effectiveness) at various screen mesh sizes and media types and sizes;
(2) study of flow characteristics and fouling rates at different suspended-solids-loading levels and size distributions;
(3) modification of screens and filtration systems to optimize flow and minimize fouling; and
(4) modification of the system design to suit normal ballasting operations onboard ships.

The project also evaluates the effectiveness of various biocides for inactivating unwanted organisms in ballast water. The most compelling reason to consider the biocides treatment for ballast water is the ease of application. The studies include biocide chemistry in seawater and development of concentration-time (CT) relationships to identify the optimum dose for disinfection. This relationship was developed by assuming that the product of disinfectant concentration (C) and time (T) yields a constant. The CT concept is widely used in the treatment of drinking water as a criterion for cyst and virus disinfection. The biocides that are being evaluated include chlorine, aldehydes, isothiazolones, and quarternary amine compounds.

Currently, there are no internationally accepted standards to evaluate the ballast water treatment technologies. Nor are there accepted monitoring tools

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for obtaining comparable data. The ballast water treatment studies are, therefore, complemented with the development of a dinoflagellate-cyst-based biomonitoring tool to determine the effectiveness of treatment options. Dinoflagellates represent potentially noxious algal species common to tropical region. They have been identified as a severe threat to marine ecosystems. They can survive transport over long distances in ballast water tanks as most of them are cyst-forming types. Australian Quarantine and Inspection Service (AQIS) regulations currently require that dinoflagellates be used as test organisms for ballast water treatment technologies proposed for approval.

In collaboration with NUS, our group has been developing a subcomponent of the program-specifically, the production of viable dinoflagellate cysts on a large scale. The major challenge in establishing such a procedure is the difficulty of producing large numbers of cysts (hypnozygotes) in a relatively short time and then excysting them back to motile cells after the ballast water treatment in order to evaluate the treatment effectiveness. We have developed a protocol to induce the formation of large numbers of dinoflagellate cysts (~ 4×10^6 cysts/L) in a short time, under laboratory conditions. The protocol also allows us to excyst the hypnozygotes that remain after biocide treatment and/or filtration. Filtration studies have been carried out using screens of various mesh sizes and under different loading conditions, in order to study the effectiveness of filters for removing dinoflagellate cysts. A laboratory-scale filtration rig, automated with monitoring and control instruments, is used for this purpose. The effect of attachment of dinoflagellate cysts onto sediments is also being studied, as this will have a considerable effect on the efficiency of filters in retaining cysts.

The studies show that screen filtration is an effective technology for shipboard application. However, off-the-shelf technologies that are currently available in the market are not designed to meet the requirements of a normal ballasting operation. The results also show that seawater turbidity and particle-size distribution have profound effects on screen performance. Systems designed for freshwater operations, where turbidity levels are much lower than in normal seaport water, will not perform effectively in highly turbid ports. The next phase of our study will be to develop a modified screen filtration system and evaluate it onboard ship for modifications.

The biocide studies are in progress in our laboratories. We have so far identified two proprietary biocides that degrade quickly in seawater. Their effectiveness against dinoflagellate cysts is being evaluated.

Measuring Ballast Water Delivery and Management Patterns in the United States: The National Ballast Water Information Clearinghouse and National Ballast Survey

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ABSTRACT: **By provision of the National Invasive Species Act of 1996 (P.L. 104-332), the U.S. Coast Guard and the Smithsonian Environmental Research Center were directed to create the National Ballast Water Information Clearinghouse. A primary charge of the Clearinghouse is to measure patterns of ballast water delivery and exchange across commercial shipping ports throughout the United States. The Clearinghouse has implemented the National Ballast Survey (NABS) to investigate these patterns. NABS is designed explicitly to measure patterns of ballast water delivery and management (primarily ballast water exchange) according to vessel class by geographic region and season of arrival. Additionally, among-year changes in ballast water management by vessel class and geographic region will also be possible. An important aspect of NABS is the ability to assess accuracy of data through use of multiple, independent data sources. In 2001, data from the Clearinghouse will be analyzed and reported to the U.S. Congress and U.S. Coast Guard for review.**

Key words: National Ballast Survey, ballast water, exchange, management

INTRODUCTION

The National Invasive Species Act of 1996 (NISA) directed the U.S. Coast Guard (USCG), in conjunction with the Smithsonian Environmental Research Center (SERC), to develop a National Ballast Water Information Clearinghouse (hereafter Clearinghouse). The Clearinghouse, located at SERC, plays a central role in the organization and analysis of national data concerning the transfer and invasion of nonindigenous species associated with the ballast water of ships.

The Clearinghouse serves as a source of national information on ballast water and invasion biology. The primary foci of the Clearinghouse are (1) the study of spatial and temporal patterns of ballast delivery and management, (2) the study of patterns and rates of nonindigenous species invasion, (3) a directory of ongoing and past research in these areas, and (4) compilation of the broad range of general topics relevant to these issues. Synthesis of these data will provide a valuable resource, which is now lacking, and will be accessible via a Clearinghouse site on the World Wide Web (http://invasions.si.edu/ballast.htm). Such a synthesis will promote comparisons between patterns of invasion and patterns of ballast water management, and testing for a reduction of invasion rate in response to various management activities.

NISA calls for a variety of measures to reduce the risk of exotic species invasions associated with release of ballast water by ships. Among these, NISA requests that all ships arriving to U.S. ports from outside the Exclusive Economic Zone (EEZ) follow voluntary guidelines for open-ocean exchange of ballast tanks that are to be discharged in U.S. waters. This management practice is intended to "flush out" ballast tanks and minimize the transfer of nonindigenous coastal species.

A key element of NISA involves tracking the effectiveness of voluntary guidelines, as measured by (1) the level of compliance with voluntary guidelines, (2) changes in the rate and patterns of ballast water delivery, and (3) reduction in the rate of ballast-mediated invasions. The Clearinghouse was created to provide these analyses on a national scale.

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Figure 1. United States Coast Guard Captain of the Port (COTP) Zone Headquarters (Great Lakes COTP zones excluded).

The Clearinghouse has developed a nationwide program to measure the current and changing patterns of ballast water delivery to U.S. ports. This program, called the National Ballast Survey (hereafter NABS), estimates the amount and source of ballast water discarded by commercial vessels and originating from outside of the nation's EEZ.

This program will result in a comprehensive analysis and biennial report to the U.S. Congress on the status of ballast water delivery throughout the country. The first report will provide a national baseline of information on current status, and subsequent biennial reports will measure trends or changes in ballast water management.

Methods

The overall objective of NABS is to measure the spatial and temporal pattern of ballast water delivery to U.S. ports from all commercial vessels arriving from outside of the EEZ. In particular, NABS measures ballast water delivery and management (including exchange) by vessel class, geographic region, season, and year; characterizes ballast water exchange according to type and degree of exchange; and provides verification of data through use of multiple, independent data sources. Specifically relevant to NISA, NABS estimates (1) the frequency of voluntary compliance with ballast water exchange guidelines authorized under NISA and (2) the effects of compliance on the pattern and rate of ballast water delivery.

Although these objectives are conceptually straightforward, there are many complexities to address in the design of NABS. One such complication involves widespread variation among

- vessel types–There are many different classes of commercial vessels, which can differ in both the ballast water delivery pattern and management (*i.e.*, exchange) practices;
- geographic regions–The use of ballast water may differ among regions, even when controlling for vessel type, depending upon cargo, port system characteristics, prevailing weather, and other factors;
- seasons–The use of ballast water may vary by season, even when controlling for vessel type (*e.g.*, due to weather);
- years–The use of ballast water may change among

years, especially reflecting long-term changes in rate of management practices such as ballast water exchange.

The diverse array of ballast water tanks within a single vessel also poses a challenge to describing ballast water management patterns. Each tank may have a different history with respect to its ballast water sources and management. Furthermore, ballast water exchange can occur in two different ways (empty-refill or flow-through) and to various degrees (*e.g.*, 50% replacement, 200% replacement of the original water).

A particularly difficult issue in describing water delivery and management involves the extent of error associated with data reporting. There are two potential sources of error: (1) error due to under-reporting by ships, and (2) the accuracy of the data that are reported. NABS is designed to estimate both types of error.

To meet the objectives outlined above, NABS is designed explicitly to describe national ballast water delivery and management patterns on multiple spatial and temporal scales. More specifically, a stratified design for data collection and analysis has been implemented and is described below.

Analytical Strata: Vessel Type, Geographic Region, Season, and Year

It is probable that ballast water management (such as the frequency of exchange) varies according to vessel class, geographic region, and season. NISA allows for implementation of regulations of ballast water exchange on any one of these strata. NABS therefore provides resolution at each of these levels to evaluate where voluntary guidelines are or are not effective.

Although NABS is intended to establish an important baseline of ballast management practices during its first two years (*i.e.*, subject of the first report to the U.S. Congress), it is important to recognize this as a "snapshot" measure of patterns. Because the frequency of ballast water exchange may change over time, NABS is designed to evaluate both current practices and long-term (*i.e.*, among-year) trends in ballast water management.

Vessel Type

There are several classes of vessel that arrive at U.S. ports from outside of the EEZ (*e.g.*, tankers, bulk carriers, container ships, general cargo vessels). These vessel types are designed to transport particular types of cargo and vary in size and ballast water capacity. The type of cargo, size, and ballast tank configuration can influence not only the quantity of ballast water released in a port, but also the overall management of ballast, including the history of ballast water and whether ballast water exchange is conducted (*e.g.*, NRC 1996).

NABS will collect data necessary to (1) characterize the ballast water delivery and management pattern for each major vessel class and (2) compare directly the amount of released ballast water and the history of this water (*i.e.*, ballast water sources and frequency of ballast exchange) among vessel classes.

Geographic Region

The coastal United States and its ports can be variously divided into distinct geographic regions. Each area differs in the amount and sources of ballast water received from commercial vessels (Carlton *et al.* 1995). Spatial differences in ballast water characteristics can result from differences in the overall number of ships, the relative abundance of different vessel types (as above), and the management practices employed. Importantly, ballast management practices (such as the amount of ballast water on board or the frequency of ballast water exchange) may vary spatially, even controlling for vessel type, depending upon weather conditions, import-export patterns, or other factors.

NABS tests for spatial patterns in the delivery and management of ballast water. Although there are many spatial scales to consider, the U.S. Coast Guard Captain of the Port (COTP) zone has been chosen as the level of spatial resolution for analyses. There are approximately 32 COTP zones in the United States (Figure 1). Each COTP zone is comprised of multiple ports and is headquartered at one of these ports. The selection of the COTP zone as the spatial scale of resolution has distinct advantages for the organization and management of data collection. More specifically, this plan allows NABS to take full advantage of the existing structure of U.S. Coast Guard operations throughout the country to obtain and disperse information about commercial shipping and ballast water.

Season and Year

The use of ballast water, as well as the capacity of vessels to safely conduct ballast water exchange, may vary temporally. First, weather patterns or cargo

(import-export schedules) may influence ballast water management on a seasonal timescale. Second, ballast water management patterns may change across years, controlling for seasonal variation. Such interannual changes may result from shifts in trade patterns as well as implementation of NISA's voluntary guidelines or from other future management programs.

NABS will test for temporal patterns among seasons and years. Furthermore, because temporal patterns may vary (interact statistically) with vessel type and geographic region, it is essential to examine temporal data within each COTP zone and vessel class (as outlined above). NABS employs this scheme to provide a comprehensive analysis of nationwide patterns, including the necessary resolution to examine the individual influences of vessel class, geographic region, and season. Furthermore, a comparison among years will be obtained for each major vessel class by controlling for seasonal patterns.

Characterization of Ballast Water Exchange and Delivery

Although vessels often carry ballast water that derives from multiple sources, NABS is designed to provide a clear accounting only for ballast water that (1) originates outside the U.S. EEZ and (2) is discharged within U.S. waters, corresponding directly to NISA's voluntary ballast management guidelines. Information on the history of ballast water in other tanks may also be of interest and relevant to invasion management. For example, relatively empty ballast tanks may carry some risk of invasion associated with residual organisms that can occur in both sediments and biofilms and thus be resuspended and discharged following entry into U.S. ports. In addition, water from domestic U.S. ports may promote the spread of nonindigenous species after initial invasions. However, these elements are beyond the present scope of NISA and NABS, and the associated risk is less well defined than that of ballast water from foreign sources (hereafter foreign ballast water).

NABS estimates the total volume of foreign ballast water released in U.S. waters, characterizes the history (origin, management, and fate) of this water, and identifies specific information about ship and voyage characteristics. A tank-by-tank history for all ballast to be released per ship is collected for analysis. This history includes the origin of ballast water, whether open-ocean exchange or some other ballast management practice occurred, type of exchange

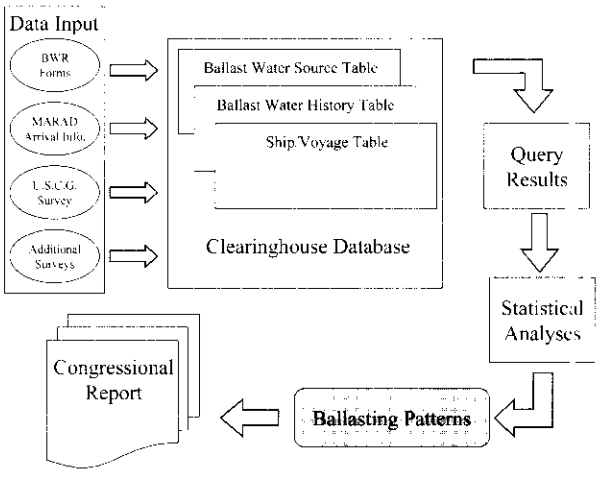

Figure 2. Functional aspects of the United States National Ballast Survey (NABS). Data from multiple sources are entered into the Clearinghouse database, queried, and analyzed to determine ballast management patterns.

(*i.e.*, empty-refill or flow-through), degree of exchange (*i.e.*, percent of water replaced), and location of exchange. It is biologically significant to distinguish between an exchange of 50% vs. 200% of the ballast water in a tank, because these differ in the efficiency of removing unwanted organisms (*i.e.*, not all ballast water exchange is of equal value in reducing nonindigenous species transfer).

Verification of Data through use of Multiple, Independent Data Sources

The success of NABS in accurately assessing current ballast water practices, and complying with voluntary guidelines under NISA, depends upon the quantity and quality of data. Three characteristics of paramount importance in making inferences include

- accuracy of the data (*i.e.*, error in measurements);
- use of data that are representative (*i.e.*, randomly selected and independent measures);
- sufficient data to provide adequate estimates (*i.e.*, reasonable statistical confidence).

NABS utilizes multiple sources to obtain accurate and representative data for analyses (Figure 2). This can be viewed as a multi-step process, involving three primary data sets, although others may become available as state and regional ballast water legislation and efforts emerge. First, the data submitted directly by the vessels upon arrival to the United States includes ballast water history for most vessels arriving at each U.S. port from outside of the EEZ (*i.e.*, Ballast Water Reporting Forms, hereafter BWR forms). Second, a

comprehensive data set that includes all arrivals at each port (U.S. Customs data as reported by the Maritime Administration, hereafter MARAD data) determines the proportion of vessels that do not submit BWR Forms (*i.e.*, rate of under-reporting). Third, a subset of all arrivals will be boarded by the U.S. Coast Guard to (1) estimate the accuracy of the BWR forms data and (2) make statistical comparisons of ballast delivery patterns by vessel class, geographic region of origin and arrival, and size. These latter data are obtained by the U.S. Coast Guard via a stratified random selection and survey of vessels arriving in U.S. Coast Guard COTP zones.

Data Sources and Types

The quantity and nature of data vary among sources, and each is intended to serve a specific purpose.

BWR Forms

The most comprehensive data on ballast water delivery and management come from the BWR forms. All commercial vessels entering U.S. waters from outside of the EEZ are expected to report to the Clearinghouse specific information concerning ship and voyage characteristics, ballast water history, and ballast water exchange. Assuming a high rate of reporting and accuracy, the BWR forms will (1) provide a dataset of up to 50,000 ships per year for all major commercial vessel types and U.S. ports and (2) be used as a primary dataset for analyses of nationwide patterns.

MARAD Arrivals Information

MARAD organizes a complete record on arrival of commercial vessels to U.S. ports. For each ship, MARAD records data on vessel type as well as other ship and voyage characteristics. These data are used to measure the rate of under-reporting associated with the BWR forms. Through direct comparison of the two data sets, the number and identity of ships (by vessel type, port, and date) absent from the BWR database can be calculated.

U.S. Coast Guard Survey

The U.S. Coast Guard provides data for multiple vessel classes by region (COTP zone) and season. These data are collected from randomly selected vessels and include ballast water history, ballast water management, and ship/voyage characteristics. In addition, a water sample is taken from exchanged tanks and analyzed for salinity content. The Clearinghouse is currently convening a workshop to explore other analyses to differentiate coastal from open-ocean water. These data are used to estimate the error rate in the BWR Forms, providing a measure of accuracy.

There is intentional overlap in the information included in the BWR form and U.S. Coast Guard survey, providing a direct comparison to check for accuracy (errors) of the BWR Forms. This survey also creates the opportunity for U.S. Coast Guard to communicate directly with ship operators, to clarify ships' practices (as represented on the BWR forms), and to address questions that may exist about the voluntary program.

Additional Surveys

There are several opportunities to obtain additional data through other surveys to augment the U.S. Coast Guard survey effort and provide a complementary, independent set of measures. For example, (1) SERC and other research institutions may collect data on ballast water characteristics, (2) some state, regional, and local agencies have expressed an interest in particular types of ballast water measurements, and (3) some agencies may be willing to assist in short-term, intensive measures of ballast water (*e.g.*, "Ballast-Water Awareness Month"). Development of these opportunities is now being pursued actively.

Data Management

A central feature of NABS is a relational database where all ballast water information is stored and managed. The management and use of data from each source can be viewed as a series of step-wise processes that involve this database. Key steps include data receipt, data entry, data validation and proofing, database queries, and statistical analyses. In addition, data management requires some regular, iterative steps involving maintenance and security.

Data Receipt

BWR Forms. Most BWR Forms are currently sent to the Clearinghouse by fax and U.S. mail. Faxes and mailed forms are catalogued, photocopied, and archived in separate physical locations for security purposes. Data are entered manually into the Clearinghouse database and undergo validation during this

process. Entered data are then proofread against original hard copies to check for entry errors.

The Clearinghouse also provides several systems for electronic and remote reception of completed BWR forms. These systems include a BWR form posted on the World Wide Web (http://www.serc.si.edu/invasions/ballast.htm), e-mail transfer of forms as attached files (ballast@serc.si.edu), and forms sent directly on disk. Forms in any of the electronic formats are checked visually for formatting errors and proofed before import into the Clearinghouse database.

MARAD Arrival Information. The MARAD data on nationwide arrivals are transferred electronically to the Clearinghouse. As above, these data are checked visually for formatting errors.

U.S. Coast Guard Survey. Data from the U.S. Coast Guard survey are collected on handheld computers or hard-copy questionnaires. Data are uploaded to District Coast Guard computers and transferred to the Clearinghouse database via modem or the Internet. Hard copies of all data files are printed, examined for format or transmission errors, and archived at SERC.

Analyses

NABS employs a variety of analyses to address questions about nationwide ballast water delivery and management patterns. The multiple data sources are queried to estimate independent estimates of ship arrivals and ballast water delivery by vessel class, geographic region, season, and year. A comparison of results among data sources allows NABS to estimate error (*i.e.*, statistical confidence) in the results from the BWR forms due to (1) inaccuracy of reported data, (2) the rate of under-reporting, and (3) potential biases associated with under-reporting. Furthermore, through analysis of these multiple data sources, NABS can report patterns of ballast water delivery that include estimates of statistical confidence as well as statistical comparisons among strata.

Analysis of BWR Form data

If all ships submitted error-free BWR forms, determining the patterns of ballast water delivery and management would be a simple task of data tabulation. In reality, not all ships submit a BWR form and errors exist among forms that are submitted. To address these problems, NABS estimates the magnitude of uncertainty due to under-reporting and calculates inaccuracy of the BWR form data. NABS uses the BWR form data to measure broadscale patterns and other supporting data sets to estimate the associated error. Error estimates come from comparisons with MARAD data (to measure under-reporting) and U.S. Coast Guard survey data (to measure data accuracy). These estimates of error are used to generate confidence intervals around BWR form data mean estimates. Given the sample size (*i.e.*, 32 COTP zones and 3840 samples per year nationally) this approach should be highly effective for estimation of ballast delivery patterns on a national basis.

NABS will also report the summaries of all data from the BWR forms by stratum (*i.e.*, vessel class x COTP zone x season). However, it may not be effective to estimate error rates for BWR form data in each of the strata, using identical methods. Unlike the error estimates for national patterns, the degrees of freedom for within-stratum estimates are relatively small and limited to the number of vessels sampled by the U.S. Coast Guard and others. The U.S. Coast Guard Survey data may be more effectively used to directly estimate within-strata compliance and error rates in some cases. Importantly, this latter approach allows for more sophisticated statistical comparison of ballast characteristics among strata.

Error Due to Inaccuracy

To estimate error due to inaccuracy, the results of the U.S. Coast Guard survey are compared directly with BWR form data. Specifically, the inaccuracy is treated as the result of two random events: (1) whether a mistake was made and (2) the magnitude of the mistake. Event 1 can be modeled as a binomial random variable. If no mistake was made, the inaccuracy is zero. If a mistake exists, the second random variable is the magnitude of this mistake and can be modeled using a normal distribution. The results of this model can generate error estimates for the tabulated results of the BWR forms.

Analysis of data from U.S. Coast Guard Survey

Although data from the U.S. Coast Guard survey play a key role in analyzing BWR forms data (as above), they also provide a valuable tool for fine-grained analyses and comparisons of ballast water characteristics. For example, these data can be used to test for statistical differences in the amount of unexchanged ballast water (or rate of compliance) for bulkers versus tankers between two regions, whereas

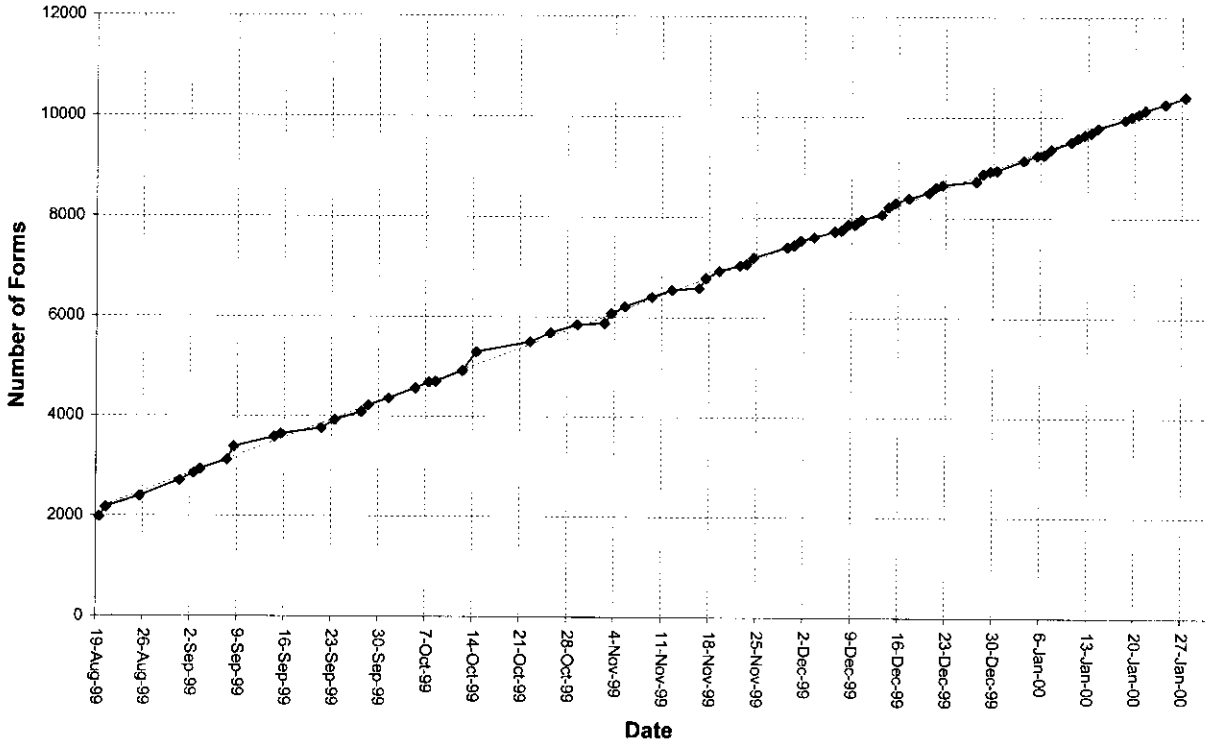

Figure 3. Number of ballast water management reporting forms submitted to the National Ballast Water Information Clearinghouse per day.

the potential for such direct statistical comparisons will be more limited with the BWR form data.

NABS uses standard parametric and non-parametric statistical techniques to test for differences in ballast characteristics among strata. For continuous data, multi-factor or nested ANOVA with multiple comparison of means are used. For dichotomous or categorical data, logistic regression and log-linear analyses are used. To test for long-term trends, ANOVA and linear regression to compare ballast characteristics among-years are employed.

Initial Results and Discussion

Although data collection began on July 1, 1999, it is still too early to publish definitive results. Data collection is planned for an additional 18 months, until at least summer 2001, at which point full scale analyses will be performed. The results from this program will be included in a congressional report prepared by the Smithsonian and U.S. Coast Guard. In the interim, the Clearinghouse is posting quarterly reports on the World Wide Web (http://invasions.si.edu/ballast.htm).

Some apparent, but preliminary, trends are beginning to emerge based on the data collected to date. It must be noted that data collected so far only span two and one-half seasons, thus no seasonal or inter-annual variation can be described. Given the extent of this national program and the temporal proximity to its start date, it is premature to assess whether participation by commercial ships will change through time.

The Clearinghouse has received approximately 10,600 BWR forms from July 1, 1999 through January 31, 2000. This equates to 1,515 forms per month. Figure 3 tracks the number of forms received per week. The slope of the curve is constant, indicating an unchanging influx of BWR forms of 51 forms per day. At this rate, the total number of forms expected for 1 year is 18,400. Assuming no seasonal variation, the constant rate of arrivals across 7 months implies no change to the number of participants in the program. As more commercial vessels learn of the reporting requirement, an increased reporting rate is to be expected, but the current findings suggest little or no change to ballast water management and delivery reporting.

Based on MARAD data from 1996 through 1998, the number of commercial vessels (greater than 300 gross tons and excluding passenger vessels) that arrive

per year is approximately 50,000, with very little inter-annual variation. Although the Clearinghouse does not yet possess MARAD data corresponding to the exact dates of the NABS data collection, precluding direct comparison, it appears there is substantial under-reporting on the part of commercial vessels. If MARAD data from recent years is a good indicator of recent vessel arrivals, approximately 40% of the vessels required by NISA to report their ballast water management and delivery practices are doing so (*i.e.*, 60% under-reporting). As stated earlier, the degree of under-reporting reported here must be verified by comparison with MARAD data that are contemporary to the period in question.

Because of the apparent extent of under-reporting to date, the Clearinghouse is undertaking additional analyses (1) to confirm this result and (2) identify the source of under-reporting by region and vessel type. A first step in this process, now underway, is to verify that the MARAD data do not overestimate arrivals. Following any corrections, if necessary, the second step will identify the extent of under-reporting by geographic region, vessel type, and season.

At present, little can be said with respect to spatial variation among and within coasts. A similar situation exists for comparisons among vessel types, and for analyses to detect temporal variation. As more data are compiled, the resolution at which analyses and comparisons can be made will increase and patterns will begin to emerge. In the long run, it is hoped that data on shipping and ballast management and delivery will be useful to those engaged in efforts to describe the rate and pattern of marine invasions to U.S. waters.

Acknowledgements

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An International Exchange of Ballast Water Research between New Zealand and Massachusetts

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ABSTRACT: Research on the ballast water problem in New Zealand since 1995 is briefly described, including the results of a two-year sampling survey of container ships and bulk carriers in the ports of Lyttelton and Nelson. Current research involving both the Cawthron Institute in New Zealand and Battelle in Massachusetts, is looking at methods of measuring the water volumes exchanged by ships in mid-ocean (by reballasting and by dilution) and the efficiency of these exchanges in ridding tanks of unwanted species. The research also includes existing and potential methods that may be used by quarantine agencies to confirm independently that mid-ocean exchanges have occurred. Other research by Cawthron, is investigating the survivorship of species in ballast tanks in greater detail, particularly on trans-Tasman Sea and trans-Pacific voyages.

Keywords: ballast water, exchange, efficiency, compliance, indicator, New Zealand, plankton

INTRODUCTION

The undesirable dispersal of marine organisms in ships' ballast water is, by nature, an international problem. In the last 10-15 years, as this environmental problem has become increasingly apparent, various maritime nations have commissioned reviews and enquiries and held national and international meetings on the subject. The International Maritime Organization (IMO) of the United Nations is involved, particularly through its Marine Environment Protection Committee (MEPC) and its Convention for the Prevention of Pollution from Ships (MARPOL). Mandatory international regulations for managing ships' ballast water are imminent, and already apply inside territorial sea areas of some maritime nations and within the jurisdiction of some ports.

When funding for research on the ballast water problem is provided, the scope is often regional, with the funds coming from ports, or local or state government. Few nations have any significant, long-term, nationally funded research programs on the ballast water problem. There are also very few instances of international collaboration, except perhaps in the Great Lakes where geography compels the involvement of neighboring nations.

This paper briefly describes how ballast water research carried out by the Cawthron Institute in Nelson, New Zealand, over the last four years evolved into a collaborative research program with Battelle Ocean Sciences, Duxbury, Massachusetts.

New Zealand is a small, geographically isolated, sparsely populated (3.7 million in 1996) island nation with a disproportionately long coastline relative to its land area. Like our much larger Australian neighbor, from whom we are separated by the 1000-mile-

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wide Tasman Sea, agriculture, forestry, minerals, and fisheries are important exports. Generally, these have very much higher volume-to-value ratios than do New Zealand's less bulky imports. Most of the 2,500 to 3,000 trading vessel visits to New Zealand each year are by ships that are fully or partly ballasted. With the exception of bulk carriers importing petroleum products and fertilizers, most bulk carriers arriving in New Zealand are fully ballasted. Collectively, all types of vessels discharge an estimated 4 to 6 million mt of ballast water annually. The Australian ports receive an estimated 150 million mt.

As is described by Taylor *et al.* (2000) in this volume, more than half of New Zealand's trade is with Asia and Australia. Several of the conspicuous nonindigenous species that have arrived recently are of Asian origin and are also established in Australia; *e.g.*, the Asian kelp, *Undaria pinnatifida*, and the Asian date mussel, *Musculista senhousia* (Furlani 1996). Considerable trans-Tasman trade between New Zealand and Australia means that foreign invaders on temperate shores of either country are likely to be translocated by shipping across the Tasman Sea.

New Zealand government funding for research on the ballast water problem has been made available in the last four years, and was perhaps partly prompted by a notable conference *Ballast Water–A Marine Cocktail on the Move* held by the NZ Royal Society in Wellington in 1995 (Lynch 1995). In 1996, the New Zealand Ministry of Fisheries (MFish) [previously part of the Ministry of Agriculture and Fisheries (MAF)] funded the Cawthron Institute to conduct research on ballast water with these goals: (1) to devise a method for routinely sampling ballast water from a wide range of ship types and (2) to examine the samples microscopically to compare the biota with such variables as (a) age of the water, (b) the size and types of tanks and ships, and (c) whether or not the ships had claimed to have exchanged their ballast water in mid-ocean.

The Quarantine Problem: Identifying Non-compliant Vessels

Quarantine agencies responsible for enforcing national regulations pertaining to discharges of ships' ballast water, *e.g.*, MFish or the Australian Quarantine Inspection Service (AQIS), need to identify noncompliant vessels. These are vessels that have not made mid-ocean exchanges in accordance with IMO recommendations, which, briefly stated, are that vessels exchange ballast in deep water (not shallower than 500 m) far from coastal influences, by emptying their tanks until suction is lost before reballasting, or, in the case of flow-through dilution, by pumping a volume of mid-ocean water equal to at least three times the vessel's ballasting capacity.

Port companies also may increasingly have vested interests in identifying vessels that constitute a quarantine risk. A *Contra Costa Times* reporter, Denis Cuff, reported that the Marine Conservation Center and Baykeeper organizations of San Francisco had notified the Port of Oakland, on 7 January 1999, of their intention to sue the Port for alleged insufficient measures to control and prevent the arrival of invasive species in ships' ballast water, thus harming San Francisco Bay fish and aquatic life. According to Michael Lozeau, executive director of the San Francisco Baykeeper, "Ocean exchange of ballast is a good interim step, but is not the final answer [to the problem]" (*Contra Costa Times* 9 January 1999).

As a quarantine measure to minimize the risk of dispersing invasive marine and freshwater species, the efficacy of exchanging ballast in mid-ocean is perhaps based more on intuitive common sense than upon hard scientific evidence. Scientists were advocating such a procedure at least a decade ago; *e.g.*, Williams *et al.* (1988). In the case of exchanges made by emptying and refilling tanks, the removal of most of the water must, arguably, greatly reduce the risk of introducing nonindigenous species–although there remains the niggling uncertainty about residual water and sediments that cannot be voided before refilling begins.

The efficacy of flow-through dilution, by pumping through a volume three times that of a ship's ballasting capacity is, however, less intuitive. The procedure is recommended by the IMO partly on the basis of pioneering Australian work undertaken by Drs. Geoff Rigby and Gustaaf Hallegraeff (1994) on the 141,475 dwt BHP Bulk Carrier M.V. *Iron Whyalla* in September 1991 (when the vessel was at anchor at Singapore) and in July 1992 on a voyage from Japan to British Columbia (AQIS 1993, Parts 1 and 2).

For both trials, methylene blue was used as a tracer to measure the dilution of the original water, and changes in the diversity and the abundance of phytoplankton during and after the exchange were recorded. Rigby and Hallegraeff (1994) concluded that pumping through three times the capacity of ballast tanks effectively diluted the original water.

There were some problems with these trials, however, notably that the dye could not be evenly dispersed inside the tanks before the exchange took place, and significant differences in initial concentrations at sampling points before the exchange indicated plug flow. Also, methylene blue stains living plankton in addition to remaining in solution. So there was the potential problem of differentiating between dilution by adsorption onto organisms and that caused by the diluting effect of the incoming water.

The flow-through of three times capacity to achieve 95% dilution is also identical with the theoretical figure derived from an exponential decay equation familiar to water engineers involved with dilution dynamics. It assumes instant and perfect mixing. The complicated internal structure of some ships' ballast tanks, often a labyrinth of smaller compartments and baffles, is, however, far removed from any perfect mixing container (Figure 1).

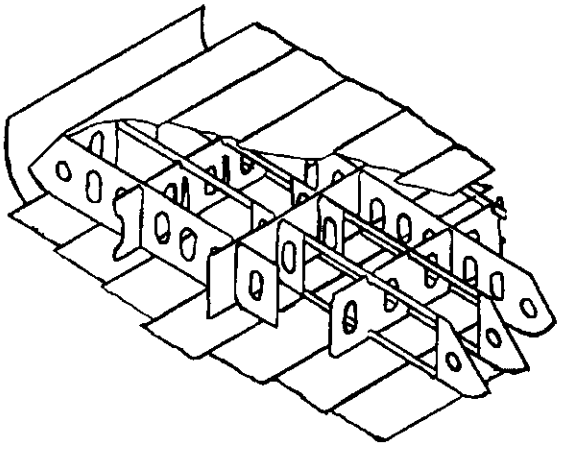

Figure 1. Internal structure of a double-bottom ballast tank with numerous compartments and baffles (Eyres 1988).

Ballast Water Research by Cawthron Before 1998

Between October 1995 and October 1997, scientists at the Cawthron Institute examined over 160 ballast tanks of all types on 75 vessels in the ports of Lyttelton and Nelson. Their procedures and the results of the water analyses are given in Hay *et al.* (1997).

On the basis of ships' records, the water from the forepeak, afterpeak, upper wing, and lower wing tanks and from the ballast holds that were sampled, was, on average, less than 10 da old. In deep tanks, the average age of the water was 20 da; while in double-bottom tanks the average was 40 da. The oldest water sampled, which was in a double-bottom tank, was reported to be 494 da old.

Sampling methods

Several water sampling methods were tried. An electric Waterra Hydrolift II inertia type pump (Waterra Ltd., 77 Mowat Ave., Suite 101, Toronto, Ontario) proved to be the most reliable water-lifting device, and ensured fast, routine sampling on any vessel with sounding pipes (more than 90% of the ships boarded had sounding pipes). Coils of 12-mm or 20-mm diam. semi-rigid, black polythene plumbing pipe, about 25-m long, were used to conduct ballast water up to the weather deck of the ship from tanks as low as 22 m below deck level. A brass or nylon foot valve (a non-return valve) was inserted into one end of the pipe coil. That end was pushed down the sounding pipe until the bottom of the tank was touched. The plastic pipe was then slightly withdrawn (about 50 cm) to prevent the valve from striking the tank bottom when the pump was in action. The Waterra pump, which has an adjustable arm that moves vertically up and down, was placed with the arm directly above the sounding pipe from which the polythene pipe was protruding. The arm was then tightly clamped to the polythene pipe just above deck level, and the pump was started. The speed of the motor and the stroke of the arm were adjusted to ensure a steady pulsing flow of water, typically 3-6 L/min. (It helps to first prime the pipe by vigorously jerking the pipe up and down manually for a few minutes before clamping the pipe to the pump arm.) By this method, we obtained 100 L water samples from all types of tanks within 15-30 min. We ran the pump by tapping into the ship's power via reefer sockets, or by using the power sockets for cranes, winches, or deck lighting. Several custom-made transformers and an array of adapters and plug types were needed to make the necessary power connections. To sample tanks less than about 6 m below deck level, we initially used petrol and electric impeller (centrifugal) pumps. Although these pumps are faster, they are more vigorous, and damaged some plankton species that were apparently undamaged by the slower inertia pump. In general, the samples obtained by using the inertia pump had a slightly wider taxonomic range of species (see Hay *et al.* 1997, fig 3.3). We therefore preferentially used the inertia pump

unless time constraints necessitated using the faster centrifugal pumps.

Results

Phytoplankton

Eighty percent of the tanks sampled contained live phytoplankton, with diatoms being the most common, followed by heterotrophic flagellates and dinoflagellates. The diversity and abundance of live phytoplankton was proportionately greater in the tanks of bulk carriers and break bulk carriers than in the tanks of container ships. Live phytoplankton were least frequent and often absent in the double-bottom tanks, especially in container ships. Presumably, this was because these tanks are used primarily for stability, and, compared with other tanks such as upper wing tanks, are infrequently emptied and refilled.

The tanks containing the highest diversity of the major phytoplankton groups were those that reportedly had been exchanged en route to New Zealand (Figure 2). This suggested that the exchange added live oceanic species belonging to groups that had previously declined or died in the darkness.

Invertebrates

Live invertebrates were found in 83% of all tanks sampled. Crustaceans were most numerous, being recorded in 80% of the tanks. Live mollusks were found in 27% of the tanks and annelid worms in 15% of the tanks (Figure 2). Compared with phytoplankton, invertebrates were proportionately more common (by 10%) inside the tanks of container ships. As with phytoplankton, the double-bottom tanks had the lowest proportion of living invertebrates. Upper wing tanks, forepeak tanks, and particularly ballasting holds contained the most live invertebrates.

There were slight differences between exchanged and nonexchanged tanks with respect to the diversity and abundance of some invertebrates (see Figure 2). Coastal harpacticoid copepods were nine times more common inside tanks that had not made a mid-ocean exchange. Certain groups of annelids, such as the Opheliide and Phyllocidae, were not represented in tanks reportedly exchanged in mid-ocean. In general, however, the diversity and abundance of invertebrates was similar in both exchanged and nonexchanged tanks. The larvae of predominantly coastal mytiloid and veneroid bivalves occurred about equally in both exchanged and nonexchanged tanks. Yet, theoretically, the larvae of these groups should be much lower or absent from the tanks purportedly carrying mid-ocean water.

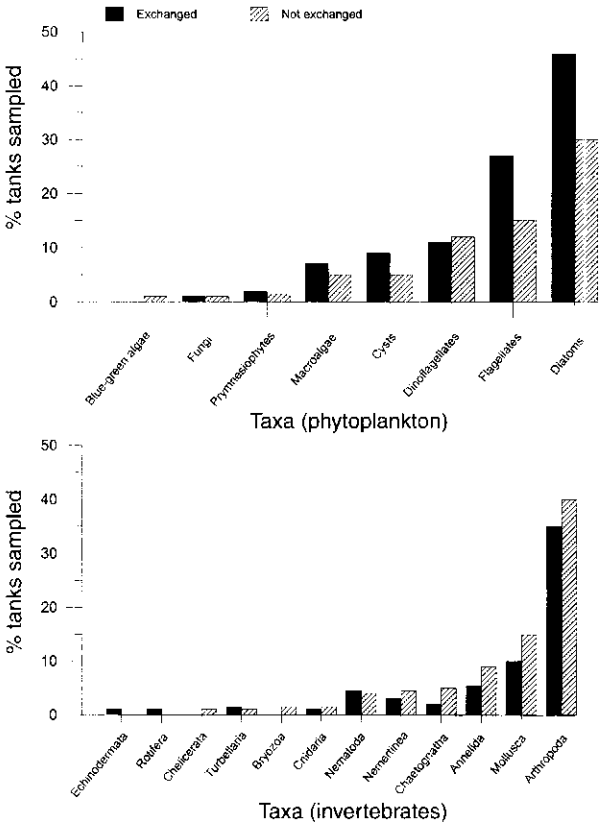

Figure 2. Percentage of tanks containing phytoplankton groups and zooplankton (including other invertebrates) phyla in exchanged and nonexchanged tanks, from Cawthron's 1995-97 ballast water sampling program.

These data suggest that in many cases the exchange procedure did not eliminate coastal species. For some taxonomic groups, recently settled juveniles may have persisted in sediments that are unlikely to be removed by mid-ocean exchange. The presence of recently metamorphosed juvenile annelid worms and juvenile mollusks also indicated the presence of semi-permanent resident populations within the sediments. These sediment-dwelling organisms may in fact be nourished and revitalized by fresh injections of mid-ocean water during exchanges.

The high percentage of coastal and oceanic species that survived suggests that any vessel discharging foreign ballast water into New Zealand waters is a significant quarantine risk, unless the ballast it is discharging has been contained by the vessel for several transoceanic voyages. For approximately half of the

samples, data were inconsistent with claims that the water had been exchanged in mid-ocean. This was either because (1) no exchange was made, (2) the exchange was only partial, thus resulting in a mixture of coastal and oceanic water, (3) coastal biota that had been transported to the mid-ocean in currents was uplifted during the exchange, or (4) somehow the biota from previous ballast loads was retained inside the tanks. Similar conclusions have been made by other researchers; *e.g.*, Locke *et al.* (1991).

Based on these early studies and those of others, Cawthron and Battelle initiated research to evaluate the effectiveness of exchange-at-sea on vessels that followed their normal exchange procedures.

The Cawthron/Battelle Study (February 1998–June 1999)

The MFish study, comprised three objectives. The first two were essentially desk-top reviews of methods and procedures currently available for measuring the volumes that are actually exchanged during mid-ocean exchanges and of procedures or water properties that might be usefully employed to test compliance. The third objective involved testing such methods and procedures aboard at least two commercial vessels travelling to New Zealand on international shipping routes. The desk-top review, completed in December 1998 (Hay and Tanis 1998), is briefly summarized below. Preliminary results and observations of research pertaining to the third objective, currently underway, are also provided.

Summary of the Literature Review

The literature review (Objectives 1 and 2) concluded that if international regulations were going to be expressed in terms of volumes exchanged, then any methods available to inspectors that enable them to measure independently the volumetric changes are useful for testing compliance. In this regard, a procedure being tested in Australia called the "Newcastle method, " which requires a master to present a ship's documentation and other logged information to corroborate claims about making exchanges, is a useful method for identifying vessels making inadequate or fraudulent claims.

Of various methods available for measuring the volumes flowing into and out of ballast tanks, magnetic flow meters were considered to have the greatest potential. Theoretically, such flow meters could be attached to the ship's ballast tanks, so that changes in a ship's ballast status may be recorded simultaneously with geographic position and time. Such information can be down-loaded to a tamper-proof data logger, which can be remotely interrogated by quarantine agencies. While such "black box" technology is technically feasible, and is used routinely elsewhere (*e.g.*, by oceanographers), the review concluded that the cost of retrofitting such equipment to the world's fleet–even to just the newer vessels–together with maintenance problems, rendered such technology impractical. Such an approach must also be weighed against the fact that mid-ocean exchange is acknowledged by IMO to be an imperfect and interim procedure until a better solution to the ballast water problem is found.

Indicators

With respect to physical and chemical features in ballast water that may be used to verify the coastal or mid-ocean origins of ballast water, we concluded that in circumstances where quarantine agencies are particularly worried about the discharges of foreign fresh or brackish water, the measurement of salinity was a very useful indicator. For New Zealand, however, where all large commercial ports are on the coast, the introduction of foreign freshwater is a relatively small quarantine risk–unlike Great Lakes ports, for example. Nevertheless, even in New Zealand, salinity is a useful measurements in cases where vessels originally uplifted river or estuarine water and reportedly made an exchange in mid-ocean. So salinity can be used to detect some cases where the exchange was inadequate or where claims are fraudulent.

The review concluded that measuring concentrations of various chemicals in ballast water is limited as a compliance tool simply because of the nonconservative nature of the tanks. Sediments with contaminants such as organic carbon, nutrients, chlorophyll *a*, and iron will be resuspended as ballast is taken aboard, lifting levels above those that would reasonably be expected for uncontaminated water, while in ballast holds, previous cargoes (*e.g.*, fertilizers or sugar) may be compromising. Measurements of dissolved oxygen and of the oxidation-reduction state of seawater, while helping to build up a profile of the water, were also deemed unsatisfactory as compliance tools. Not enough is known about the rate of decline of dissolved oxygen in ballast tanks and the effects of resuspending sediments and the biota they contain. While low redox potentials may tend to suggest that

waters are anoxic, in practice the concentration of dissolved oxygen may be quite variable. Oemcke and van Leeuwen (1998) found that oxygen-saturated water from a ship's ballast tank (6.3 mg/L) had a redox potential of 42.7 mV which would normally suggest anoxia given that seawater typically has a value of more than 200 mV. Oxygen is not the only electron acceptor (oxidizing agent) in the ocean. Nitrates can accept electrons and be reduced to ammonia, and sulphates can be reduced to sulphide, while minerals such as iron can be reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state. It can be shown experimentally how the addition of ferrous compounds to water supersaturated with oxygen can cause an immediate decline in redox potential (*e.g.*, from 276 to 27 mV), without depleting dissolved oxygen (Oemcke and van Leeuwen 1998).

Chemical concentrations might become a useful variable in a multivariable check on the probability of whether a particular load of ballast water is a result of reballasting or three-times-flow through dilution. McKeown and Mills (1998) reported that the U. S. Coast Guard (USCG) had chosen nitrate as an indicator of whether or not ships had made mid-ocean exchanges because nitrate concentrations were an order of magnitude lower in the open ocean compared to coastal and inland waters. How useful this variable proves to be as a tool for testing compliance is not yet known. Obviously, if ships have nitrogen-rich sediments in their tanks, and the sediments and bacteria are resuspended during the exchange process, then the usefulness of the test is compromised. Also, vessels may make exchanges beyond the 12 mi-territorial limit in water more than 500 m deep, which is more characteristic of coastal than of open-ocean water, and has, as a result, higher concentrations of nitrate than would be expected from a truly mid-ocean exchange.

Recent research by Hall *et al.* (1998) in New Zealand does not support the USCG study. On the M.V. *Tasman Enterprise*, travelling between Devonport, Tasmania and Tauranga, New Zealand, two pairs of previously fully emptied ballast tanks (244 and 239 m^3 capacity) were filled by gravity flow with Tasmanian coastal water. The water was sampled and analyzed daily on the voyage. Day-to-day variations in concentrations of nitrate, ammonia, and phosphorus–with no clear trend over time–were found. The concentrations of these nutrients varied between the two tanks (0.9–4.1 µg/L of nitrate nitrogen in one tank and 28–87 µg/L in the other), even though they had been simultaneously filled with the same source water. The authors therefore concluded that previous ballast water in the tanks may be important in determining nutrient concentrations.

Optical characteristics

The review concluded that the preliminary results of a feasibility investigation by Battelle into the usefulness of optical characteristics to determine whether an exchange has taken place were extremely promising. Comprehensive worldwide sampling is required. Based on the preliminary results of about 50 coastal and mid-ocean samples, Battelle has recommended that the USCG continue with its technical investigation of the optical methodology and is advising the USCG on future developments. Ideally, research workers should travel a trans-Atlantic or trans-Pacific route, simultaneously sampling both ballast tanks and the open ocean. The ballast tanks should be sampled at regular intervals throughout the balance of the voyage to detect any changes over time. Samples of original harbor water should also be collected for analysis and characterization. This type of sampling along with a diverse sample set of strategically selected coastal and open-ocean water samples will form a strong foundation for the decision algorithm.

Battelle has also recommended to the USCG new instrumentation to permit onboard processing of ballast water. This is envisioned as a self-contained instrument, roughly briefcase size. It will include some type of sampling interface either to bring samples up from the tanks or to probe the tanks for *in situ* measurements. The instrument should be fully programmed and automated to determine whether the ballast water sample is predominantly ocean or coastal.

The optical methodology and conceptual instrumentation will require some development. However, once developed, it will provide measurement capabilities sufficient to enforce regulatory compliance.

Field Test of Methods

The third objective of the Mfish study is to measure the biological effectiveness of mid-ocean exchange and to search for any water feature that can be usefully used to differentiate coastal and mid-ocean waters inside the exceedingly nonconservative environment of ships' ballast tanks. There are three main tasks:

- to measure the actual dilution of the original ballast water after mid-ocean exchange;
- to compare this physical dilution with the removal of organisms in the original water;
- to monitor the physical, chemical, and optical properties of the original and replacement water on trans-oceanic voyages with a view to finding one or more that will differentiate the water types.

Although some of this work is similar to the research previously carried out by Rigby and Hallegraeff (1994) on the M.V. *Iron Whyalla*, we reasoned that such important work warranted repeating on different types of vessels (especially container ships), in different types of tanks, and on different ocean routes.

Dye trials

Preliminary work at Cawthron indicated that the fluorescent water tracer dye Rhodamine WT was well suited as a tracer for measuring the physical dilution of original ballast water after an exchange. In that preliminary study, we measured how fluorescence over a range of rhodamine concentrations from 1 to 0.0001 parts per million (ppm) was affected by such variables as time, salinity, turbidity, the addition of iron II and III compounds, and by the presence of large numbers of phyto- and zooplankton.

These measurements were compared against dilution curves derived by serially diluting rhodamine in 0.45-µm filtered seawater, river water, brackish water (17‰), and deionised freshwater. Dye concentrations and water types were triplicated in 250-ml bottles. Fluorescence was measured with a Turner Designs 10-05 Fluorometer (www.turnerdesigns.com).

We decided that a concentration of 1 ppm was probably the most concentrated dye solution that could realistically be used in ballast tanks. Even at this concentration, the dyed ballast, when discharged, would appear faintly pink, while the volumes of dye necessary could be prohibitively expensive (*e.g.*, 10 L of dye in a 10,000 m^3 tank). For ballast water work, we would recommend initial concentrations in the range of 10^{-1} to 10^{-2} ppm. A concentration of 10^{-4} ppm approaches the limits of resolution of the fluorometer.

Across the concentration range that we tested, there was a linear, log/log response between concentration and fluorescence. The curves for seawater, river water, and deionised water were very similar and not significantly different at the 95% level. Filtering the sea, river, and brackish water samples, which had high concentrations of phyto- and zooplankton, had no effect on fluorescence.

When we added the tracer at concentrations of 10^{-1} and 10^{-2} ppm to dense cultures of phytoplankton (*Gymnodinium* and *Protoceratium*), the readings remained consistent over a week and were unaltered by filtering out the organisms at the end of the experiment. We found that the fluorescence of 250-ml samples of the various water types, when stored in amber bottles in the dark, remained constant for up to four weeks. Left in the light, the most dilute solutions began to lose fluorescence after about a week.

Iron trials

Experiments on the effect of iron indicated that at high concentrations of ferric chloride (*e.g.*, 1 mg/L) there was up to a 30% reduction in fluorescence. Ferrous sulphate, however, had a lesser effect. At lower concentrations (*e.g.*, two orders of magnitude less at 1 µg/L), the addition of the iron compounds had no detectable effect on fluorescence. Adding iron filings to seawater to produce a light brown coloration (similar to weak tea) had no significant effect on fluorescence.

Dye trials on ships

On the basis of our preliminary laboratory trials, we concluded retrospectively that rhodamine would have been a suitable ballast water tracer in most of the 160 ballast tanks we had previously examined—especially in those tanks that were routinely filled and emptied. Such frequently filled tanks typically contain clear water, unlike infrequently used tanks, where the water may be either stained bright red by rust or have turned sulphurously black.

Three trials on a New Zealand coastal vessel, M.V. *Spirit of Vision*, in a forepeak tank with 115 m^3 capacity, demonstrated how important it is to ensure that the dye is thoroughly mixed in the tanks, well before any exchange takes place. We found that this was best achieved by adding the dye to the bottom of the tank as filling commenced, and by filling the tank to less than capacity in the first few hours so that water is able to slosh about. Later, by profiling the tank and comparing the fluorescence with the theoretical value for complete mixing, we found that the water tracer dye in the ballast tank was homogeneous.

We found that the dye dilution at the end of each voyage, after a three-times-flow through exchange, as

indicated by declining fluorescence, was in the range of 89-97%–which is indeed close to the theoretical value of 95%. On one voyage, the tracer was diluted 35% at the top of the tank and 95% at the bottom of the tank, which suggested that the two water masses were stratified by the new, incoming ocean water layering above the original harbor water. This stratification persisted until a water volume about twice the capacity of the tank had flowed through. By the time three volumes had flowed through, however, the remaining dye tracer was homogeneously dispersed inside the tank and the stratification had disappeared. On this trial, the concentration of rhodamine declined from 0.12 ppm before the exchange to less than 0.01 ppm at the end of the voyage. We calculated this as indicating 96.7% dilution of the original water.

With respect to the biota, however, we have some interesting preliminary results from the exchange trials on the *Spirit of Vision*, which are described by Taylor *et al.* (this volume). Theoretically, a three-times-volume exchange should cause a 95% decline in the density of the indicator taxa uplifted at source. Indicator taxa were defined as phyto- and zooplankton species that did not occur in mid-ocean plankton samples taken at the same time as the exchanges were conducted. On neither of two trials in New Zealand coastal waters did this occur. This is attributed to inadequate mixing between the original and exchanged water volumes, the possibility that the exchange resuspends coastal species that have previously accumulated in tank sediments, and perhaps an ability on the part of some species to move against the flow and thus remain inside the tank. There is also the possibility, however, that some of the indicator taxa were in fact uplifted during mid-ocean exchanges, even though they were not detected in the mid-ocean plankton samples.

In February 1999, our efforts to measure the efficiency in terms of dilution and the biological effectiveness of mid-ocean exchanges were scaled up to 1,400m^3 tanks on a converted parcel chemical carrier, M.T. *Iver Stream* (170 m length; 32,570 dwt), travelling between Japan and New Zealand. Three-way comparisons of the old and new ballast water and of ambient mid-ocean water at the exchange location were made during flow-through exchanges, and the chemistry, optical properties, and biota of the ballast water were monitored throughout the entire journey.

On the *Iver Stream*, Rhodamine WT dye concentrate was added to emptied ballast tanks via Butterworth hatches on deck. These hatches permitted us to pour dye concentrate directly into the empty tanks. The amount of dye added was calculated to provide a final concentration of approximately 0.1 ppm when the tanks were filled and the dye was thoroughly mixed. A check for stratification of the dye, which was made by taking samples of the surface, mid (7 m), and bottom (14 m) layers of the tank 24 hr after they were filled, indicated complete mixing.

Mid-ocean exchanges resulted in a dye dilution of greater than 95%. On one exchange, off the mouth of the Sepik River (north coast of Papua New Guinea), the salinity of the incoming water changed from oceanic (34-35 ppt) to slightly brackish (32 ppt), despite the ocean depth at that location being approximately 900 m. A layer of lower salinity water at the top of the tank demonstrated that stratification was occurring during the exchange. After twice the tank capacity had been exchanged, the stratification disappeared. This result suggests that salinity imbalances may be important with respect to the effectiveness of flow-through exchanges, especially if they are incomplete.

On the *Iver Stream* voyage, coastal and open-ocean water and ballast samples collected for analyses of their optical characteristics demonstrated that the marked reduction in concentration of dye tracer coincided with significant changes in the optical signature. After each exchange, the optical signature of the ballast water shifted from that of typical coastal water to that of oceanic water. This shift was not observed in the control (nonexchanged) tank.

With respect to the biota in the tanks, preliminary results from our monitoring of events on the *Iver Stream* show that the rate of dilution (as measured by dye dilution and the optical signature) in the exchanged tanks was associated with a similar decline in the abundance of the dominant indicator taxa (as defined above): adult copepods, 95-100%; larval and juvenile polychaetes, 97-100%; dinoflagellates, 97-99.9%. An important consideration, however, was differences in the change in abundance of viable organisms in the control tank during the time the exchanges were made: adult copepods, 14-79%; larval and juvenile polychaetes, 100%; dinoflagellates, 61-77%. For most groups, mortality appeared to increase significantly after five days. This was associated with a warming of the ballast as the vessel entered the tropics, in addition to the other factors

affecting survival (*e.g.*, oxygen depletion and absence of light). Several taxa, comprising predominantly coastal species (*e.g.*, bivalve and barnacle larvae), were uplifted in relatively high numbers during exchanges.

Conclusions

Dye tracers such as Rhodamine WT reliably measure the dilution efficiency of mid-ocean exchanges, but the logistical constraints of adding the tracer to the tanks before an exchange, especially where sounding pipes are the only feasible access point, means that tracers are unlikely to be useful compliance tools. Their main application is likely to be in calibrating indirect measures of dilution (*e.g.*, optical signatures).

The flow through dilution method of mid-ocean exchange has been shown to have an efficiency in excess of 95%. However, further monitoring of vessels at sea is needed to verify if salinity imbalances are a problem. When making exchanges in deep water (>500 m), the salinity of the new water relative to the old should be considered as a factor determining the rate at which the old water is diluted during the exchange.

The dilution of phyto- and zooplankton during mid-ocean exchanges appears to be heavily dependent on the flow dynamics and the mixing characteristics in the ballast tanks. The tolerances of the organisms to the tank environment will be a key factor affecting measures of the rate of dilution of the biota. Identifying and quantifying the factors affecting survivorship is a priority for further research. Therefore, a wide range of phyto- and zooplankton taxa should be used as indicators of the biological efficacy of exchanges. The usefulness of larval invertebrates as indicators is limited, however, because they can occur in both coastal and open ocean water.

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Future Research on Ballast Water Treatment—A Technologist's View

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ABSTRACT: **Since the mid 1980s, an international research effort focused on the development of ballast water treatment options has been steadily increasing. The effort has resulted in the development of a number of management and treatment options that can be applied to ballast water. Ballast water can be treated on-shore, in port, or on shipboard during ballasting, in-transit, or during deballasting. There has been considerable discussion and debate on the merits of the various options; this paper makes a contribution with an assessment of the current treatment technologies and suggestions for further research. To select the most suitable treatment and management options for ballast water requires a well-informed assessment of a wide range of variables. Some can be rejected or refined on the basis of impracticality, impossibility, and proven poor performance. However, to thoroughly assess the options for ballast water treatment and management will require the development of ballast water treatment standards. The setting of standards for treatment will enable the comparison of treatment options on the basis of cost, safety, environmental risk, and efficacy in a way that is not possible in the present environment. Furthermore, the development of standards will influence the direction of ballast water research by placing a focus on the questions that must be answered to set appropriate standards.**

Key words: ballast water, treatment, management, standards

INTRODUCTION

Research into the treatment of ballast water has gained considerable momentum since the experiments on ballast exchange of Williams *et al.* in the 1970s (Williams *et al.* 1988). Laboratory research has been conducted on methods to inactivate a range of organisms, including algal cysts (Oemcke 1999a; Hallegraeff *et al.* 1997; Montani *et al.* 1995; Bolch and Hallegraeff 1993; Ichikawa *et al.* 1992), other algae (Oemcke 1999a, 1998; A. Jelmert, pers. comm.), *Undaria pinnatifida* spores (Mountfort 1997), and zooplankton (A. Jelmert pers. comm.; Mountfort 1997). Field studies have included tests on the use of filtration (Parsons *et al.* 1997; T. Waite, pers. comm.), heat (e.g., Rigby *et al.* 1998; Prentice and Thornton 1997; Rigby and Hallegraeff 1994; D. Mountfort, pers. comm.), hydrocyclones plus UV irradiation (A. Jelmert and H. Nilsen, pers. comm.), and discharge to sewer (A. Cohen, pers. comm.), and are proposed for filtration plus UV irradiation (note: the author is involved in this proposal). However, it remains the case that research into the treatment of ballast water is in the early stages of development.

Several reviews have been published on technologies for ballast water treatment (*e.g.*, NRC 1996; Carlton *et al.* 1995; Gutteridge, *et al.* 1993; Laughton *et al.* 1992) with recent reviews by Oemcke (1999a, b). This paper summarizes the findings of the latter review, with particular emphasis on the interactions between the nature of the shipping industry and the efficacy of disinfectants in particular circumstances. The requirement for an overall framework, within which to compare ballast water disinfectants, and some of the research needed for this system are discussed. Some of the disinfectant options reviewed in Oemcke (1999a, b) are shown in Table 1.

The treatment and management of ballast water can be conducted at a number of stages of a ship's voyage (see Carlton 1985 or Hayes and Hewitt 1998). It can be conducted on shore or on ships, and before, during, or after trips on which ballast water is carried. Figure 1 shows a summary of the locations at which ballast water management and ballast water treatment can be conducted. This figure includes the options identified by Carlton *et al.* (1995), except non-discharge of ballast and extending voyage length. Management techniques can be used to control species transfer without the addition of water disinfection technologies. The treatment options would

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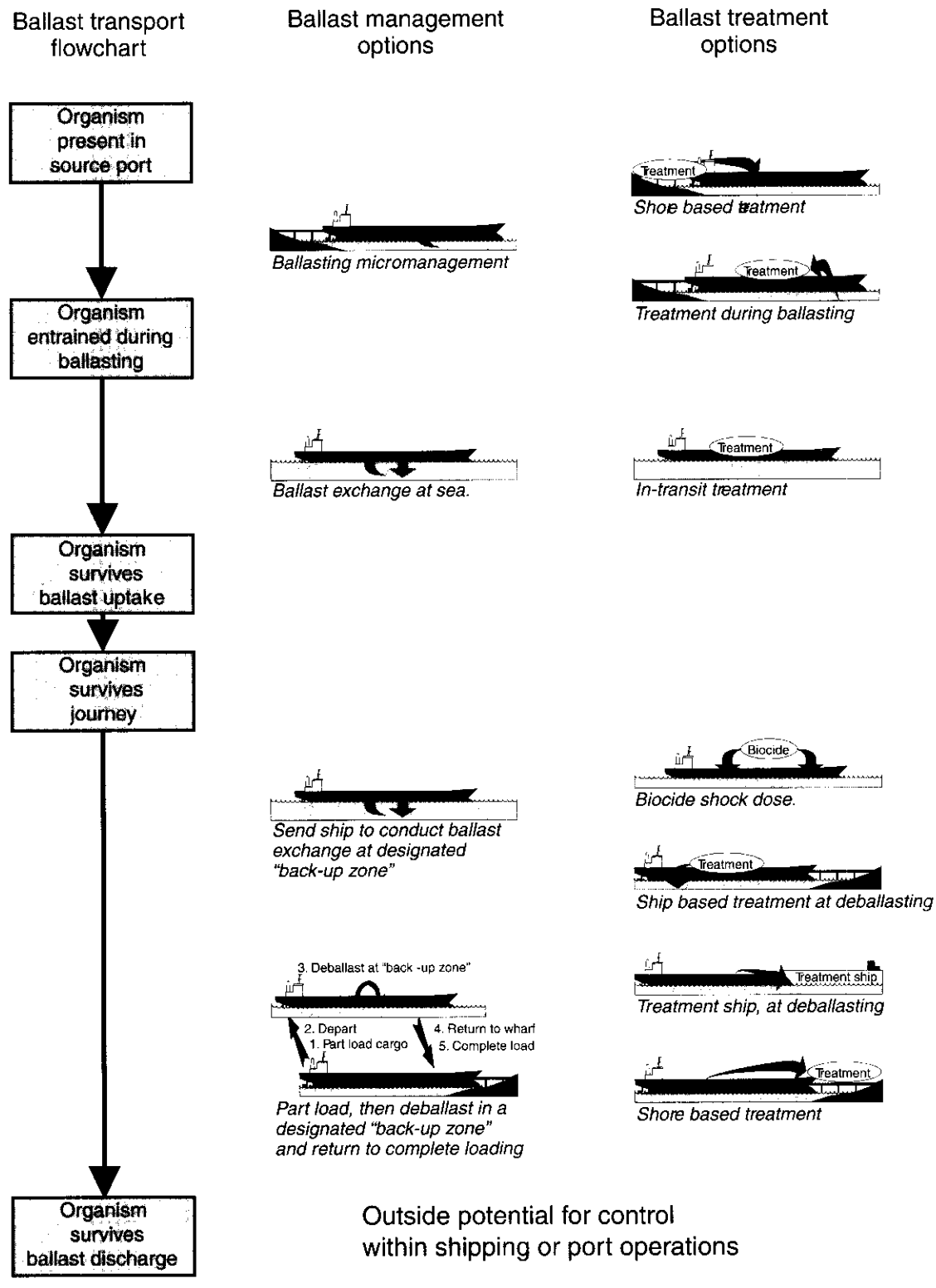

Figure 1. Ballast water treatment options within the shipping and ports industries.

require the addition of physical or chemical disinfectants to ballast water.

The feasibility of the use of various management and treatment strategies and/or technologies for ballast water treatment is affected by a wide range of technical issues, including the following.

- Cost to conduct (on a comparable basis, for example US$/mt).
- Time required for completion of treatment. Some methods will not be able to be completed within the duration of a voyage, depending on the route.
- Technical impediments. A particular impediment to various treatments, as described below, is the ability to achieve adequate mixing.
- Availability of proposed technologies at the required scale.
- Long-term toxicity.
- Efficacy of disinfectants. For the ballast treatment options, a range of disinfectants could be efficacious, but the disinfectants vary with the stage of a voyage.

In order to move forward, it is necessary for the industry and regulators to be able to select ballast water treatments that work and are cost-effective. There are competing issues, where managers may tend to regulate conservatively and industry will want the lowest possible combination of cost and risk to meet regulations. This paper reviews the technical issues for treatment at the various stages of a ship's voyage, and proposes the development of a framework for comparing options that will assist in making these management decisions.

Port-based Treatment Systems (Shore or Treatment Ship)

Treatment on land is often favoured by regulators and managers, as the quality of treatment is often considered more controllable than with any other system. Treatment on land can be conducted at a shore-based plant or by a treatment ship (*e.g.*, Carlton *et al.* 1995; Gutteridge *et al.* 1993). The technological requirements for effective treatment at port-based facilities can be met in many ways and would be site specific. Several different types of filters can be used to remove a large number of organisms (*e.g.*, sand filters, pressure filters, screens, membranes), pH can easily be adjusted to give optimum conditions for disinfectants, and oxidant residuals can be removed.

The difficulties, real or perceived, of implementing port-based treatment appear to have limited consideration of this option, and it is likely that in many cases implementation will be very difficult. The use of potable water is very unlikely to be cost effective due to the high standards of treatment and high value of fresh water supplies (Oemcke 1999b). Discharge to sewer is unlikely to be widely effective because not all sewage is disinfected, and much is only disinfected to the level required for the removal of bacteria; many ports are distant from sewage treatment plants large enough to take their flows; and there is anecdotal evidence that highly saline water (particularly saline shock loads) upsets nutrient removal and sludge settling in biological sewage treatment systems. It may have a role in areas where ballast discharges are predictably low and is being trialed in San Francisco (A. Cohen, pers. comm). One likely benefit from discharge to sewer is the removal of cysts in primary sedimentation processes.

Table 1. Physical and chemical methods reviewed for ballast water treatment by Oemcke (1999a, b)

Physical treatment	Chemical treatment
filtration	Chlorine
cyclonic separation	other halogens (bromine and iodine)
ultraviolet irradiation	chloramine
heat	chlorine dioxide
ultrasound	ozone
magnetic fields	hydrogen peroxide
electric fields	organic compounds
mechanical damage	copper ions
	copper + silver ions
	ionising radiation
	photochemistry
	oxygen deprivation
	pH adjustment
	salinity adjustment
	tank coatings

Options for the shore-based treatment of ballast water, separate from potable water and sewerage systems, have been proposed. Gutteridge *et al.* (1993) presented concept designs for a shore based system and several authors have discussed various alternatives (*e.g.*, Carlton *et al.* 1995). A system of cyclonic separation and UV-irradiation has been proposed for VLCCs (very large crude carriers) where ballast is purchased from a port-based supplier, or alternatively from a specially equipped tender (H. Nilsen and A.

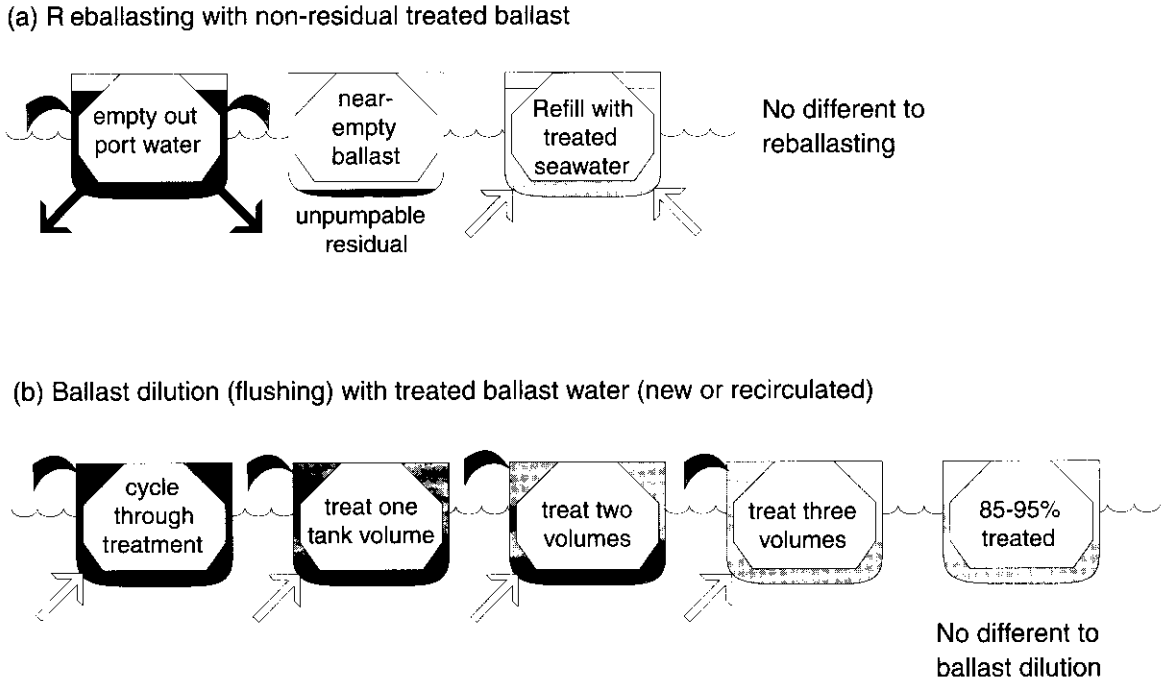

Figure 2. Representation of the use of nonresidual disinfectants for in-transit treatment of ballast water, showing that, in general, they are no different from ballast exchange.

Jelmert, pers. comm.). VLCCs have very high costs for ballast exchange and well-defined trade routes, which makes a system of purchasing treated ballast water more likely to be attractive on financial grounds compared with other potential markets within the shipping industry.

In-transit Treatment

In-transit treatment must be capable of achieving an effective disinfection residual throughout the vessel during transit. Options that produce no residual, such as filtration, cyclonic separation, high power ultrasound, magnetic treatment, and UV cannot be utilized for in-transit treatment, as the organisms must be moved to the treatment plant. The hydraulic inefficiencies of ballast tanks mean that all water will not pass through the treatment plant. Figure 2 helps to illustrate how the use of nonresidual in-transit treatment is no more effective than ballast exchange at sea. This can also be illustrated by assuming that a pumping system ensures that 90% of the ballast water in a ballast tank passes through a (nonresidual) in-transit treatment system, which then inactivates 99.99% of organisms. In this case, 10.01% of the organisms within the ballast tank survive treatment due to hydraulic inefficiency, even though the disinfection system inactivates 99.99% of the organisms that pass through it.

Ideally, in-transit treatments will be broadly biocidal and capable of being mixed into ballast water during ballasting. Treatments with an ability to obtain an evenly distributed residual include ozone, chlorine, chlorine dioxide, bromine, hydrogen peroxide, pH adjustment, heat, proprietary biocides, and copper sulphate, among others. For oxidizing biocides (*e.g.*, ozone, chlorine, bromine, hydrogen peroxide, and chlorine dioxide), chemical reduction of the residuals at the sediment-water interface and areas of corrosion are likely to adversely affect the most cost-effective dosing methods (Oemcke and van Leeuwen 1998a, b). It may be possible to arrange chlorine dioxide dosing to make it effective, although benthic organisms in sediments will probably be difficult to remove and local areas of corrosion may have a negative effect.

A large body of data suggests that dinoflagellate cysts are very difficult to remove from ballast water with any biocide other than heat (*e.g.*, Oemcke 1999a; Hallegraeff *et al.* 1997; Montani *et al.* 1995; Bolch and Hallegraeff 1993; Ichikawa *et al.* 1992). It is conceptually possible to pre-treat by filter or cyclone to remove these resistant organisms prior to applica-

tion of the biocide. Pre-treatment would need to be conducted during ballasting prior to in-transit application of biocide, a two-stage treatment process that increases treatment plant complexity and adds cost.

Currently, a promising option for in-transit treatment to remove cysts of dinoflagellate algae is heat. The ship's engine cooling system and exhaust are areas where free waste heat can be obtained, giving this option the opportunity to be relatively cost neutral compared with ballast exchange at sea. However, potentially important pathogens (*e.g.*, *Aeromonas salmonicida*, *Vibrio cholerae*) will not be affected, and a wide range of organisms remains to be tested. Waste heat will not be suitable for short voyages, due to the time required to transfer adequate heat into the water, and may not work in cooler waters due to heat loss to the environment (Rigby *et al.* 1998). No detailed examinations on the corrosion potential have been conducted. Although Rigby *et al.* (1998) believe this will not be a problem, it requires investigation before heat treatment is adopted. Heat may affect the stress loadings of the vessel by causing differential expansion of the ship superstructure (Carlton *et al.* 1995), which also requires attention. The use of additional waste heat from the exhaust may improve the process, and the use of recirculation, rather than the ballast dilution approach, may improve the efficacy of heat treatment (D. Mountfort, pers. comm.), although it is potentially more expensive, due to the heat exchangers.

Shipboard Treatment during Ballasting

Shipboard treatment during ballasting has important advantages over treatment during deballasting. Water can easily be taken in through the ballast pumps during ballasting, whereas during deballasting it is discharged through multiple outlets. It should be noted that 5–20% of the ballast on board is often deballasted during a ships' approach to port, particularly from the topside tanks (Hayes and Hilliard 1996). Further, ballasting is often conducted at a slower rate than deballasting (*e.g.*, Hayes and Hilliard 1996). This will reduce the size of shipboard treatment plants required during ballasting compared with treatment during deballasting, as treatment plant size is roughly proportional to flow rate.

Solids separation (*e.g.*, screening or hydrocyclones) is the simplest system for ship-board treatment. It requires that the organisms removed by the screens can be returned to the source port, to avoid

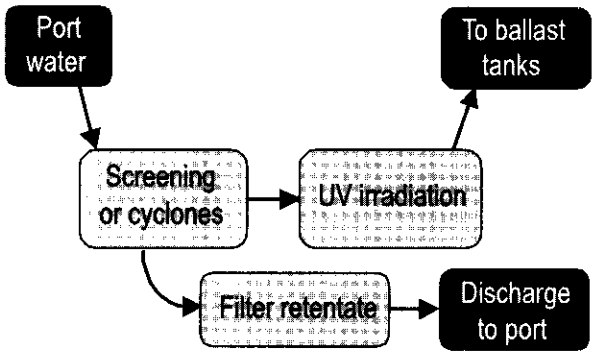

Figure 3. The use of nonresidual biocides for ballast water disinfection during ballasting, using UV irradiation as an example.

expensive treatment of the screenings. Screening can handle sediment loads if appropriate units are selected and designed appropriately. For treatment of larger organisms in ballast water, a 50-µm screen appears to be appropriate, and for the removal of dinoflagellate cysts, 20-µm micron screens will be necessary (Oemcke 1999a, b). Due to their high density, dinoflagellate cysts should be removed by hydrocycloves (Anderson *et al.* 1985), and new systems are claimed to be effective for removal of a range of organisms and particles to less than 10µm (H. Nilsen, pers. comm.).

Ultraviolet irradiation (UV) has considerable potential as a secondary ballast water treatment, following solids separation during ballasting (Oemcke 1999a, b; 1998). UV will require pre-clarification to be effective, both to reduce turbidity and remove UV-resistant dinoflagellate cysts (Oemcke 1999a). The example of a shipboard ballast water plant, for treatment during ballasting (Figure 3), shows how a UV plant might be configured for ballast water disinfection.

Ozone is unlikely to be an applicable treatment during ballasting, due to the high doses or fine filtration required to remove resistant organisms (Oemcke and van Leeuwen 1998a). Chlorine is similarly constrained. However, a system that uses membrane filtration followed by chlorination is proposed for cruise ships (Carless 1998). This system has potential as cruise ships use small amounts of ballast water, so although the cost burden of high technology options is high, their simplicity is beneficial. Furthermore, membrane filtration alone will remove all organisms except for viruses. This approach suggests that some combinations may have potential for ships using

Table 2. Refined list of ballast water treatment alternatives for shipboard treatment adapted from Carlton *et al.* (1995) by Oemcke (1999a, b)

Treatment option	Effectiveness against classes of organism: Zooplankton and fish	Macroalga spores	Dinoflagellate cysts	Sub 20mm organisms	Attached bacteria
Organisms present in source port					
1. Ballast "micromanagement"[a]	Partially effective, should be pursued as risk reduction strategy				
2a. Filtration during ballasting (50 µm)[b]	✔	✘	✘	✘	partial
2b. Filtration during ballasting (20 µm)[b]	✔	✘	✔	✘	✔
2c. Filtration during ballasting[b] 50 µm + cyclone)	✔	✘	✔	✘	?
2d. Cyclonic separation alone[b]	research	research	✔ probably	some	research
3. Enhanced mechanical damage at pump[c]			requires research		
4. Ultraviolet Irradiation[a]	✔	research	note 2	research	✔?
5. High power ultrasound[a]	Maintain 'watching brief' for a few years until technology develops				
6. Other new technologies[a]	Maintain 'watching brief' until technology develops				
7. Chlorine dioxide[a]	Effectiveness unknown, requires research				
Organism survives ballast uptake					
8. Heat treatment in-transit[c]	✔	✔	✔	partial	✘
9. Chlorine dioxide in-transit[a]	Effectiveness unknown, requires research				
10a. Ballast dilution (3 volumes)	> 85-90% effective for pelagic, effect of dead spots must be researched				
10b. Reballasting	>90% effective for pelagic, implementation difficult for many ships				
Organism survives journey					
10c. Backup exchange zones	Same as options 10a and 10b except for potential risk to backup zone				
10d. Part load then deballast (return to sea or backup zone)	100% effective within port as no ballast is discharged, potential risk to backup zones				
11. Non-discharge of ballast water	100% effective where viable				
12. In situ extermination	Difficult to achieve mixing or find suitable disinfectant				

[a] Screening and/or cyclonic separation will be necessary as a pretreatment; [b] 20-µm screens and cyclonic separation likely to be effective; 50 µm filtration or UV alone ineffective; [c] assumes adequate time and heat transfer are available

small volumes of ballast (*e.g.*, cruise ships), although they may not have potential for larger vessels.

High-power ultrasound is a new technology that may eventually be effective for ballast water treatment. The technology is still in the developmental stage, so very few good data are available on costs per unit of water treated in large installations, or on the effects of interfering substances or the limitations of the technology (*e.g.*, suspended solids). The relationship between laboratory tests and full-scale plants cannot be determined until full scale plants are built and evaluated for disinfection applications. A "watching brief" is, therefore, the appropriate approach to this technology at present as it will probably take several years before developments reach the stage where testing for efficacy against ballast water organisms is appropriate.

Chlorine dioxide is an effective cysticide that may be appropriate for treatment during ballasting. The main concerns with chlorine dioxide are cost and the possible environmental toxicity of by-products.

Biocidal Shock Dosing

The use of biocides to treat ballast during a vessel's approach to port, especially vessels that have been identified as a risk by a port state, is a conceptually attractive option, but has a variety of difficulties and concerns. These include identifying a chemical that works, identifying a chemical that is safe to han-

dle and not prohibitively expensive, identifying a chemical that is safe to discharge to the environment, and determining a method for thoroughly mixing the chemical into the ballast tanks.

Loaded vessels carrying no ballast on board (NOBOB) entering the Great Lakes of North America have the potential to be dosed with biocide to inactivate the organisms in nonpumpable ballast residuals remaining in empty ballast tanks. Lubomudrov *et al.* (1998) and Moll *et al.* (1998) recommend glutaraldehyde, which they believe will have time to break down before its eventual release to the environment, and could be dosed at a station by properly trained staff. It must be noted that these chemicals present a high level of risk to the health of staff handling them and may be too unsafe for the use of these chemicals. RNT (1997) recommend the use of glycolic acid or glutaraldehyde. This approach may be suitable for areas like the Great Lakes, where ships enter without ballast and then trade within the system, and can be dosed during river passages en route to the lakes; however, the literature suggests that high doses will be required.

Ballast Management Options

There are several management options in Figure 1 and Table 2, which, in addition to the treatment options, complete the picture of potential ballast water treatment technologies. Much of the current effort in treatment technologies is motivated by the inadequacies of these options in terms of both safety and efficacy for the removal of organisms. However, it may be possible to improve the efficacy of these practices, particularly ballast exchange, and they will have an important role in the control of introductions from ballast water. Further research is necessary (Oemcke 1999a, b).

Model of Ballast Treatment Systems

As discussed above, the treatment of ballast water on shore (or a treatment ship) will depend on local site constraints, land availability, infrastructure availability, and local discharge regulations; a very wide range of technologies is suitable. Designers and operators of treatment facilities should know the options that can be applied for treatment and be able to make decisions based on what is known about the efficacy of a very wide range of treatment technologies and alternatives for organism removal (discussed in more detail in Oemcke 1999a). However, shipboard treatment is a more highly constrained system that requires careful consideration of the alternatives.

Carlton *et al.* (1995) proposed a list of potential ballast water treatments, which is refined to the 12 currently strong options for shipboard treatment and shown in Table 2. Their five options for prevention of organism intake have been refined to a single option called "ballasting micromanagement" in Table 2, and the model for filtration is refined to two levels of screening (20-μm and 50-μm). Options from the list of Carlton *et al.* (1995) that have been omitted are those that recommend in-transit treatment with nonresidual biocides or biocides that need to be evenly and reliably distributed to be effective. The use of shore facilities to provide treated water, use of potable water, discharge to sewage, and options specific to sediments have been omitted from the list as they are not considered widely viable. Tank coatings and oxygen deprivation are not effective treatments.

Comparing Ballast Treatment and Management Options

Comparing ballast water treatment options is the most important issue confronting technologists involved in ballast water management. The previous sections have focused on a review of the options available for the treatment of ballast water. As indicated, a number of alternatives can be rejected on the grounds of technical feasibility, lack of broad applicability, or toxicity. Furthermore, it is clear that the financial issues vary across the industry. However, in general the ballast water treatment alternatives cannot be objectively compared, either between the potential locations for treatment or technologies.

Treatment options are typically compared on the basis of cost to reach an agreed standard, within any other existing constraints. At present two *de facto* standards are being used for ballast water treatment, but they are not recognized as being standards. I consider these to be standards only because they are the only widely used basis for comparing disinfectants and management schemes.

The first *de facto* standard is based on the efficiency of ballast exchange processes (ballast dilution or flushing and reballasting), which is somewhere around 90%, and often much lower. An efficiency of 85% has been treated as acceptable by the U.S. Coast Guard (Reeves 1997). Hallegraeff (AQIS 1998) suggests that ballast dilution is about 85 to 95% effective. The second *de facto* standard is that implied by

the current approach to ballast disinfection, where 100% control of the resting cysts of certain species of dinoflagellate algae is the target of treatment.

These two *de facto* standards are incompatible with each other, and mean that quantitatively comparing the treatment processes and management options is impossible. This is because

- the exchange efficacy standard is biased towards the selection of ballast exchange as an option, having grown out of the use of that option; and
- there is no basis for requiring any treatment to remove or inactivate all algal cysts, as no treatment other than heat is available.

Criteria for the treatment of ballast water are required to determine if the processes are achieving an appropriate level of removal. To do this, it is suggested that the following criteria be fulfilled:

1. Develop an understanding of the organisms that are desirable to remove from ballast tanks, which is the subject of considerable research. This does need to be a complete understanding, but can grow with the scientific contribution.
2. Select indicator organisms. For example, *Vibrio cholerae*, resistant motile/pelagic unicellular marine alga or protozoa, sexual stages of *Undaria pinnatifida*, *Gymnodinium catenatum* hypnocysts, *Dreissena polymorpha* pelagic veligers, and *Asterias amurensis* larvae may be considered appropriate at present.
3. Determine a desired level of removal, based on the available risk assessment tools.

With a set of indicator organisms and a desired level of removal, ballast water treatment and management options can be selected on the basis of their ability to remove these organisms to the required level. The treatment and management options can then compared on an objective basis such as cost, environmental hazard, time required, space required, *etc.*, for treatment to the mandated level.

A preliminary dataset can be used to establish treatment requirements in the short term, with scientific advances used to refine the criteria used for selecting between options. Without this system, it will be difficult to resolve the competing claims of proposed solutions, research will remain relatively unfocused, and managers will be constrained by a lack of performance requirements in setting objectives. The establishing of standards will begin a debate on standards that can be resolved in the long term by research. The process for setting standards in the water supply industry is similar. Great gains were made by the removal of basic faecal contamination, with an international process of refining standards and targets an ongoing feature of the water supply industries research effort.

For the ballast water treatment industry several current fields of research will help to refine the system of standards over time. These include the following:

- Efforts to understand the dynamics of the survival of organisms present in ballast tanks (*e.g.*, Hall *et al.* 1998; Ruiz and Hines 1998; Taylor-Wood *et al.* 1997).
- Efforts to understand or estimate the inoculum of an organism that is necessary to infect a new site (*e.g.*, Ruiz and Hines 1998).
- Improved understanding of risks; for example, quantitative probabilistic approaches (Hayes 1998; Hayes and Hewitt 1998); semi-quantitative approaches (Hilliard and Raaymakers 1997; Hayes and Hilliard 1996); and quantitative approaches (Carlton *et al.* 1995). The qualitative probabilistic approaches will be the most precise risk assessment, but probably require enormous investment.

The Required Level of Assessment

Comparisons should be conducted on the basis of individual ships and their trading patterns, on the required level of organism removal for that trading pattern, and over the life of the ship. Costs of ballast water treatment and management techniques cannot be generalized for the whole shipping industry due to variation in ship design, ballast pumping rates, ship purpose, and trading routes. Treatment and management options should also be compared over a lifetime, not over a single trip, to reduce distortion of cost comparisons.

Depending on the management or treatment technology, the cost of ballast management is related to either the total ballast capacity and trip length or to the size of ballast pumps, each of which vary significantly. Ballast water treatment methods will vary in their efficacy depending on the conditions to which they are subjected. For example, lower doses are effective for UV irradiation of algae as trip length increases and higher temperatures are possible for longer trips when heat treatment is used. Some organisms will be inactivated without treatment, depending on temperature changes during trips or length of trip.

It will be valuable to understand whether the concentration of organisms in ballast water is important, or if the number of organisms discharged is more important, in order to establish a sensible set of treatment criteria. If it is purely the number of organisms, then treatment systems for different vessels will necessarily differ. A 90% effective treatment (*e.g.*, exchange) for a ship discharging 1,000 mt of ballast water will release the same volume of untreated water as a 99.9% effective treatment (*e.g.*, filtration + UV irradiation) on a ship discharging 100,000 mt of ballast water.

Moving Forward

The development of effective ballast water treatment and management techniques requires the development of a set of logical treatment standards. Without such standards, the options for treatment and management cannot be compared objectively and the debate about solutions to ballast water will remain unfocused, as it is presently.

Science can be further developed following the setting of standards. This will most likely include some of the following.

- A step forward in the debate about risk assessment methodologies, as management questions increasingly become the focus of risk assessment. For example, questions about the level of risk assessment required will be coupled with an assessment of the ability to respond adequately. This is likely to result in a shift in resources towards developing solutions rather than quantifying risks to a very precise level.
- The interrelationship between infectious dose and treatment will be explored in detail, as the costs of meeting tight standards will begin to become apparent.
- Understanding the issue of concentration and dose will become important, as it will have an impact on the cost of treatment across the industry.

Specific Technological Recommendations

To progress forward on the development of technologies listed in Table 2, the following specific recommendations are put forward, in no particular order.

Ballast Treatment

1. Screening/cyclone pilot tests. Pilot testing should be conducted for screening at 20μm–50μm, to determine its ability to handle sediment loads and blooms of plankton and phytoplankton. This pilot testing will find the operating constraints so that systems can be appropriately designed and enable full-scale cost data to be accurately determined.
2. UV laboratory tests. Further laboratory testing on UV disinfection is required. This testing should target species of phytoplankton and protozoans that will pass clarifiers and for which disinfection efficacy data are not currently available. It must account for dark repair and the effect of storage on inhibiting photorepair mechanisms.
3. UV pilot tests. Pilot testing for UV+screening/cyclone systems should be pursued in addition to further laboratory testing. There are enough data demonstrating that UV has considerable potential for ballast water treatment during ballasting, and full-scale design data need to be gathered. The pilot testing must aim to test the limits of the UV system.
4. Ultrasound. High-power ultrasound should be viewed cautiously by ballast researchers until the costs and operating constraints of full-scale systems are understood. This technology should be watched, as it may have potential for treatment during ballasting.
5. Heat treatment testing. In-transit heat treatment needs to be researched to determine where this will be an appropriate solution. Modelling of flow processes may enable systems to be designed that improve the transfer of heat into the ballast tank, and can be designed into new vessels.
6. Chlorine dioxide laboratory tests. Laboratory-scale tests of chlorine dioxide should be considered for addition to any tests being conducted on cysts, to determine if it is effective for their treatment. It is a highly effective cysticide, and may have potential for ballast treatment, either shipboard or land-based.

Ballast Management

1. Ballast exchange dye tracer tests. Testing of the effectiveness of ballast dilution and reballasting at sea should be further pursued. Tracer studies have had problems with evenly distributing dye into the water, and it would be preferable to use a method that injects a pulse of dye into the incoming ballast water during the exchange process. Data from well-conducted tests may be

useful to help design more effective systems or to eliminate this option from consideration.

2. Modelling the effectiveness of ballast exchange. Modelling of a ballast tank using a flow-analysis package and including the internal structures of the tank is necessary to evaluate the effectiveness of dilution and to design good dilution systems for new vessels, if this option is appropriate.

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UV Disinfection of Ballast Waters: Effects of Organism Size on System Scaling

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ABSTRACT: Ballast water has been demonstrated to be one of the principal vectors for unintentional introduction of non-native species. Although a number of treatment options, both physical and chemical, have been proposed, UV sterilization remains an especially viable and environmentally benign approach. Developments in UV technology using high-powered excimer sources in the germicidal region of the UV spectrum (developed to treat bacteria in metal-working fluids) have enabled the efficient delivery of doses capable of inducing acute and latent phototoxicity in a wide variety of organisms. In this investigation, we evaluate the efficacy of high-intensity UV irradiation at applied doses of 10-100 mWsec/cm² in controlling planktonic organisms likely to be entrained in ballast water. These include larval and adult crustaceans, larval bivalve mollusks, larval fish and microalgae. Since this technology will likely be combined with some physical filtration for shipboard installations, the relationship between organism size and effective UV dose becomes an important consideration. We present preliminary data for a representative range of organisms using an experimental flow cell.

Key words: ultraviolet light, filtration, system scaling

INTRODUCTION

The introduction of non-native aquatic species into the North American waterways has been occurring for as long as transoceanic travel has existed. In fact, representatives from virtually every group of aquatic organisms have been introduced somewhere in the world (Morton 1997). The scale of exotic species introduction is enormous: 136 nonindigenous species are known from the Great Lakes and at least 43 of these have arrived since 1960 (Mills *et al.* 1993). Of the 150 nonindigenous species that have been discovered in San Francisco Bay, at least 21 of these have colonized the Bay since 1973 (Carlton *et al.* 1990). In Coos Bay, Oregon, exotic species number approximately 80, and in Chesapeake Bay approximately 15.

How to curtail the invasion of non-native species from North American rivers and lakes is a critical question. It has repeatedly been shown that ballast water is the principle vector for unintentional introduction of these nonindigenous organisms into U.S. waters (Wiley 1997; Reeves 1997; Carlton and Geller 1993), and likely facilitated the introduction of zebra mussels (*Dreissena polymorpha*), to North America. Support for this argument was given by Carlton and Geller (1993) in a study where ballast water from 159 cargo ships that had traveled from Japan to Oregon were sampled, discovering 367 distinct taxa representing 19 phyla. This illustrates that ballast water is a medium in which a diverse community of organisms can survive and even thrive during transport over many miles.

There are some controls that can minimize the chance of successful colonization by an exotic aquatic species. A currently favored technique is the

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exchange of ballast water at sea. The objectives of this technique are to flush out organisms and bring the salt concentration of the ballast water to approximately 30 ppt (Reeves 1997; Wiley 1997), limiting the survival of salt-intolerant organisms. However, potential complications of ballast exchange still exist. Because of the amount of ballast that would have to be removed at one time, it may be unsafe for the larger ships to exchange enough water. Ballast exchange may also be waived in poor weather, it may be ineffectively performed, or there may still be significant survival of exotic species protected in sediments remaining in the tank even when the exchange is performed within the bounds of existing legislation (Reeves 1997; Wiley 1997). The direct treatment of ballast water may be a more desirable option.

In 1990, the U.S. Congress enacted P.L. 101-646, the Non-Indigenous Aquatic Nuisance Prevention and Control Act. In 1992, the Marine Board of the National Research Council began the "Assessment of Ship Operations [Ballast] Technologies for Controlling the Introductions of Non-Indigenous Organisms". The International Maritime Organization has created a Ballast Water Working Group with a mandate to develop international guidelines governing both ballast water exchange and the development of methods for treatment of ballast water. Treatment options include both shipboard and shore-based operations and a variety of potential treatments are currently under consideration. The principal categories of treatment under particular scrutiny are acoustics, biocides, deoxygenation, electric pulse/pulse plasma, filtration, magnetic, thermal, and ultraviolet light. Although many of these techniques are currently in use in water treatment, several are impracticable for shipboard operation. For example, the mechanical and energy requirements for heat sterilization of ballast water are substantial. Initial attempts to demonstrate solutions have, therefore, focused on what is both feasible and practical. A recent study by Battelle (1998) concluded that "UV treatment is currently the best suited for secondary treatment of ballast water."

Rationale for UltraViolet (UV) Radiation

The ability of ultraviolet (UV) radiation to inactivate water-borne microorganisms has made it an excellent choice as a disinfectant in the drinking water and wastewater treatment fields. Despite the fact that the potential for water treatment with UV radiation has been known since the beginning of the century, UV light as a practical means of treating ballast water has only recently received attention (Wright *et al.* 1997). The most important advantage UV has over other disinfectants is its ability to treat pathogenic organisms without the addition of chemicals that may produce toxic byproducts. In this way, treatment can be environmentally friendly and still remain cost effective.

One aspect of the toxicity of UV radiation involves photochemical damage to DNA within the cells of microorganisms. Nucleic acids in living cells absorb light in the UV wavelengths (240–300 nm), inducing dimerization of the thymine bases due to a C5, C6 doublebond breakage. This distorts the sugar-phosphate DNA backbone rendering the cell incapable of replicating (Liu *et al.* 1995; Water Environment Research Foundation 1995; Russo and Russo 1993; Skeldon 1991). This process dominates for smaller organisms and can be modeled as a photochemical reaction between light and the DNA molecule.

UV radiation has been proven effective against vegetative and sporous forms of bacteria (0.2-5.0 mm), viruses and bacteriophages (0.02-0.2 mm), and many other pathogenic microorganisms (Sobotka 1993). Some of these include *E. Coli* (Mechsner *et al.* 1991) *Legionella* sp. (Yamamoto *et al.* 1987; Liu *et al.* 1995) *Bacillus subtilis* (Sommer and Cabaj 1993) hepatitis A virus (Battigelli *et al.* 1993; Wiedenmann *et al.* 1993), Coxsackie virus B-5 (Battigelli *et al.* 1993), rotavirus strain SA-11 (Battigelli *et al.* 1993), and the F-specific RNA bacteriophages, MS2 and jX174 (Battigelli *et al.* 1993; Wiedenmann *et al.* 1993; Havelaar *et al.* 1991).

Since many microorganisms have the ability to reactivate or repair the DNA lesions caused by UV light over time (Russo and Russo 1993; Mechsner *et al.* 1991; Yamamoto *et al.* 1987) and there is no residual toxin in the system, the standard UV treatment system used on waste or drinking water produces a sufficient dose to cause the immediate death of the pathogen. Objectives for ballast water are similar but distinct. The larger organisms may require higher doses for sufficient photochemical effects to accumulate to produce an outright kill. For these organisms, photobiological effects, such as disruption of germinal organs, damage to eyes, or other effects, may provide a more efficient path to the objective of a ballast

TABLE 1. Relative sizes of microorganisms found in water

Microorganism	Approximate Size	Examples
Viruses	.02 – 1 mm	Hepatitis virus, 0.02 mm; HIV, 0.08 mm
Bacteria, cocci (spherical) and bacilli (rod-shaped)	0.25 – 5 mm	*Pseudomonas*, 0.5 – 0.62 mm *Vibrio cholerae*, 1 – 10 mm
Protozoans	1 – 80 mm	*Myxosporeans*, 5 – 30 mm Microsporidians, 1 – 10 mm
Fungi	1 – 100 mm	Aphanomyces
Cyanobacteria (blue-green algae)	0.2 – 2 mm	*Microcystis elebans*, 2-6 mm *Spirulina subsalsa*, 0.4-4 mm *Chroococcus limneticus*, 6-12 mm
Phytoplankton (includes diatoms, dinoflagllates, cryptomonads, macrophyte spores, and other pico-, nano-, micro-, and colonial phytoplankton)	2 mm – 2 mm	*Skelatomina*, 7-15 mm *Thalassiosira eccentrica*, 40-120 mm *Cryptomonas, pseudobaltica*, 18-30 mm *Chroomonas amphioxera*, 10-19 mm *Euglena proxima*, 18-25 mm *Pfiesteria*, 5-450 mm (cyst stage, 7-60 mm) *Gymnodinium* (red tide species), 20-25 mm *Gonyalaux* (red tide species), 28-43 mm
Zooplankton	30+ mm	Zebra mussel veligers, 30 – 65 mm Adult calanoid copepods, 1.6 – 12 mm Various crab and shrimp zoea, 5 mm Starfish (*Asterias rubens*) larvae, 2 mm
Fish eggs	0.5 – 5.0 mm	
Larval fish	2+ mm	

water control system–preventing the nonindigenous species from colonizing its new ecosystem.

The optimal light source for a ballast water control system is likely to be very different from the optimal system for drinking water treatment. In addition to larger target organisms, the presence of suspended particles, such as dirt and algae, will reduce the range of UV in water. Microorganisms may be shielded by these particles and, thus, not be affected by the radiation (Skeldon 1991; Taylor 1988). These obstacles could be prevented through a physical pretreatment process, such as filtration (Liu *et al.* 1995; Skeldon 1991; Taylor 1988).

In the Great Lakes Ballast Technology Demonstration Project supported by the Northeast-Midwest Institute and the Lake Carriers Association, initial emphasis has been placed on a physical filtration system. In this regard, a major practical consideration is the smallest particle size capable of being filtered at the very large flow rates involved with ballast water loading. Such a system can be regarded as a pre-treatment. Secondary treatment (like UV) must be capable of effectively dealing with particles too small for physical filtration and should include bacteria. The discovery of cholera in shellfish and fish in Mobile Bay, Alabama (McCarthy and Khamdaty 1994) was traced to ballast water in vessels that had recently visited South American ports. In advance of practical demonstration, it was generally felt that the lowest cut-off size for effective physical filtration is 100-200 mm. Recent results (see reports from Parsons and Cangelosi in these proceedings) suggest this value is between 25 and 50 micrometers. These encouraging results still require an effective secondary treatment since many pathogens are considerably smaller (see Table 1). *Vibrio cholerae*, for example, is typically 1-10 mm.

Preliminary experiments using a medium-pressure mercury lamp and notch filters indicated that monochromatic UV light was toxic to late-stage dressenid larvae. Pre-settlement larvae exposed to 254- and 280-nm light at 100 mWsec/cm^2 completed settlement, but subsequently all died. Control larvae had 100% survival (Wright *et al.* 1997). Recent developments

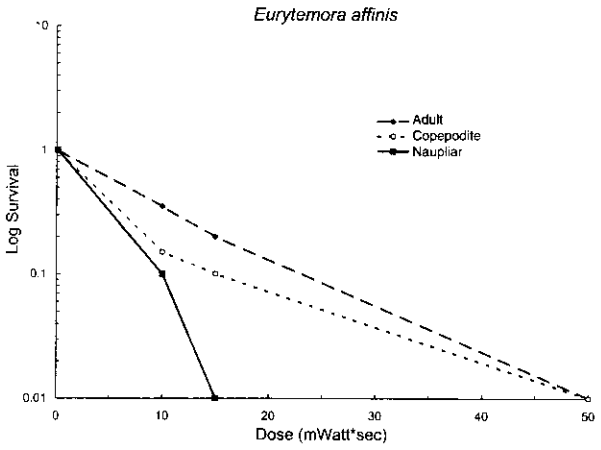

Figure 1. Dose-response relationship for copepods *(Eurytemora affinis)* exposed to excimer UV light.

in light sources suggest that a system tuned to a narrow spectral bandwidth optimized for ballast water is feasible. An excimer UV lamp currently manufactured by T^3I has been demonstrated to affect a three log removal of bacteria from industrial cooling fluids having the opacity of milk (Bissing *et al.* 1998).

Experimental Methods and Results

For the work described here, we have configured our experimental scale UV reactor to test the efficacy of excimer UV on aquatic organisms. In this system, test organisms are pumped through quartz tubing to triplicated exposure ports in the lamp housing. A fourth loop with no UV access serves as a control. Another test rig delivers 150 gallons per minute, either as a once-through pass or as part of a closed loop system. The once-through operation would simulate (at small scale) a ballast water loading operation, and the closed-loop would simulate a continuous treatment system that might be employed on board a vessel under way. Dose is a function of lamp output and exposure time (and is adjusted through flow rate). For the initial experiments presented here, the small-volume test stand was used in a once-through configuration.

The work reported here is a selection of data from a pilot project exploring the effectiveness of excimer UV light on a variety of planktonic organisms representative of those that might be entrained in an industrial intake or ballast water in an estuarine environment, such as the Chesapeake Bay. Some species that are not indigenous to the Chesapeake Bay have also been included, either because of ease of culture or because of their status as a nuisance species spread via ballast water.

Latent phototoxicity can be quantified only in terms of the biological effect achieved as a function of UV dose, the product of UV flux ($mWatt/cm^2$) and exposure time (sec), when organisms are held in culture over several days or through different developmental stages. The quantification of latent effects places a substantial burden on experimental controls. Tests were performed on a range of organisms including, but not limited to, zebra mussels (*Dreissena polymorpha*), grass shrimp (*Palaemonetes pugio*), copepods (*Eurytemora affinis*), *Artemia salina,* Sheepshead minnow larvae (*Cyprinodon variegatus*), and algae (*Chorella vulgaris*). Representative data are shown in Figures 1-4.

Copepods

Copepods were maintained in culture at the Chesapeake Biological Laboratory using ambient Patuxent River water adjusted to a salinity of 12 ppt. Cultures were fed regularly with *Isochrysis galbana* at densities of $>10^5$ cells/ml. Cultures were concentrated to exposure densities using 32-mm mesh Nitex sieves, sufficiently small to filter out all larval stages. Where needed, adults were filtered from the remaining culture using a 212-mm mesh sieve. Following irradiation, copepod suspensions were concentrated using 32-mm mesh sieves to 1/20th of their volume and larval counts made of each of three aliquots from each test replicate. Organisms were scored live or dead according to their response to physical stimulus, and numbers of live animals were compared with controls (see Figure 1).

Three different life stages were exposed to UV irradiation: stage 1-3 naupliar larvae (< 60mm), stage 1-3 copepodites (150-250 mm), and adult copepods (>500 mm). It can be seen that as the applied dose was increased, a stronger effect was measured. Similarly, higher doses produced a more acute effect. Differences in numbers of naupliar larvae between the controls and UV exposed samples represent the sum of naupliar mortality and reproductive delay or failure. That is, no new larvae hatched in the UV-exposed samples, suggesting an interruption of reproduction even at low doses.

Artemia salina

Artemia salina were commercially obtained cysts and were maintained in a dry condition until used. A

maximum of 5gm of *Artemia* was used in about 3 L of water. Water temperature was maintained between 25 and 30°C, and salinity between 5 and 15 ppt throughout the hatching period or for at least twenty-four hours. Light was maintained at about 2000 lux with constant aeration (without creation of foam) throughout the hatching period. The optimum pH for the hatching medium is between 7.5 and 8.5. After 24 hr, aeration was turned off and settling allowed for 5-10 min, because the empty shells will then float. Since *Artemia* nauplii are phototactic, one corner of the bottom of the incubating tank was illuminated to simplify separation of unhatched cysts and collection of nauplii. With an oven baster or a siphon, concentrated *Artemia* nauplii were siphoned out into the *Artemia* net bag or into a 37-um-mesh sieve, for proper separation. The nauplii in the bag were rinsed in a gentle stream of water. Figure 2 shows increased killing at higher UV doses. Latent effects are difficult to determine due to changes in the control samples.

Fish Larvae

Sheepshead minnow larvae (*Cyprinodon variegatus*) were collected from local estuarine sites. The minnows usually reach sexual maturity 3-5 mo after hatching, with standard lengths of about 27 mm for females and 34 mm for males. They were held at temperatures of 25-30°C, 20-30 indiv. in each rearing tank and fed Tetramin daily. When adults reached sexual maturity, they were kept in a temperature controlled system at 18-20°C. To initiate spawning, 75-80% of the water was changed and the temperature

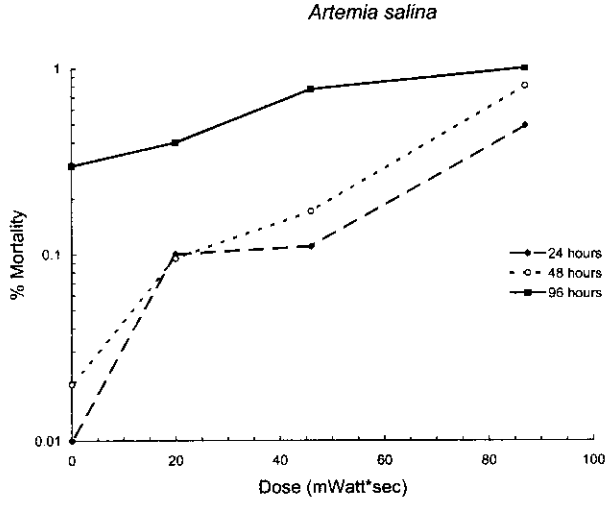

Figure 2. Dose-response relationship for brine shrimp *(Artemia salina)* exposed to excimer UV light.

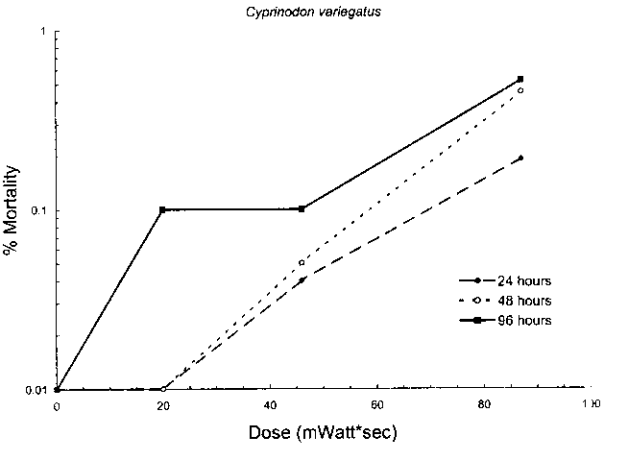

Figure 3. Dose-response relationship for sheepshead minnows *(Cyprinodon variegatus)* exposed to excimer UV light.

raised to 25°C with a photoperiod of 14-hr light and 10-hr dark. Adult females generally lay 10-30 eggs per spawn. To obtain embryos for a test, adult fish, generally five females and three males, were transferred to a 15-gal aquarium with the appropriate photoperiod, temperature, and salinity, 7-8 da before larval fish were needed. The spawning tank was fitted with spawning mats where the eggs were laid and adhered. Spawning usually began within 24 hr. Embryos spawned over a 24-hr period may hatch over a 72-hr period, so it is advisable to obtain eggs over several days to ensure that a sufficient number of newly-hatched (<24 hr) larvae will be available to initiate a test. When the mats contained a sufficient number of eggs, they were removed from the tank, rinsed in high-saline water with methylene blue (to prevent fungal infections), and incubated for 6-7 da at 25°C with gentle aeration. Water was changed daily. Approximately 24 hr before hatching, the salinity of the seawater was changed to that of the test salinity. Water chemistry was monitored over the hatching period. Larvae were fed newly hatched *Artemia* daily. The size of the minnows irradiated ranged from 0.4 to 0.9 cm. Higher doses were required for killing larger organisms than was needed for smaller organisms. Yet, even for these large organisms, a strong latent effect was observed for moderate doses (Figure 3).

Algae

The estuarine alga *Chlorella vulgaris* was cultured at the Chesapeake Biological Laboratory culture facility from in-house stocks. These were grown up as a 1L culture in sterilized filtered (16 ppt) water fortified with f/2 nutrient media. The culture was diluted to

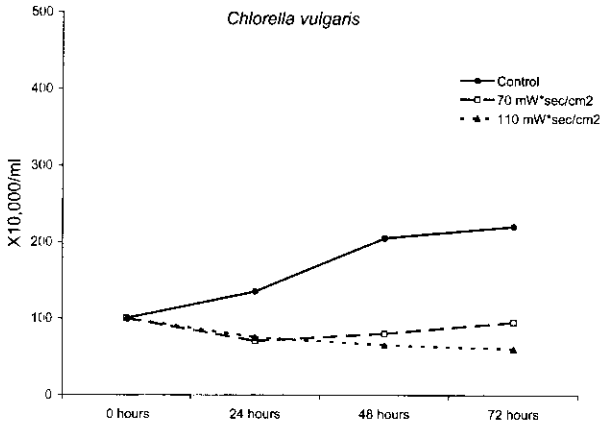

Figure 4. Dose-response relationship for green alga *(Chlorella vulgaris)* exposed to excimer UV light.

5L with filtered estuarine water (16-ppt salinity) prior to the experiments. The approximate starting cell density was 2 x 10^6 cells per ml. Following the exposure treatments, each 600-ml glass beaker containing 400 ml *Chlorella* culture was allowed to grow under continuous fluorescent light. At daily intervals, samples were taken for cell counting and microscopical examination, extraction of chlorophyll pigments with acetone, and direct *in vivo* chlorophyll fluorescence determination. The results of all three monitoring methods were consistent. The direct cell counts show significant inhibition of growth at doses of 70 mWsec/cm^2 and 110 mWsec/cm^2 relative to the control. The 70 mWsec/cm^2 treatment, however, showed signs of recovery after 48 and 72 hr (Figure 4). The total extractable chlorophyll reflected this inhibition only at the highest dose, indicating that the chloroplasts at the 70 mWsec/cm^2 treatment level remained viable. The simplest method, *in vivo* fluorescence measurements, corresponded well with total extractable chlorophyll and provides a facile approach to monitoring the effects of UV light on phytoplankton chlorophyll production. This technique may be used in future studies as a biological actinometer of UV flux or could be developed into a rapid evaluation technique analogous to the measurement of total coliforms used to benchmark drinking water disinfection.

Summary

We have initiated an investigation on the latent and acute phototoxicity of UV radiation on organisms typically found in ballast waters. The current data suggest that organism size and morphology can affect the required UV doses. The doses required to kill larger crustaceans and fish may be an indication of photoprotection provided by carapace pigments and the greater mass of integument in larger organisms. There are also qualitative data suggesting that biological response is affected by the intensity, wavelength, and integrated dose of UV. The monochromatic output of excimer lamps can be tuned to optimize the efficiency of these processes. Latent phototoxicity (at 96 hr post irradiation) has been demonstrated at two-orders-of-magnitude lower doses than is required for immediate mortality.

Acknowledgements

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Progress in the Management and Treatment of Ship's Ballast Water to Minimize the Risks of Translocating Harmful Nonindigenous Aquatic Organisms

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ABSTRACT: **Billions of organisms are transported around the world in some 10 billion mt of ballast water that are carried annually on ships. An increasing number of these organisms are becoming established in new locations, resulting in significant ecological damage worldwide as well as posing a threat to human health. A range of ballast water management guidelines and regulatory practices have been introduced by various countries in an attempt to minimize the risks of establishment from these inoculations. The International Maritime Organization is developing a set of international regulations, the use of which is likely to enter into force early in the new century. A range of potential ballast water treatment options have been suggested to kill biota or minimize the threat of water discharges; however, it is unlikely that a single treatment process will be developed that will eliminate all unwanted organisms in ballast water. Rather, a range of options appropriate for each particular ship and voyage, aimed at minimizing the risk, will emerge. Ocean exchange is currently the primary option that is being used. While this process can be effective in many cases, there are limitations which are likely to restrict its universal use. Ballast water heating utilizing waste engine heat has been demonstrated to be effective for killing many organisms and shows considerable promise for the future. A shipboard demonstration of filtration and testing of a hydrocyclone/ultraviolet irradiation system are in progress and will provide data to assess the feasibility of these options. A range of other biocidal and chemical processes have been or are being tested; however, the majority of these have been rejected at this stage on cost, effectiveness, practicality, or environmental grounds. Research and demonstration aimed at assessing these and other options are continuing**

Key words: ballast water, harmful aquatic organism, treatment, exchange, water heating

INTRODUCTION

Countless billions of marine organisms are transported around the world in the 10 billion mt of ballast water carried annually on ships to maintain their safety and stability at sea. Quantities and number of discharges continue to increase in major ports around the world with the use of larger ships, shorter transit times, and increases in world trade. The likelihood of these organisms surviving the voyage and becoming established in a particular port depends on many factors, including the types and nature of organisms, length of voyage, and environmental conditions at the ballasting and deballasting ports.

The consequences of nonindigenous organisms becoming established in a particular area can be quite devastating and have resulted in significant ecological and environmental damage worldwide as well as posing a threat to human health. The possible link between ballast water and dispersal of marine organisms was first suggested by Ostenfield (1908) and Peters (1933). However, interest in ballast water discharges as a global environmental cause for concern has gained considerable impetus over the last decade with the documented establishment of a number of nonindigenous harmful aquatic organisms around the world.

Perhaps the most publicized of these is the zebra mussel (*Dreissena polymorpha*) in North America. First discovered in Lake St Clair in 1988 (Griffiths *et al.* 1991; Johnson and Padilla 1996), it has now become established in more than 50% of the waterways in the United States and is estimated to cost

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some US$500 million annually in nuisance costs resulting from blocking of power plant water intakes and water treatment, as well as fouling of fishing nets, boat hulls and buoys (Weathers and Reeves 1996). The toxic dinoflagellate, *Gymnodinium catenatum*, strongly linked to ballast water discharges, became established in the Derwent and Huon estuaries of Tasmania, Australia in 1972 coincident with the establishment of a new woodchip mill. This species has been responsible for the regular closure of shellfish beds to harvesting as a result of high Paralytic Shellfish Poisoning (PSP) toxin levels (Hallegraeff and Bolch 1992; McMinn *et al.* 1997).

Ballast water has been suggested as the vector for the introduction of the American Atlantic comb jelly, *Mnemiopsis leidyi*, in the Black Sea in the 1980s (Vinogradov *et al.* 1989). Since 1990, it has been responsible for a huge decline in commercial fisheries in the Black Sea where it has affected anchovy, sprat, and horse mackerel fisheries and also in the Sea of Asov, where anchovy and Asov kilka catches have been severely reduced (Vinogradov *et al.* 1996). It has now spread to the eastern Mediterranean Sea (Kideys and Neirmann 1994). Recent studies have suggested that cholera introduced in ballast water could present a health risk in some areas (McCarthy and Khambaty 1994). In particular, the survival of cholera by association with algal blooms and colonization of the chitinaceous exoskeletons of some marine organisms suggests that the role of ballast water in this case may be associated with the transport of intermediate hosts in addition to free cholera cells (Epstein 1993; Colwell and Spira 1992).

Carlton *et al.* (1995) have estimated that more than 3000 species are transported around the world by ship each day, and some 40 recent invasions have been mediated by ballast water (Carlton and Geller 1993). It is important to note that although significant emphasis has been placed on ballast water in relation to the translocation and establishment of unwanted aquatic organisms, other vectors such as hull fouling on ships and pleasure craft as well as organisms carried in oysters and fish for the aquarium trade may play a significant role.

As a result of these biological invasions, a range of ballast water management guidelines and regulatory practices have been introduced by various countries in an attempt to minimize the risks of new species becoming established. At the international level, the International Maritime Organisation (IMO) through its Maritime Environmental Protection Committee (MEPC) has been developing draft regulations with a target date for the adoption of a mandatory international instrument for ballast water management early in the new century.

These guidelines include a number of recommended management and treatment procedures that need to be considered by the ship's Master in order to comply with the requirements prior to discharging ballast. Not all of these options are suitable or appropriate for use on all ships, and some are still being tested and developed. Research and development programs have been established in Australia, New Zealand, the United States, Germany, Canada, Japan, Israel, Ireland, Wales, Sweden, and Norway to investigate introductions and to support these regulatory efforts by testing the efficacy of various options and to identify and test appropriate alternatives.

This paper reviews the suite of management and treatment options that have been considered or are being utilized or developed for potential implementation both locally and at the international level.

Management, Treatment and Target Species–Some Considerations

A single practical, safe, environmentally acceptable, and cost-effective solution that will kill all living organisms, or those that could potentially germinate from material present in all ballast water contained in the wide range of ship's tanks, is unlikely to ever become a reality. Rather, an overall strategy based on a range of management and treatment options to minimize the likely risks of organisms becoming established (once discharged) is currently and is likely to remain the generally accepted approach. This is certainly the approach that is being developed in Australia and several other countries as well as forming the current basis for the IMO regulations.

Another aspect relates to the types of organisms at which the control measures are aimed. Without some identification of particular species of concern, it is difficult to assess the likely efficacy of any ballast treatment option. The Australian approach has focused on a number of target species. The initial list of target species was developed to include a number of "model organisms" that were thought to have been introduced via ballast water into specific locations with significant environmental, aquaculture. or human health consequences, and had the potential

to become established in other ports if some control measures were not taken.

One particular model organism selected for the early research work was the toxic dinoflagellate, *G. catenatum*, which has resulted in the establishment of a costly toxin monitoring program in Tasmania to protect human health and to identify closure periods for harvesting of affected shellfish. *G. catenatum* produces resting cysts that can survive in sediments for many years and become established when appropriate conditions exist. Hence, it was considered that a study of its behavior under ballast tank conditions and various ways of killing both the motile and cyst forms of this organism would also provide a reasonable basis for assessing likely effectiveness of various treatment options on a number of other organisms.

Some researchers and countries do not consider that a targeted organisms approach is appropriate, since one ship alone can discharge many hundreds of different species that have the potential to cause problems if they become established. No doubt this debate will continue, but without some basis for testing and comparing the effectiveness of various treatment options, or for assessing compliance, it will be difficult to progress beyond accepting treatments that offer complete sterilization. Target lists can be changed from time to time to take into account changing conditions or observed introductions from other locations. It is interesting to note that *de facto* target species are sometimes used as a basis to handle a specific problem, even though the overall approach to management is regarded as non-target oriented. Examples include the use of salinity increases by flushing with ocean water in North America to minimize the risk of new *D. polymorpha* introductions and the banning of ballast discharges in New Zealand ports for ships arriving from specified Australian ports (namely Port Philip Bay in Victoria and Tasmanian ports) because of concerns over possible introductions of *Asterias amurensis*.

In general, the risk-based approach seeks to assess the potential risk of one or more organisms becoming established within a port as a result of the water discharged. If the risk is below an identified level, the ship would not be required to undertake any treatment. For example, in a port where *A. amurensis* did not exist and its establishment would create major problems, there would be little risk involved for a ship discharging ballast water from ballast taken on in a port where this organism is known to be definitely absent. This is a very simplified case since, for example, the ship could contain residual sediments from a previous port where the organism is prolific.

There are several types of risks that need to be considered in assessing the overall risk of a particular organism becoming established. In addition to the biological risk (which is based on a multitude of factors related to organism presence, uptake, survival, discharge, establishment and impact), other risks such as social and management need to be taken into account. In Australia, a Decision Support System (DSS) based on a combination of all of these risks is at present being developed and will be used by the Australian Quarantine and Inspection Service (AQIS) in managing and implementing their ongoing ballast water program (Hayes and Hewitt 1998). This basic approach, albeit in a very basic form, has been used by AQIS for several years in implementing and managing its voluntary guidelines.

Initially the DSS will be implemented at a low level of sophistication since only limited information on input data such as biological presence and survivability will be available. However, the models being developed allow for the level of accuracy to be improved as further research and monitoring results become available.

Management Options

For any form of voluntary or mandatory ballast water controls to be acceptable, it is essential that these controls involve management and treatment options that are

- safe
- technically effective in killing the organism(s) of concern,
- cost effective
- environmentally acceptable, and
- practical.

In the past, the handling of ballast water has been essentially based on the need to achieve loading and unloading of cargoes in the most expeditious and safe way (consistent with a pre- established ballasting/deballasting plan) with little regard for the local environmental conditions. As a result of observations and research over the past decade, a series of precautionary management practices have been developed to assist in minimizing the risks of organism invasion (Carlton *et al.* 1995; AQIS 1998; Rigby 1994). Attention to these precautionary management practices during ballasting or deballasting in some

cases may provide a proactive approach that will be much simpler and more cost effective than one of the treatment options.

In principle, these techniques are aimed at minimizing the risks of the uptake of organisms, thereby reducing the quantity discharged and the probability of survival and establishment. Some of the main options that have been suggested are briefly outlined below.

Minimization of ballasting during presence of target species

Some organisms proliferate in a particular location at specific times, and avoidance of ballasting at these crucial times can minimize the number of organisms taken into ballast tanks. For example, clearly visible blooms of toxic algae could be avoided (Hallegraeff and Bolch 1992). These blooms are often limited to relatively short periods, especially during crucial periods when, for example, permanent resting cysts are present in the water column.

Minimization of ballasting at night

Many benthic species rise in the water column at night (Carlton *et al.* 1995) therefore avoidance of ballasting at these times may be beneficial.

Minimization of ballasting in areas where sewer and industrial discharges occurs

Human pathogens may be discharged in some port locations where ballasting takes place.

Minimization of ballasting in global hot spots

This approach, suggested by Carlton *et al.* (1995) is similar to the first option and suggests that it may be possible to identify (via an international advisory network) regions where ballast water ought not to be taken on or where hot spots specific to a particular species exist.

Minimization of sediment uptake in shallow ports or dredging areas

Sediments present in ballast water during ballasting can present a problem in some ships, as they may settle in some of the ballast tanks and provide a habitat for organisms. Many large bulk carriers ballast in deep ports and this is less of a problem except perhaps in periods of high rainfall. However, it can be a cause for concern in shallower ports with minimum under-keel clearance. There are many aspects of sediment behavior that are not well understood, and further work is required to identify more clearly the true role that sediments play in the translocation of organisms. In some cases, although sediment may be present in ballast tanks, it is not necessarily discharged at the time of deballasting. Suctioning from high in the water column may assist in minimizing sediment uptake in some locations (Taylor 1996).

Confinement of ballast to specific tanks

Some ships have the capability of retaining all or part of the ballast water in non dedicated tanks that can be later discharged at sea when alternative water is taken into other tanks (Rigby 1994).

The practicality and effectiveness of some of these management options are quite limited as the ship's Master often has little scope to vary ballasting times or patterns, as this needs to be synchronized with unloading schedules. It is also sometimes necessary to ballast and deballast within the same port, so the ability to select locations and times is again limited. Some ship's schedules change on leaving a particular port, and the ship eventually ends up in a port different from that originally planned. These types of changes may have a bearing, in particular, on the effectiveness of the strategy of avoiding hot spots.

Combined management strategies

A combination of one or more of the above options, together with a number of other procedures, has formed the basis for overall integrated management strategies that have been suggested or included in guidelines introduced by various countries. The AQIS guidelines (AQIS 1998), for example, suggest that a number of the above options be adopted (wherever appropriate) in addition to the use of ballast exchange or some other treatment option and a consideration of the risk associated with discharging the ballast water in the deballasting port. The latter should take into account the conditions existing in the ballasting and deballasting ports for the target organisms of interest. The computerized DSS currently under development by AQIS will take all of these components (together with a more detailed assessment of biological, social, and management risks) into account. Carlton *et al.* (1995) have have recommended a series of procedures based on consideration of the above precautions together with

considerations of ballast exchange at sea, backup zones for vessels that have been unable to exchange at sea, some form of risk assessment, and quarantine procedures for vessels identified as having high risk.

Treatment Options

A number of treatment options have been suggested as potential candidates either to completely kill or to significantly reduce the total number of organisms or the number of species present in the ballast water (Carlton 1990; Rigby *et al.* 1991; Rigby 1995; NRC 1996). These suggested treatments in most cases are essentially based on technologies or processes currently in use for industrial or domestic water treatment, and may not be effective or appropriate for treatment of ballast water. Only limited laboratory or ship-based trials have been undertaken to assess their effectiveness.

As distinct from some of the conventional processes, which are carried out in purpose-built equipment where design and operating conditions can be closely controlled, effective treatment of all the ballast water on a ship presents a range of differing problems. A typical "Cape Size" bulk carrier, such as the BHP-owned *Iron Whyalla*, with a loaded deadweight of 141,475 mt carries some 50,000 mt of ballast water in 10 sets of topside and double-bottom tanks as well as a forepeak and afterpeak tank. Indeed, each of the double-bottom tanks contains some 50 or so separate compartments (each open to the adjacent compartment for access and water flow); the whole ship contains many hundred small separate compartments.

Ballasting often takes place with both ballast pumps operating at a combined flowrate in the vicinity of 4,000 mt hr^{-1}. The internal construction of the tanks is complex, with a range of longitudinal, stiffener, and side-frame steel sections to maintain the ship's strength. The tanks are suitably placed to maintain the stability of the ship. The consequence of this variety of tanks and structural and ballast water piping arrangements is that access to individual tanks and the ability to undertake specific treatment options in a controlled manner may be limited. Treatment processes can potentially be undertaken during ballasting, during the voyage, or while the ship is deballasting.

Table 1 lists the main potential treatment options that have been suggested. For the purposes of this discussion, ballast water exchange options have been included in the treatment options rather than in the management options, as the process does involve a change in the contents and composition of the water in the tanks. Special attention will be given to those options that are currently in use, have the most potential to be adopted for widespread use, or are being tested or demonstrated at present.

Table 1. Possible options for ballast water treatment

Physical	Biocidal chemical or other
	Ultraviolet irradiation
Ballast water exchange/ salinity increase	Ultrasound
	Electrical discharge
Heating/exchange	Oxygen deprivation
Filtration/separation	Chemicals
	ozone
	chlorination
	organic acids
	copper/silver systems

Ballast water exchange

The exchange of original ballast water with ocean water in some form or other serves as the basis of control measures being utilized by several countries and is currently the only treatment option being used by the shipping industry. The basis of this form of treatment is that water from the deep ocean (generally considered to be free of the organisms of concern) is exchanged for the original water taken on during ballasting. The near-surface-dwelling organisms of the deep ocean form a group quite distinct from those organisms living in coastal waters where ballast water is first taken on (Carlton 1990).

In addition to exchanging all or part of the original water and organisms, this option can be effective as a natural biocide by increasing salinity levels in brackish waters to a point where some fresh water species are not able to survive. This form of treatment is the basis of the exchange controls on ships entering the St. Lawrence Seaway in North America in an attempt to control the spread of *D. polymorpha*.

The effectiveness of ballast exchange in replacing the original water and organisms will depend on the efficiency of the exchange, together with the exchange considered necessary to have the desired effect. For example, an increase in salinity will not generally require complete exchange of the ballasted

Table 2. Efficiency of water exchange for various ocean exchange options.

Option	Mode of Exchange	% Water Exchanged	Indicative cost[2] cents/mt	Reference
1	Continuous flushing, 0.5 tank volume	39.3	0.8	Rigby & Hallegraeff 1994
2	Continuous flushing, 1 tank volume	63.2	1.5	" "
3	Continuous flushing, 2 tank volumes	86.5	3.1	" "
4	Continuous flushing, 3 tank volumes	95.0	4.6	" "
5	Continuous flushing, 4 tank volumes	98.2	6.1	" "
6	Empty / refill(10 mt residual per db tank)	99.8	1.5	Calculated
6a	(calculated for 50 mt residual water)	99.2	1.5	
7	Non - dedicated tanks empty /refill	100	1.5	Rigby 1994
8	Empty/refill	95.0	1.4	Miller 1998
9	Dilution/flushing	90.0		IMO MEPC1996 1998
10	Sequential empty / refill	>99		Wonham *et al.* 1996
11	Continuous flushing / heating	>99	≈4.5	Rigby, Hallegraeff and Sutton 1998
12	Ocean exchange for salinity increase of brackish water;			
	(ocean 35‰, brackish 15‰)	75	1.2 (empty / fill) 1.8 (flush)	
12a	(ocean 35‰, brackish 5‰)	83	1.3 (empty / fill) 2.4 (flush)	

[2] based on a typical Cape Size bulk carrier carrying 50,000 mt ballast water (fuel costs estimated for diesel fuel 35c/l) assumed no gravity filling/emptying.

water (see Table 2, options 12, 12a), whereas the elimination of all original organisms would require complete replacement of all of the ballasted water (and sediments).

Two basic options exist for ocean exchange as shown in Figure 1 (Rigby 1994; Rigby and Taylor 1994). Reballasting is an effective way of replacing the original water with fresh ocean water. The efficiency of displacement will depend on the design of the ship's ballast tanks, quantity of water pumped and the pumping system design. Typically, this efficiency will vary in practice from 90-99.8% (Table 2, Figure 2). The lower efficiencies result from higher quantities of water remaining in the tanks after emptying. In many ships, such as the *Iron Whyalla*, secondary stripping using an ejector system can reduce this quantity to quite low amounts.

One of the potential hazards associated with this mode of exchange is that safe limits of bending moments and stresses on the ship can be exceeded in some cases (Rigby and Hallegraeff 1994). A sequential procedure where tanks are emptied and refilled in a defined sequence can assist in minimizing excessive stresses and bending moments (AQIS 1993a). It is essential that any of the ocean-exchange options be undertaken only if safe conditions can be maintained. Ultimate responsibility for the safety of the ship resides at all times with the ship's Master, and use of procedures where the safety of the ship and its crew may be affected should be approved by the Classification Society and the Flag State Administration.

Ballast exchange, or continuous flushing, avoids the problem of exceeding safe bending moments or stresses, since the tanks remain full at all times. The efficiency of water exchange of this option depends on the number of tank volumes exchanged (Rigby and Hallegraeff 1994; Table 2, Figure 2). Typically, an exchange equivalent to 1 tank volume will result in approximately 63% of the original water being replaced, whereas an exchange equivalent to 3 tank volumes will replace approximately 95% of the original water (Table 2, Figure 2). The efficiency of water exchange using this technique is lower if the ship is not at sea (for example, in a port), as the mixing is less efficient (Rigby and Hallegraeff 1994). Reballasting compared to ballast exchange is generally more cost effective and can achieve a higher level of original ballast replacement in a shorter time. However, safety restraints may often dictate use of

the ocean exchange option. A combination of both reballasting and ballast exchange may be appropriate for some ships, especially where ship stresses are of concern when emptying some specific tanks (Rigby and Hallegraeff 1994).

It is noted that not all ships will be able to carry out the ballast exchange option as there may be no provision on the ship for overflow of the water. Minor modification to facilitate this option in new ships would not be of serious concern in new ship designs. Modifications to the basic flushing arrangements originally suggested by Rigby and Hallegraeff (1994) using alternative piping arrangements are possible (Armstrong 1997; IMO MEPC 1996, 1998), and may result in slightly different efficiencies of exchange. It needs to be pointed out, however, that differences in ballast tank, piping, and pumping designs are likely to have a much bigger influence on the efficiency than the superficial improvements in efficiency that are likely to result from these modifications.

An interesting option, currently being practiced by one shipping company, involves a 120,000-dwt bulk carrier and the use of dedicated and non-dedicated tanks to completely eliminate the original ballast water (Rigby 1994). In this case, the original ballast water (35,000 mt) is taken on in the Nos. 2, 3, 5, 7, and forepeak and afterpeak tanks. Once at sea, in an appropriate area, tanks Nos. 1, 4, and 6 are filled while the original tanks are emptied sequentially. The overall result is that none of the original water is discharged in the receiving port (Table 2, Figure 2, option 7), thus giving a 100% solution to possible organism translocation. Once again it needs to be stressed that this option is only likely to be possible on a limited number of ships.

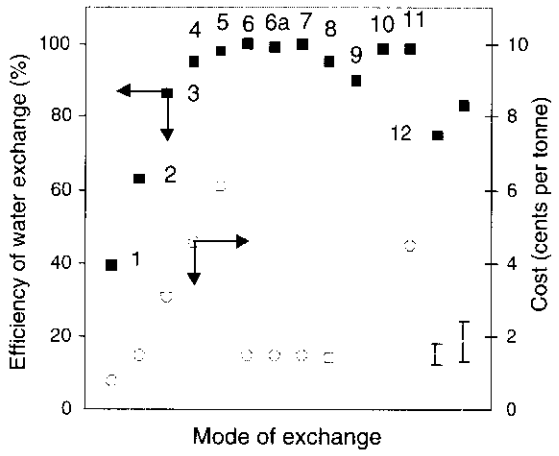

Figure 2. Efficiency and cost of ocean exchange for various options.

The operating cost of carrying out ballast exchange is essentially that of the additional fuel required to operate the generators and pumps over the period of exchange. Table 2 and Figure 2 show some indicative fuel costs based on a typical Cape Size bulk carrier carrying 50,000 mt of ballast water), assuming a cost of 35¢/liter. Additional pump maintenance costs for the period have not been included. These costs do not take into account any additional costs that might be incurred by personnel working outside normal hours or for delays that could be involved if the ship was required to carry out exchange other than during its regular voyage route. These costs can be very substantial and

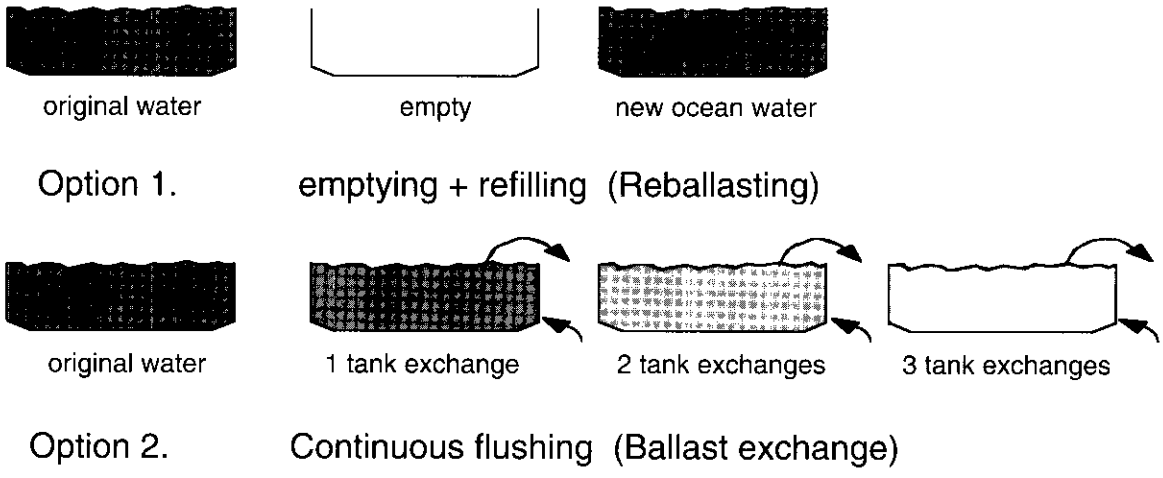

Figure 1. Basic options for ocean exchange of ballast water.

need to be borne in mind when developing overall strategies.

The efficiency of removal of organisms as distinct from ballast water is a complex issue, which will be affected by the nature and behavior of organisms in the tanks, the design of tanks, mixing within the tanks, and the types and behavior of sediments. For example, some fast-swimming zooplankton could feasibly remain after several tank volumes had been replaced, as indicated by recent sampling studies on the *Iron Whyalla* (Sutton *et al.* 1998).

Shipboard-based microscopic examination of organism removal during ballast exchange and heating trials on the *Iron Whyalla* showed that the efficiency of removal of phytoplankton from the water was similar or higher than for water exchange (Rigby and Hallegraeff 1994; Rigby *et al.* 1996b). Flushing trials by Ruiz and Hines (1997) in wing tanks showed a 60% water exchange on the basis of salinity and less than 90% on the basis of coastal plankton communities. In another trial, the exchange of 3 tank volumes resulted in water exchange efficiencies of 70-100% on the basis of salinity and greater than 95% on the basis of the presence of coastal organisms. In a recent trial undertaken by Brazil on the product carrier M/V *Lauras*, a water exchange efficiency of 90% was achieved with a phytoplankton exchange of 96%. Chlorophyll *a* exchange was estimated as 86% (IMO MEPC 1998). Many other studies showing survival of organisms have been reported following ballast exchange in some form or other; however, in many cases there has been no quantitative assessment of actual ballast exchange and so it is difficult to compare differences between water exchange and biological exchange. For example, Locke *et al.* (1993), using freshwater zooplankton and salinity as indicators (for ships originating from fresh and brackish water ports), estimated that for 24 vessels entering the Great Lakes region the efficiency of zooplankton exchange was 67% and for water exchange was 86%.

The significance and role of sediments in relation to ballast exchange has been poorly researched. Analyses of sediments removed from tanks have identified the presence of harmful organisms. For example Hallegraeff and Bolch (1992) have reported the presence of toxic dinoflagellate resting spores in various types of sediments. It is feasible for sediments to remain in ships for many years. However, in many ships sediment is confined to areas well away from locations where the main flow of water occurs and hence may not play a significant role in organism dispersal. Work currently in progress is aimed at examining some of these effects in more detail (Rigby *et al.* 1999a).

While ballast exchange is currently the primary option available to ship's Masters for treatment of ballast water, there are some limitations and potential disadvantages as far as organism translocation is concerned. For example, MacDonald and Davidson (1995) found that reported ballast exchange increased the diversity of diatoms and dinoflagellate species during trips from mainland Europe to Scotland. These observations illustrate that caution needs to be exercised in assessing the most appropriate management or treatment option that might be used. Given the fact that many organisms do not survive on international voyages (Rigby and Hallegraeff 1994), it might be more appropriate in some cases to rely on natural mortality as a means of minimizing the risks rather than introducing a new community via ballast exchange.

Ballast water heating and flushing

The potential for inactivating toxic dinoflagellate cysts and killing other organisms in ballast water by heating has attracted recent interest as an environmentally responsible and potentially cost-effective treatment option. Initial laboratory work (Bolch and Hallegraeff 1993) indicated that heating *G. catenatum* dinoflagellate cysts to temperatures of 40-45°C for very short periods of time (30-90 sec) resulted in death. Lower temperatures at these short treatment times were less effective in killing the organisms.

Waste heat from a ship's main engine, which is currently pumped overboard as a waste product, can potentially provide a cost-effective source of heat. However, if this option is to be used, heating needs to take place during transit, as the ship's engine is not usually in operation during ballasting or deballasting. An analysis of available heat on the *Iron Whyalla*, indicated that the most appropriate means of utilizing this heat would be to flush the rejected hot ocean water (approx. 42°C) through the ballast water tanks in sequence, allowing the excess water to overflow from the ballast tanks. In addition to heating the ballast water, the flushing effect of the hot water would also significantly reduce the amount of originally ballasted water (and organisms) present at the end of the heating/flushing period.

A preliminary biological examination of this oceanic water heated by the engine's cooling system on a voyage on the *Iron Whyalla* (Rigby and Hallegraeff 1994) showed that no phytoplankton or zooplankton survived, suggesting that treatment in this manner had potential. However, without significant modification to the main ship's engine and waste-heat-recovery systems, there is insufficient heat available to reach a uniform ballast water temperature of 40°C in all tanks. Temperatures in the vicinity of 35-38°C are likely to be possible (after 24 to 30 hr flushing of each tank) on a voyage from Japan to Australia.

On the basis of these data, a series of further experimental tests (Rigby 1994) identified that most phytoplankton algae tested including *Skeletonema costatum*, dinoflagellates *Amphidinium carterae*, *G. catenatum* and *Alexandrium catenella*, and the golden-brown flagellate *Heterosigma akashiwo*, tested in the vegetative stage could be readily killed at temperatures as low as 35°C and treatment times in the range of 30 minutes to several hours. In addition, significant mortality was also achieved with *G. catenatum* and *A. catenella* cysts using longer incubation times (several hours) at temperatures as low as 35 to 37.5°C, with total mortality achieved at 38°C after 4.5 hr.

In order to test the practicality and effectiveness of this treatment option, two shipboard trials on the *Iron Whyalla* were undertaken between Port Kembla in New South Wales and Port Hedland in Western Australia and between Mizushima in Japan and Port Hedland. It was necessary to install some additional piping and valves to enable these trials to proceed.

On-board microscopic observation of heated water samples (Rigby *et al.* 1999b) showed that none of the zooplankton (mainly chaetognaths and copepods) and only very limited original phytoplankton (mainly dinoflagellates) survived the heat treatment. The original organisms were reduced to flocculent amorphous detritus. Subsequent culturing efforts on the heated ballast-tank samples produced growth of only some small (5 µm) diatoms and colorless ciliates that are considered to be of little consequence. Although no toxic dinoflagellate cysts were present in the tanks, based on earlier laboratory experiments, it is probable that these would have been effectively killed by the temperatures achieved during the heating trial, since essentially all of the water reached 37-38°C.

In addition to the effects of heating, this approach is also very effective in exchanging the original ballast water at the same time. Observation showed that 90-99% of the original plankton taken on during ballasting was removed by flushing (Table 2, Figure 2, option 11).

The temperatures reached during these trials would be insufficient to kill bacteria such as cholera. It is possible to heat ballast water to higher temperatures, for example, by heat exchange with the high-temperature piston-cooling-water circuit, by injection of steam, or by heat exchange with hot flue gases (Edyvean and Snedden 1985; Sobol 1995). However, these options require circulation of water to and from the ballast tanks during the voyage, can potentially cause operational problems or lack the amount of energy necessary to heat all the water, and are therefore less attractive than the flushing option. Nevertheless, future ship designs could consider some of these options.

Heating of ballast water as described above also has the added advantage that organisms contained in sediments would also be subjected to these temperatures (in fact higher temperatures are experienced at the bottom of the tanks where the ballast water is pumped into the base of the tanks).

The heating/flushing option is best suited for international voyages where there is adequate time to heat all of the tanks. It would not be possible to do this on a short coastal voyage.

Very few ships would be able to undertake this treatment option without some modification to the ballasting piping arrangement. Nevertheless, the option could ultimately provide one of the more attractive options for future ballast water management in order to comply with international requirements.

Filtration and Hydrocyclones

Filtration of ballast water to separate unwanted organisms is an option that has attracted some interest, especially in the United States where a large-scale shipboard trial on the bulk carrier M/V *Algonorth* has been in progress for some time.

Many of the organisms of concern in ballast water could be removed by filtration. Filtration at around 50 µm would be effective for the removal of most of the zooplankton and 20-µm filters would remove dinoflagellate cysts. Filtration of sea water is used routinely in many applications, including off-

shore oil platforms. Problems have been experienced in these operations with filter materials becoming coated with lipids and mucilage from plankton (Edyvean and Snedden 1985). Recent improvements with filter technology involving continuously cleaning and backflushing filters have reportedly overcome some of these problems. Apart from the cost and technical performance of the filtration system, there are several other issues that need to be considered. These include, for example, the storage and handling of the concentrated sludge and the significance of sediments that may exist in the ballast tanks.

The *Algonorth* trial involves a deck-mounted containerized filtration test unit that treats ballast water held in one of the upper wing tanks which has a capacity of 220 m^3. Multi-level filtration using different size filters is being investigated at water flowrates up to 400 mt per hr. The project involves both biological and mechanical testing protocols. Although detailed information on the costs of filtration will depend on the results of the trial, indicative increased freight costs between US 2-20 cents/mt have been suggested (Parsons *et al.* 1997).

Hydrocyclones have also been proposed as a means of separating organisms from ballast water. Initial work by Jelmert (A. Jelmert, pers. comm.) using a "Lakos" hydrocyclone, and more recently in conjunction with a Norwegian hydrocyclone manufacturer who has developed a new concept, has demonstrated an 80% removal efficiency at 7µm. It has been proposed that the concept would include an ultraviolet irradiation treatment step after the hydrocyclone. Further testing of the biological capacity and removal efficiency of the combined system under development is in progress.

Ultraviolet Irradiation

Ultraviolet (UV) radiation is effective in destroying a range of microorganisms and is used routinely in the treatment of industrial water. However, its application for removing or inactivating many of the higher organisms and cyst stages of protozoa, microalgae, and macroalgae of interest in ballast water is yet to be demonstrated. UV radiation was shown to be ineffective for inactivation of six species of dinoflagellate cysts (Montani *et al.* 1995). These experiments involved placing the organisms under a lamp for a measured period but did not measure the light intensity. Oemcke (1998a) demonstrated that UV can be effective for inactivation of the cyst-producing dinoflagellate *Amphidinium* sp. Photoreactivation has been shown to be disabled by storage in ballast tanks after irradiation, and elevated temperatures increase the inactivation. Irradiation and dark storage were shown to be effective for disinfection of *G. catenatum* vegetative cells, but initial tests with cysts indicate that inactivation will be much more difficult (Oemcke 1998b).

Some of the issues that need to be addressed in assessing the potential use of UV irradiation for long-term ballast treatment include fouling of the UV quartz sleeve and the attenuating effect of sediments in the water (probably requiring pre-treatment by filtration at 20-30 µm). Oemcke and van Leeuwen (1998) have recommended a pilot-scale demonstration of combined filtration and UV irradiation so that the large-scale feasibility and costs can be assessed.

Accurate costing of this and other potential combined treatment options will not be possible until further work is completed. However, in order to gain an appreciation of the potential costs of some of these options for comparison with current practices involving ocean exchange, it is useful to examine one possible scenario. Previous work for treatment of ballast water at 4,000 mt hr^{-1} involving separation (AQIS 1993b), together with comments from various workers associated with hydrocyclone and ultraviolet irradiation treatment, would suggest that the capital costs associated with retrofitting this type of equipment could be in the vicinity of US $2–2.5 million.

If this cost is considered for a bulk carrier operating on an international route, say from Australia to Japan (with a voyage round trip time of approximately one month) then it would discharge ballast water 12 times per year. If a capital recovery factor of 0.15 is assumed (interest rate of 8% over a 10-yr period) the capital cost component of the treatment (for 50,000 mt of ballast water) would be US 50-62.5 cents/mt ballast water. Operating costs would be in addition to the capital cost component. To put this cost into perspective, it needs to be compared with the costs for ballast exchange noted previously of US 1.4-4.5 cents/net (noting that these costs do not include a capital component).

Ultrasound

Ultrasound (using frequencies in the range from 15-100kHz can destroy micro-organisms in water by

means of localized mechanical stresses resulting from cavitation (Shankie and Riach 1995). Although some success has been achieved killing *D. polymorpha* veligers, it was concluded that such a system would not be practical for large-scale power plant intake application (NRC 1996). Given the technical uncertainties about the likely effectiveness of ultrasonics for killing organisms of interest in ballast water, together with the problems and cost associated with scaling-up existing systems to the size required for large ships, it appears unlikely that this option will become significant as a ballast water control measure.

Electrical Shocks and Pulses

Electric shock experiments by Montani *et al.* (1995) using 100 Volts AC for 5 sec were found to inactivate dinoflagellate cysts. Bolch and Hallaegraeff (1993) achieved inactivation of *A. catenella* cysts with applied voltages greater than 5 V/cm^2, but 7% *G. catenatum* cysts survived after treatment at 7.5 V/cm^2. However, this latter work demonstrated that the effect was due to the generation of chlorine and an increase in temperature rather than the electric shock.

Various forms of electric pulses have been demonstrated to either kill or stun brine shrimp, *Artemia salina*. Initial investigations with dinoflagellate cysts have not been encouraging and it appears unlikely that this option will attract much interest for large-scale ballast water treatment.

Chemicals and Other Options

A number of other biocide and chemical treatments have been suggested as potential candidates to kill ballast water organisms. At the current stage of demonstration and development, all of these have been rejected based on ineffectiveness, practicality, cost, or effect on the environment. Some brief comments concerning a selection of these options follow.

Ozone is used extensively for disinfection of fresh water; however, recent work by Oemcke and van Leeuwen (1998) has suggested that ozone is unlikely to be appropriate for ship board treatment of ballast water due to the possibility of increased corrosion and the difficulty in maintaining an effective disinfection residual as a result of the presence of sediments and dissolved iron.

Chlorine and hydrogen peroxide are both effective for destruction of some ballast water organisms, including dinoflagellate cysts (Rigby *et al.* 1993; Bolch and Hallaegraeff 1993). However, the high concentrations required to provide effective treatment mean that the costs would be prohibitive for the large quantities of ballast water involved. Problems associated with the safety and residual concentrations of chlorine also make these options unattractive.

Recent work by Voigt and Gollasch (1998) has shown that low concentrations (50 ppm) of a peroxygen based liquid formulation involving specific activators have been effective in treating larval and adult stages as well as resting stages of *A. salina*. Further research work is in progress to pursue this treatment option.

Other chemicals including bromine, iodine, and organic acids (such as gluteraldeyhde, glycolic acid and paracetic acid) have been suggested. While these chemicals are not uncommon in laboratory use for disinfection, their efficiency and use for treating ballast water remains questionable.

A synergistic combination of copper and silver ions has been used in hospital water systems for the control of bacteria. Although suggested as a possible candidate for ballast water treatment, tests carried out by Lloyd's Register (1995) on ballast water organisms showed that neither bacterial nor phytoplankton viability appeared to be significantly affected by the treatment.

A reduction in dissolved oxygen levels (by injection of nitrogen or by use of an oxygen scavenger chemical) although effective for starfish larvae, is ineffective for killing *Undaria pinnatifida* spore (Mountfort 1997). Dinoflagellate cysts are known to survive for long periods under anaerobic conditions (Anderson *et al.* 1988). This option has therefore not been considered as a serious possibility for use in ballast water management.

Conclusions

A number of precautionary management practices have been developed to minimize the uptake of organisms in the water during ballasting. While these practices can provide a proactive approach that is much simpler and more cost effective than other treatment alternatives, variations in local conditions at the time of ballasting and practical limitations mean that the overall effectiveness of management options alone is somewhat limited. Nevertheless, an understanding of the possible options, and imple-

mentation where appropriate will form an important part of the overall ongoing strategy to minimize the spread of nonindigenous aquatic organisms.

Several treatment options aimed at killing or removing the harmful organisms present in the ballast water are presently in use, or are being developed and tested for use in conjunction with the management practices.

Ballast water exchange at sea will continue to be the primary option for most ships for many years to come. However, safety and practical restrictions, together with limitations on suitable exchange locations, are likely to limit the universality of this option. Heating of the ballast water using waste engine heat is a promising and cost-effective option that is likely to gain increasing acceptance in the international arena in the near future.

Filtration and hydrocyclones coupled with a secondary treatment option such as UV irradiation are currently being tested at practical scales of operation. These options will be more costly than either ocean exchange or heating and the technical efficiency for removal of organisms is yet to be reported and overall effectiveness assessed.

Other alternatives, including the use of biocides or chemicals, have generally been excluded to date based on cost, effectiveness, practicality, or environmental grounds. However, ongoing research and development will continue to provide the necessary data and confidence to assess the effectiveness of these options.

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Development of an Aquatic Nuisance Species Barrier in a Commercial Waterway

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ABSTRACT: **The National Invasive Species Act (NISA) of 1996 authorized the U.S. Army Corps of Engineers to carry out a demonstration study of an aquatic nuisance species dispersal barrier in the Chicago Sanitary and Ship Canal. The objective of the study is to slow or prevent the dispersal of invasive species via the canal. This century-old, man-made canal is the only aquatic link between the Mississippi River and the Great Lakes drainage and forms a two-way avenue for invasive species dispersal. The canal is used for transportation of freight between Lake Michigan and the Illinois Waterway, and to carry wastewater away from Lake Michigan, Chicago's drinking water supply. Recreational vessels frequent the canal, but it is not used for water skiing or swimming. No migratory species traverse this man-made canal; however, the barrier is expected to affect native as well as invasive species. A multi-agency advisory panel ensures representation of the myriad interests in the canal and development of the barrier. The panel members identified potential methods and recommended an initial approach. Due to the commercial uses of the canal and its importance to Chicago's drinking water, physical barriers and canal closure are not practical alternatives. The demonstration study will begin with installation of a micro-pulsed DC electric barrier designed to deter fish, rather than stun them. The study will add methods to target other species as funding allows. Monitoring of the barrier performance will help determine effectiveness of each method. Conceptually, the full-scale barrier will consist of a two-barrier, redundant system in a restricted reach of the canal. Construction of the electric barrier should begin in the late spring of 2000.**

Key words: aquatic nuisance species, barrier, canal, dispersal, Great Lakes; Mississippi River, Chicago River.

INTRODUCTION

The Chicago Sanitary and Ship Canal (San-Ship Canal) forms the sole aquatic link between the Great Lakes and Mississippi River drainage basins. This important transportation corridor also stands as an open portal to invasive species presently inhabiting either the Great Lakes or the Mississippi River. To slow or stop the spread of invasive species between the Great Lakes and Mississippi River drainage basins, the U.S. Army Corps of Engineers (USACE) and other agencies are examining methods to create a dispersal barrier in the San-Ship Canal. The National Invasive Species Act (NISA) of 1996 authorized up to $750,000 for the Corps to carry out a dispersal barrier demonstration study in the Chicago Sanitary and Ship Canal. In fiscal year (FY) 1998, $500,000 was appropriated for this work; an additional $300,000 has been scheduled for the Corps in FY1999.

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HISTORY OF THE CHICAGO RIVER

Originally, the Chicago River flowed into Lake Michigan (Figure 1). During wet seasons, a shallow wetland called Mud Lake connected the Chicago River and Des Plaines River. Mud Lake was navigable by canoe for a few weeks of the year. Joliet and other early explorers recognized the potential for creating a permanent aquatic connection between Lake Michigan and the Des Plaines River to facilitate the transportation of goods and travelers to and from the Midwest.

The Illinois Michigan Canal (IM Canal) was the first attempt to form a permanent waterway between Lake Michigan and the Illinois River. The IM canal was essentially the predecessor of the San-Ship Canal. The IM canal was operated by pumping water from the South Branch of the Chicago River 15 ft up into the headwaters of the canal. The pumping reversed the flow of the river for much of the year. Inflow from Lake Michigan improved water quality of the heavily polluted river and carried the contamination away from Lake Michigan, Chicago's drinking water. During wet periods, however, the river still

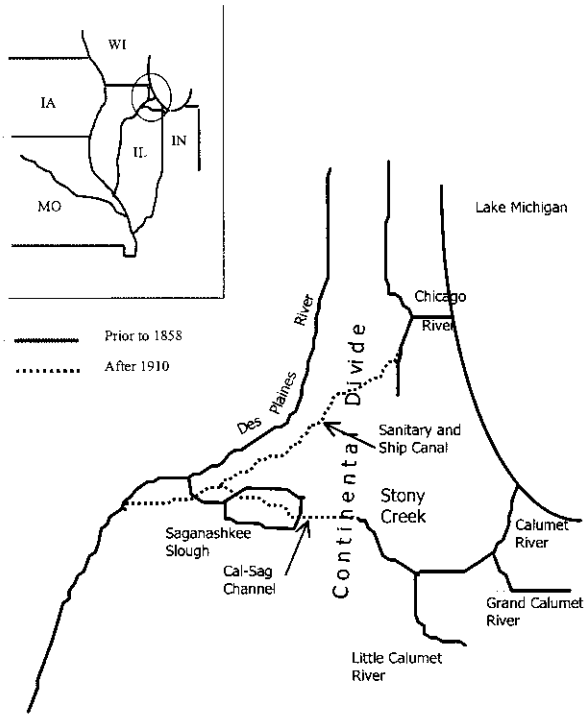

Figure 1. Configuration of the Chicago and Calumet rivers before and after development of the Sanitary and Ship Canal and Cal-Sag Channel.

flowed into Lake Michigan, tainting the City's water supply. When the Chicago Sanitary and Ship Canal opened in 1910, the flow reversal became essentially permanent. Pumping was no longer required to maintain flow through the canal. The elevation change between Lake Michigan and the Des Plaines River was sufficient to create a flow out of the lake.

Other rivers and canals now connect to the San-Ship Canal. Like the Chicago River, the Calumet River originally flowed into Lake Michigan. The Calumet River now connects with Saganashkee Slough to form the Cal-Sag Channel. Once complete, this connection reversed the flow in the Calumet River such that it flows out of Lake Michigan. There are three other connections between Lake Michigan and the San-Ship Canal. The North Shore Channel at Wilmette, Illinois helps maintain water quality in the north branches of the Chicago River. Both the Grand Calumet and Little Calumet River connect to the Cal-Sag Channel. All water from these various sources must pass through a single narrow reach near Lemont, Illinois where the canal cuts through native limestone. At this point, the 50-m wide and 7.5-m deep canal has perpendicular walls and a flat bottom (Figure 2).

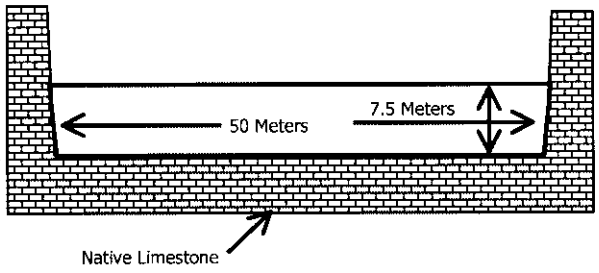

Figure 2. Cross-section of the Chicago Sanitary and Ship Canal at the proposed barrier site.

Water Quality and Flow

Historically, poor water quality in the canal formed an effective barrier to inter-basin range expansion of Great Lakes or Mississippi River species. Over the last two decades, the Metropolitan Water Reclamation District of Greater Chicago has invested millions of dollars to improve wastewater treatment and water quality in the canal. The improvements have contributed to significant increases in dissolved oxygen and reduced ammonia-nitrogen. In turn, the diversity and abundance of fish species in the canal system have increased (Figures 3 and 4).

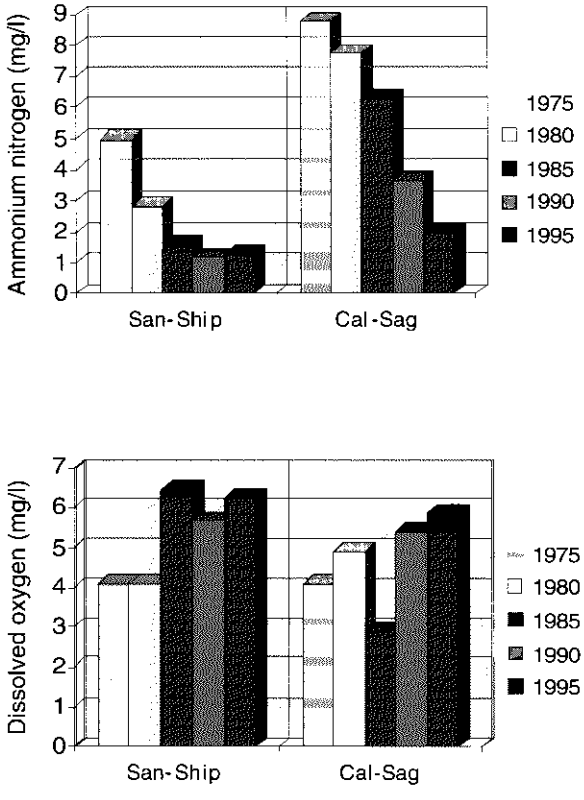

Figure 3. Water quality parameters in the Chicago Sanitary and Ship Canal and the Cal-Sag Channel, 1975-1995.

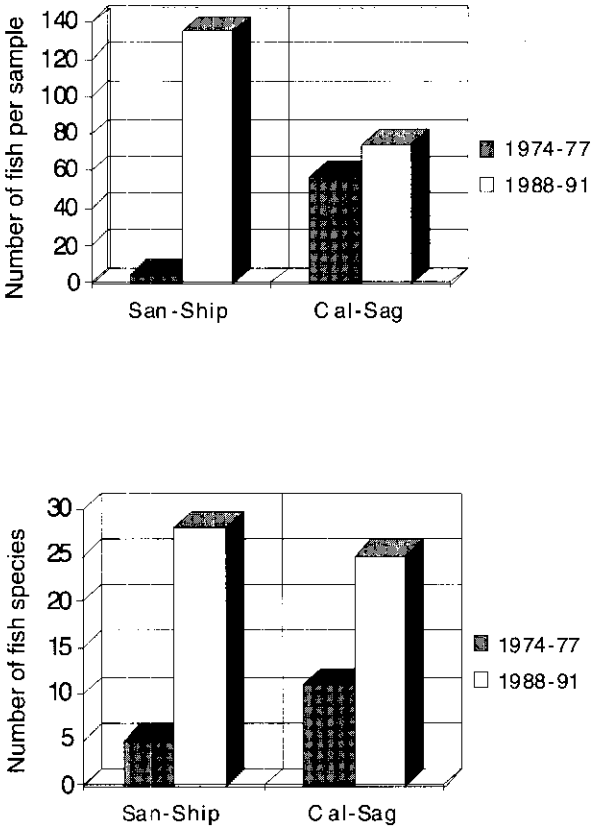

Figure 4. Fish species abundance and diversity changes in the Chicago Sanitary and Ship Canal and the Cal-Sag Channel, 1975-1995.

The average annual discharge from Lake Michigan is fixed by court decree at cfs. This volume includes what is used for drinking water as well as water that leaks through breakwalls or passes during operation of the locks. Diversion gates at Chicago Harbor, O'Brien Lock and Dam, and on the North Shore Channel at Wilmette control the volume of Lake Michigan water diverted down the canal to maintain water quality. Depending on rainfall, there can be a ten-fold variation in flow volume and velocity in the canal. Flow volume ranges from 2000 to 20,000 cfs with corresponding velocities of 0.5-5 ft per sec.

Species of Concern

Today the Chicago Sanitary and Ship Canal is a two-way avenue for inter-basin spread of invasive species. As occurred with the zebra mussel (*Dreissena polymorpha*), other invertebrates and fish in the Great Lakes can spread into the Mississippi basin. Native and introduced species in the Mississippi drainage can spread into Lake Michigan and the other Great Lakes. These invasive species will directly compete with and could prey upon existing biota.

Table 1. Recent invasive species present in either the Great Lakes or the Mississippi River basins. * Species already found in Lake Michigan or the Calumet River.

Great Lakes	Mississippi River
Spiny waterflea* *Bythotrephes cederstroemi*	Striped bass *Morone saxatilis*
Fish hook waterflea* *Cercopagis pengoi*	Hybrid striped bass* *Morone saxatilis x M. chrysops*
Eurasian ruffe *Gynocephalus cernuus*	Grass carp* *Ctenopharygodon idella*
Round goby* *Neogobius melanostomus*	African spiny wateflea* *Daphnia lumholtzi*
Tubenose goby *Proterorhinus marmoratus*	Black carp *Mylopharyngodon piceus*
White perch* *Morone americanus*	Bighead carp *Hypophthalmichthys nobilis*
New Zealand mud snail *Potamopyrgus antipodarum*	
Three-spine stickleback* *Gasterosteus aculeatus*	

Table 1 lists invasive aquatic species that could spread or are in the process of spreading from the Great Lakes or the Mississippi basin to invade a new, major drainage. Hybrid striped bass (*Morone saxatilis x M. chrysops*) are present in low numbers in Lake Michigan. Grass carp (*Ctenopharygodon idella*) have been captured in Lake Calumet, only 7 mi from Lake Michigan and *Daphnia lumholtzi*, an African spiny water flea, has been found in the Calumet River. In 1999, a round goby (*Neogobius melanostomus*) was caught below the confluence with the Des Plaines River, at RM 290.25 downstream from the site identified for the demonstration study (P. Thiel, pers. comm.).

Boat Traffic on the Canal

These two major uses of the canal–commercial navigation and wastewater conveyance–constrain the dispersal barrier options. The barrier cannot physically close the canal and should not significantly interfere with barge traffic or water flow. At the location identified for the barrier study, approximately 50 percent of the flow is treated effluent.

In addition to conveying wastewater, the canal system is an important transportation corridor for freight and recreational vessels. Chicago Lock is the

Table 2. Waterborne traffic in the Chicago Sanitary and Ship Canal, Cal-Sag Channel and Des Plaines River during 1995. Total includes tows, passenger boats/ferries, recreational vessels, cargo vessels, US Government vessels and contractors, commercial fishing boats. Other excludes the recreational and tow vessel numbers. Source: USACE 1996

Lock	River	Vessels				Kilotons
		Total	Recreational	Tows	Other	
Chicago	Chicago	52,983	38,790	118	14,075	201
O'Brien	Cal-Sag	21,883	17,517	3,746	620	12,849
Lockport	Des Plaines	4,102	1,103	2,705	294	14,865

nation's busiest, passing 50,000 to 60,000 vessels annually. The vast majority of the vessels are recreational and sightseeing craft. At O'Brien Lock, and farther downstream at Lockport, barge tows comprise a greater portion of the traffic (Table 2).

Due to the narrow width and perpendicular walls of the San-Ship Canal, the tows often navigate the canal by running the barges immediately along the wall. Insubstantial structures extending out from the wall are subject to damage or removal as the barge passes. Structures substantial enough to fend off the barge as it moves along the wall could deflect the tow out into the channel potentially causing a collision.

Advisory Panel

The diversity of uses and users of the canal system required stakeholder involvement in the development of the invasive species dispersal barrier for success of the project. The USACE formed a Dispersal Barrier Advisory Panel. Currently, panel members represent more than 25 federal, state, regional, municipal, commercial, and environmental groupsor agencies and academia (Table 3). Expertise on the panel includes field and research biologists, engineers, regulators, barge operators, and commercial water users.

The panel members characterized an ideal barrier as cost effective, quick to implement, protective of public health, environmentally sound, having little residual downstream effect, continuous, fail safe, redundant, broad spectrum, applicable to other systems, and providing long-term effectiveness. They then listed types of barriers that could be effective against an array of aquatic species ranging from zooplankton to fish. The list included chemicals (toxicants and oxidants), nitrogen stripping, low dissolved oxygen, chlorine, electricity, acoustics, lights, hydraulic jets, weirs, and screens. The panel considered modifying operational aspects of the canal system such dams, closure of the canal, and flow reversal. The panel also considered the effectiveness of biological controls such as predators, disease, and parasites.

Potential methods were ranked based on available technology, predicted or known organism response, and feasibility. Commercial navigation and wastewater uses of the canal system essentially preclude physical barriers such as screens or weirs and flow reversal.

Conceptual Approach and Recommended Strategy

The panel recommended the barrier use a redundant, "fail-safe" approach. Figure 5 illustrates the proposed barrier concept. The two independent barrier systems would be placed some distance apart, perhaps 300-500 m or more to avoid interference and allow sampling and monitoring of the canal reach between the two arrays. This canal reach could

Table 3. Membership of the Chicago Sanitary and Ship Canal Aquatic Nuisance Species Dispersal Barrier Advisory Panel.

Federal	State
U.S. Army Corps of Engineers	Illinois Department of Natural Resources
U.S. Fish and Wildlife Service	- Illinois Natural History Survey
U.S. Environmental Protection Agency	- Department of Conservation
U.S. Geological Survey	- Office of Water Resources
- Biological Resources Division	Illinois Environmental Protection Agency
U.S. Coast Guard	Illinois Pollution Control Board
Great Lakes Fishery Commission	Illinois-Indiana Sea Grant
Regional & Industry	
Metropolitan Water Reclamation District of Greater Chicago	Commonwealth Edison
	Smith-Root, Inc.
Illinois River Carriers Association	DuPage County Forest Preserve
Michigan State University	Great Lakes Commission
Loyola University	Northeastern Illinois Planning Commission
Great Lakes Sportfishing Council	Friends of Chicago River
University of Windsor	Canal Corridor Association
Minnesota Department of Natural Resources	Wisconsin Department of Natural Resources

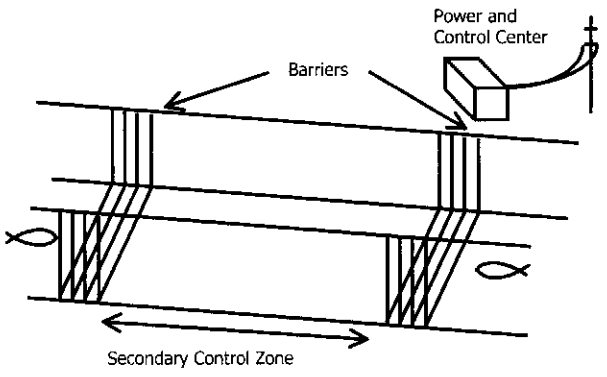

Figure 5. Conceptual layout of an aquatic nuisance species barrier in the Chicago Sanitary and Ship Canal.

eventually provide an area for application and detoxification of chemical controls.

The long-term objective of the barrier demonstration is to create a barrier that is that is effective against all types of organisms. Nonplanktonic organisms will likely be easier to deter than planktonic ones. Organisms that are strong swimmers could sense the presence of the barrier; the organism would then return in the direction from which it came. A planktonic organism would merely be flushed through the barrier, regardless of the level of discomfort. Methods that deter fish may not be effective against zooplankton, so a variety of barrier approaches will have to be employed. It is expected that native species will be affected as well as invasive species.

It will be easier to obtain a permit for non-chemical approaches than for methods that affect water quality. In the near term, barrier methods used in the demonstration study will rely on fish behavioral response. The panel recommended application of chemicals as a stopgap measure or possibly for seasonal use only.

The demonstration study will occur in phases. The first phase will target active swimming organisms (fish), beginning with an electric barrier. Next, the study will examine methods that would complement the effect of the electric field, such as an acoustic or visual barrier, water jets, or a combination of methods. As various methods prove successful at the first location, they could be applied at the second barrier site. Over the long term, the study will investigate the potential for creating a barrier for planktonic organisms.

Construction of the barrier is scheduled to begin in 2000. The project will start with a Smith-Root, Inc. micro-pulsed DC electric array. Electric barriers of this type prevent upstream migration of lampreys in Great Lakes tributaries and confine vegetation-controlling Grass carp in Western irrigationcanals. The electrodes will be railroad rails. Recessing the rails into the canal walls will avoid damage from barge traffic. The pulsators and back-up generator will reside in a small equipment shed on shore. The effect of the electric field will affect the entire water column and will extend about 7 m up and downstream from the array.

Monitoring, Safety, and Next Steps

Once the electric array is constructed, monitoring will assess the effect on fish at the barrier site. Fish present in the canal will be captured and tagged according to the location of capture, *i.e.*, upstream or downstream of the array. The effect of the array on movement of the fish will be determined through recapture.

Due to the low voltage and direct current used for the barrier, electric shock is not a concern. A boy on an inner tube, a dog, and a horse have passed through the arrays in the irrigation canals without adverse effect. Signage and lights posted well in advance of the barrier site will warn of the presence of the barrier. Swimming and water skiing do not occur in the canal, but it is conceivable that a person could fall overboard near the array. Egress ladders or similar equipment installed in the canal walls will provide a means of exiting the water.

The study will continue investigating other barrier methods that will complement or, if proven effective, could substitute for the electric array. One attractive approach requiring further investigation involves an infrasound acoustic array. This method poses no safety concerns, is commercially available, and has been successfully used in Europe to guide fish past water intakes and canal openings. During development of the first barrier, potential sites for the second, redundant barrier will be identified.

Conclusion

If successful, the dispersal barrier will prevent the passive spread of invasive species via the Chicago Sanitary and Ship Canal. It will not control human-mediated spread of invasive species, for example, as can occur through bait bucket introductions. The barrier project is an important part of a comprehensive program to prevent the introduction and spread

of aquatic nuisance species throughout North America. However, education of recreational and commercial water users and cooperation among natural resource agencies, commerce, and the public are critical components for a successful nuisance species prevention program.

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Implementation of the National Invasive Species Act of 1996 (NISA)

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Key words: NISA, ballast water, regulation, U.S. Coast Guard, exotic species, nonindigenous species, aquatic nuisance species

BACKGROUND

I am going to discuss the background of the National Invasive Species Act and bring you up to speed on how we have arrived at the current situation.[2] We are still in the comment-and-review period of the proposed rule that was published April 10, 1998.

One of the major issues that we deal with is the definition of nonindigenous species, which are species transported to other than their native habitat. In the absence of natural predators or natural controls, they can often out-compete native species, reduce biodiversity, and become nuisance species. Most of you are already aware of many of the examples of nonindigenous species that have become issues nationally and internationally. The "poster child" for us has been the infestation of zebra mussels in the Great Lakes. We were warned as early as the 1920s that the zebra mussel was a potentially invasive species, but that invasion did not occur until the 1980s. The zebra mussel problem in the Great Lakes became the driving force behind most of the policy that was implemented in the late 1980s and early 1990s.

NON-INDIGENOUS AQUATIC NUISANCE PREVENTION AND CONTROL ACT

In the late 1980s, Senator Glen proposed legislation that became the Non-Indigenous Aquatic Nuisance Prevention and Control Act (NANPCA) of 1990. NANPCA addressed the Great Lakes issue and called for a shipping study to see if this was a local or a national issue. Under NANPCA, we originally worked with the Canadian Coast Guard and issued voluntary guidelines for ballast water control management. We also worked with the International Maritime Organization (IMO) to try to come to an international resolution of this issue, which is not just a national problem.

In 1993, we promulgated mandatory regulations for the Great Lakes, requiring an open-ocean exchange or equivalent for all vessels carrying ballast water into the Great Lake system. The Great Lakes system is unique in that there is a bottleneck: all vessels entering the Great Lakes from outside the Exclusive Economic Zone (EEZ) must come through the St. Lawrence Seaway. Enforcement can be carried out at Massena, NY, between the locks on that seaway. NANPCA also provided funding for research, which many of you have already been involved with.

NATIONAL INVASIVE SPECIES ACT

As a result of the shipping study and other issues that came to the forefront, such as finding cholera in the ballast water of ships in Mobile Bay in 1992 and the green crab infestations, the National Invasive Species Act (NISA) of 1996, which amended and modified NANPCA 1990, was issued. NISA requires the Coast Guard to promulgate voluntary guidelines for all vessels entering U.S. waters from outside the EEZ. By presidential proclamation, the EEZ is 200 miles from our coastline in most areas. As previously noted, we are in the comment-and-review portion of this proposed rule. If you wish to look at the comments, they are available on the docket management services website at http://dms.dot.gov/, docket number USCG-1998-3423. Originally, I was going to speak more about the proposed rule itself, but a lot cannot be released until the final rule comes out.

We are also working with the Aquatic Nuisance Species Task Force to develop voluntary guidelines

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[2]Information in this paper current as of 1/1999

for recreational vessels. This is not just a problem with ships entering the United States: the interstate transport of recreational vessels affects a lot of our water bodies.

On the international level, we are participating in a working group within IMO. Currently, we have a proposed annex to the International Convention for the Prevention of Pollution from Ships, 1973, as modified by the Protocol of 1978 (MARPOL 73/78), that will address the aquatic nuisance species problem; a stand-alone treaty is also under consideration. One of the issues involves the measures it would take to bring it into effect. We would have to have 50% of the world's shipping tonnage in support of it as an annex to MARPOL. The U.S. Congress has given its tacit approval to MARPOL, therefore both avenues of that instrument are being considered at this time. Copies of the draft annex to MARPOL are available. Please check the IMO Documents section of our webpage www.uscg.mil/hq/g-m/mso4/first.htm for information on how to obtain them.

We recognize that the technology to control or eliminate transport by ballast water is not yet available. We accept ballast water exchange as a control measure: it reduces the risk but does not eliminate it, and it is also not possible in all situations or for all classes of vessels. We have structural and stability concerns with some ships. We do not want them to crack in half and create a major oil spill or loss of life.

I do want everyone to keep in mind that while ballast water is a major path of introduction, it is not the only path. We are also looking at issues with the ship's hull. Tributyltin (TBT) is a component of the paint used on ship hulls and does reduce the fouling on the hull, but TBT itself is of concern. When TBT paints are taken off, nonindigenous species may be transported. An international effort is underway to find replacements that are not as harsh to the environment.

We recognize there is no silver bullet for ballast water treatment. We would accept a toolbox approach in which a shipping organization could propose a ballast water control method and not be limited to just one technology or method.

The Aquatic Nuisance Species Act and the Marine Environment

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Key words: aquatic nuisance species, ANS Task Force, NISA, management plan

INTRODUCTION

The primary focus of this presentation is to highlight the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (amended as the National Invasive Species Act in 1996 or NISA) and how it applies to the marine environment. Prior to 1990, there were numerous scientists trying to get people to recognize that invasive aquatic species posed a serious problem. Although invasives were having serious economic and ecological impacts, very few people were paying attention. In the marine environment, prior to 1990, there were limited control activities taking place and very little research being conducted.

Starting slowly in the 1970s and 1980s, the voices of concern began getting louder. We began to see activities such as the control of sea lampreys in the Great Lakes and concerns about grass carp moving into the west. President Carter issued his Executive Order on exotic species, which also helped to increase awareness. The zebra mussel functioned as a poster child of sorts in that it was responsible for getting the attention that led to the passage of the Act. Not only was it causing serious environmental impacts, but was having substantial economic impacts to the water and power infrastructure in the Great Lakes.

If the impact had only been from an ecological perspective, we may not have been successful in getting Congress to pass the Act. The fact that zebra mussels shut down the water intake to the city of Toledo, Ohio, which ran uninterrupted for over 120 years, was certainly a factor in gaining attention. Senator John Glenn, Ohio and his primary staff person, Allegra Cangelosi, are owed a debt of gratitude and thanks, as are other early invasive species pioneers who were responsible for developing the legislation. When the legislation was initially drafted, it focused on the Great Lakes, as invasives were viewed as primarily a Great Lakes issue. That language was broadened somewhat before the Act was passed and in the recent amendment to the Act, the National Invasive Species Act of 1996, the focus was broadened even more.

The Act is comprised of two primary components: the ballast water management component and the development of the Aquatic Nuisance Species (ANS) program. The ANS Program has three primary elements, prevention, detection, and monitoring, and control. The ballast water program includes the establishment of mandatory ballast water regulations in the Great Lakes, and a requirement for the Armed Services to have a ballast water management program. Additionally, the National Atmospheric and Atmospheric Administration (NOAA) and the Fish and Wildlife Service (Service) are coordinating a program to provide funding for demonstration projects for new technologies for ballast water management. There is currently a demonstration project underway in the Great Lakes to look at various technologies. Other studies that have recently been completed include the Ballast Exchange Study, that will soon be out in final form and available for public review. There is also a requirement under the Act to develop a research protocol to ensure that research done with invasive species is not a contributing factors to furthering their spread. Guidelines for recreational activities are also being developed to ensure that these activities do not contribute to the spread of aquatic invasives.

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A significant feature of the Act is the provision that provides for cost-share grants for States to implement State and Interstate ANS Management Plans. Currently five states have completed ANS Management Plans and about another dozen or so are in development. If we are successful in getting more states engaged in this issue we will certainly be more effective in trying to address the problems.

The ANS Task Force was established by the 1990 Act and held its first meeting in 1991. It is co-chaired by the Director of the Fish and Wildlife Service and the Administrator of NOAA. It is a coordinating entity that is comprised of federal agencies and ex-officio members. Its primary responsibility is to coordinate the implementation of the mandates or requirements of the Act. The U.S. Coast Guard has the primary responsibility to address the ballast water mandates. The Act specifically identifies the Fish and Wildlife Service, NOAA, the Coast Guard, the Environmental Protection Agency, the Army Corps of Engineers and the Animal and Plant Health Inspection Service (APHIS) in the Department of Agriculture as members. The State Department was added by consensus of the other Federal members to ensure that international aspects and components of the program were addressed.

The Act provides for ex-officio members to participate, initially specifying the Great Lakes Commission as a member. When the Act was reauthorized, other ex-officio members were added. The Lake Champlain Program, the Chesapeake Bay Program and the San Francisco Estuary Program were designated as ex-officio members during the reauthorization. When ex-officio members were originally invited, groups such as the American Water Works Association, and the American Public Power Association were invited to be members. These additions were primarily because the focus was on zebra mussels and the Great Lakes region. We are now going through a process of reviewing ex- officio membership to determine if we need to add to that membership looking at it from both an issue basis as well as a geographic basis.

From the beginning, the task force has functioned on a consensus basis. I think to date we have never had to vote on an issue. All of the Task Force meetings are open to the public and are usually held about three times a year. Our next meeting was held in association with the International Zebra Mussel Conference in Duluth, Minnesota. Many of the responsibilities of the Task Force are carried out through various committees. To address regional concerns, we have a Great Lakes Panel, a Western Regional Panel and are in the process of putting together a Gulf Coast Panel. Other committees have been established including a Brown Tree Snake Control Committee, a Risk Assessment Committee, and a Ruffe Control Committee. We are currently in the process of establishing a Green Crab Control Committee. These groups are responsible for developing control plans, and identifying potential funding to implement them. One of the early studies that was conducted by the National Research Council called "Stemming The Tide," began identifying a list of things that could be done to address some of the ballast water issues. Other things that have implications for coastal areas include the green crab control program, which is about to get up and running, and also a mitten crab control program.

As with many projects or legislation, finding adequate funding is always a problem. While the authorization level for the Act is around $33 million, only about 44% to date has been allocated to carry out the mandates of the Act. One of the difficulties from the very beginning was that each agency represented on the Task Force falls under a different sub-committees in the Congressional Budget process. Often, one agency will get funding and another agency will not. This situation has been especially difficult because much of the work is interrelated. With the potential of the new Executive Order on Invasive Species coming out, there should be stronger support by the administration to look at cross program budgeting. When the Task Force was initially established, very little funding was available and each agency contributed a staff person to participate. As the Task Force has grown and gotten more involved, many of the Federal agencies now are coming to the table with additional money. For example, NOAA is providing funding to the Task Force on an annual basis to be used to hire an outreach person. There are also other positions that the other agencies have provided to assist the Task Force in carrying out its responsibilities.

To summarize, a quote by E.O. Wilson expresses why we all should be concerned with invasive species: "extinction by habitat destruction is like death in an automobile accident, easy to see in excess. Extinction by invasion of exotic species is like death by disease. Gradual, insidious, requiring scien-

tific methods to diagnose." The Secretary alluded to this in an earlier talk comparing it with the Exxon Valdez oil spill and what it did to raise the level of national attention. The invasive species issue is bubbling below the surface where it is often out of sight and out of mind. I believe the tide is turning and we are poised to move forward into a new decade where we will have increased opportunities to address the threat from invasive species.

Why Ballast Water Discharges Should be Regulated Under the Clean Water Act

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Key words: ballast water, clean water act, management

I. THE PLAIN LANGUAGE OF THE CLEAN WATER ACT REQUIRES NPDES PERMITS FOR BALLAST WATER DISCHARGES

The Clean Water Act (CWA) prohibits "the discharge of any pollutant by any person" except as in compliance with specified sections of the Act, including the permitting provisions of § 402. 33 U.S.C. § 1311(a). The phrase "discharge of a pollutant" is defined to include "any addition of any pollutant to the navigable waters from any point source." 33 U.S.C. § 1362(12). Vessels are specifically defined as point sources in the CWA. 33 U.S.C. § 1362(14). Moreover, the CWA specifically includes "biological materials" in its definition of pollutants. 33 U.S.C. § 1362(6). The discharge of ballast water from vessels is a discharge of pollutants because ballast water is known to contain invasive plant and animal species as well as bacteria and viruses associated with human sewage. All of these pollutants qualify as "biological materials" within the meaning of the CWA. Additionally, ballast water is likely to contain other pollutants, such as oil, chipped paint, sediment, and toxins contained in ballast sediment.

Under the CWA, vessels qualify as point sources. Accordingly, when they discharge pollutants, they are required to have National Pollutant Discharge Elimination System (NPDES) permits. Although EPA has purported to exempt "discharge[s] incidental to the normal operation of a vessel" from the requirement to obtain a permit, 40 C.F.R. § 122.3(a), nothing in the CWA gives EPA the power to create categorical exemptions. *Natural Resources Defense Council v. Costle,* 568 F.2d 1369, 1377 (1977) (*Costle*). While EPA is given substantial deference in interpreting the CWA, it cannot rely upon regulations that are clearly contrary to the express statutory requirements. *Chevron v. Natural Resources Defense Council,* 467 U.S. 837 (1984), *City of Chicago v. Environmental Defense Fund,* 114 S.Ct. 1588 (1994).

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The CWA does contain certain limited exemptions relating to the need to obtain NPDES permits for ballast water and other discharges incidental to the normal operation of vessels. None of these exemptions can reasonably be construed as permitting the blanket exemption contained in 40 C.F.R. § 122.3(a). First, the CWA excludes incidental discharges from vessels made in the "contiguous zone" and the "ocean" from having to obtain an NPDES permit. 33 U.S.C. § 1362(12)(B). These terms have clear statutory definitions: the "contiguous zone" begins three miles from shore and extends seaward to twelve miles from shore; and the "ocean," is any portion of the high seas beyond the contiguous zone. 33 U.S.C. § 1362(9) and (10). Thus, the effect of this exemption is that incidental discharges (such as ballast water) made outside of three miles from shore are not required to have NPDES permits. It cannot, however, reasonably be construed as applying inside the three mile contiguous zone boundary.

Second, the CWA specifically excludes two types of discharges from its definition of "pollutants." 33 U.S.C. § 1362(6)(A). The Act states that neither discharges of "sewage from vessels or a discharge incidental to the normal operation of a vessel of the *Armed Forces,*" are to be considered pollutants. *Id.* (emphasis added). As a result of the second aspect of this exclusion, discharges incidental to the normal operation of Armed Services vessels are not required to have an NPDES permit. However, this exemption is specifically limited to Armed Services vessels; EPA cannot reasonably expand it to apply to all vessels, as it has done in 33 C.F.R. § 122.3(a).

It is important to note that, in exempting both sewage discharges and incidental discharges from Armed Services vessels, Congress specifically provided alternative programs for control of such discharges under other sections of the CWA. *See* 33 U.S.C. § 1322(b) (addressing sewage discharges) and (n) (addressing incidental discharges from Armed Forces vessels). The fact that there is no similar statutory or regulatory provision, which addresses incidental, dis-

charges from non-Armed Services vessels under the CWA further highlights the Congressional intent that ballast water discharges be regulated under § 402 of the CWA.

The Act is clear that ballast water releases that contain biological materials qualify as point source discharges of a pollutant and that such discharges require NPDES permits under § 402. 40 C.F.R. § 122.3(a) runs directly counter to this plain statutory requirement and should therefore be repealed.

II. Existing Case Law Unequivocally Indicates that EPA Does Not Have the Discretion to Exempt Incidental Discharges from the Requirements of the CWA

In *Costle,* the D.C. Circuit addressed the question of whether EPA could exempt agricultural return flows from the requirements of the CWA. 568 F.2d 1369 (D.C. Cir. 1977). The court unambiguously stated that the EPA did not have the authority to exempt discharges from the requirements of § 402. Finding that § 402 permits were central to achieving the stated goals of the CWA, the court found that "[t]he wording of the statute, legislative history, and precedents are clear: the EPA Administrator does not have authority to exempt categories of point sources from the permit requirements of §402." *Id.* at 1377; *see also NRDC v. U.S. E.P.A.*, 966 F.2d 1292, 1305 (9th Cir. 1992); *Carr v. Alta Verde Industries Inc.*, 931 F.2d 1055,1060 (5th Cir. 1991); *Sierra Club v Abston*; 620 F.2d 41, 44 (5th Cir. 1980); and *U.S. v. Earth Sciences, Inc.*, 599 F.2d 368, 372 (10th Cir. 1979).

In reaching its result, the *Costle* court relied on both the language of the statute itself and its underlying legislative history. As noted by the court, the House Report addressed the effect of § 301 in the following terms:

Any discharge of a pollutant without a permit issued by the Administrator under section 318, or by the Administrator or State under 402 or by the Secretary of the Army under 404 is unlawful.

568 F.2d at 1374, citing H.Rep.No.92-911, 92d Congress, 2d Session 100 (1972), reprinted in Legislative History at 787. The court further noted that there were: innumerable [other] references in the legislative history in the legislative history to the effect that the Act is founded on the "basic premise that a discharge of pollutants without a permit is unlawful and that discharges not in compliance with the limitations and conditions for a permit are unlawful."

Id. at 1375.

In promulgating 40 C.F.R. § 122.3(a), EPA acted in direct violation of the straightforward rule established in *NRDC v. Costle.* EPA has created a categorical exclusion in a statutory scheme that permits of none.

III. Benefits of Clean Water Act Regulation

Control under the CWA would have two components. First, EPA would be required to develop technology-based controls based on the "best available technology that is economically achievable" (BAT). Before EPA were to set this standard, the permit issuers (typically the states under the CWA) would be required to exercise their "best professional judgement" in trying to anticipate what the BAT standard would be when it were to come out. Thus, all ballast water dischargers would immediately become subject to technology-based controls.

As importantly, the permit issuers would be required to ensure—on a case-by-case basis—that the relevant dischargers would comply with water quality standards. Given that few (if any) states have water quality standards that directly address the issue of invasive species, the key short-term issue here would be compliance with the antidegradation policy. Under this policy, no discharge can be permitted if it will impair any "existing use" of the relevant waterbody. 40 C.F.R. § 131.12. Existing uses are defined to include any species that have inhabited a particular waterbody since November 28, 1975. 40 C.F.R. § 131.3(e). Thus, under the antidegradation policy, the permit issuer would be required to perform an analysis—as a precondition to permitting a discharge of ballast water to occur—that would be designed to preclude the possibility that any invasive species present in the ballast water might outcompete any existing (i.e., native) species.

IV. Update

As of June 20, 2000, EPA has still no action in response to a petition filed by environmental groups that asked the agency to rescind its regulatory exemption for ballast water discharges. However, the State of California has developed a "total maximum daily load" for exotic species under § 303(d) of the CWA that may have the effect of severely restricting ballast water discharges in that State.

Quantitative Biological Risk Assessment of the Ballast Water Vector: An Australian Approach

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Abstract: This paper reviews the need for ballast water risk assessment and describes three approaches: environmental matching that does not rely on species information, qualitative species-specific assessment, and quantitative species-specific assessment. The paper summarizes the progress to date on the ballast water risk assessment being developed by the Centre for Research on Introduced Marine Pests for the Australian Quarantine and Inspection Service. The risk assessment adopts a quantitative species-specific approach but also includes a simple hazard assessment based on environmental matching of the donor and recipient ports. In the first instance, the risk assessment will be applied to a target list of marine pests. Risk calculations are made via a series of modules that deal with discrete elements of the introduction cycle (donor port infection status, vessel infection scenarios, journey survival) up to and including survival in the recipient port. This endpoint has been selected to provide decision makers with a quantified measure of invasion risk with reasonable bounds of uncertainty. This is currently not possible for more complex endpoints such as the likelihood of establishment, or the expected economic and environmental costs of introduced species. The assessment maintains a precautionary approach in the face of uncertainty. This has been achieved by building different levels of assessment into some of the modules, allowing a progressively more accurate estimate of risk with additional data. If the requisite data are unavailable, the assessment defaults to the previous level of analysis or adopts a conservative stance.

Key words: introduced species, ballast water, quantitative risk assessment

INTRODUCTION

Ballast water is one of several ship-related vectors responsible for the modern dispersal of aquatic organisms around the world. At least 14 marine species are known "with reasonable certainty" to have been introduced into Australia through ballast water discharges (Jones 1991). Globally, over 104 marine and freshwater species are thought to have been introduced by this mechanism (Carlton 1985)–with significant environmental and economic costs (Carlton 1996). Recent evaluations of the rates of invasions into well-studied estuarine systems indicate that introductions are accelerating, possibly associated with the increase in ballast water movements around the globe since the late nineteenth century (Carlton 1996; Cohen and Carlton 1998; Hewitt *et al.* 1999). The scale and increasing rate of this problem, the seemingly irreversible nature of introductions, and the potentially devastating impact of exotic species (*e.g.* the zebra mussel, *Dreissena polymorpha*, invasion of the North American Great Lakes and the Atlantic comb-jelly, *Mnemiopsis leidyi*, invasion of the Black Sea), make ballast water introductions one of the most important environmental issues of the late twentieth century.

Australia has responded to the ballast water threat by establishing a National Ballast Water Management Strategy. This strategy seeks to "avoid the adverse economic and environmental impact of unwanted aquatic marine organisms by minimizing their risk of entry, establishment and spread ... whilst not unduly impeding trade" (Paterson 1995). The Australian Quarantine and Inspection Service (AQIS) is the lead governmental agency responsible for implementing this strategy. To do this, the Australian Ballast Water Management Advisory Council (ABWMAC) was established to aid AQIS in directing research and development. A risk-minimization approach, which is

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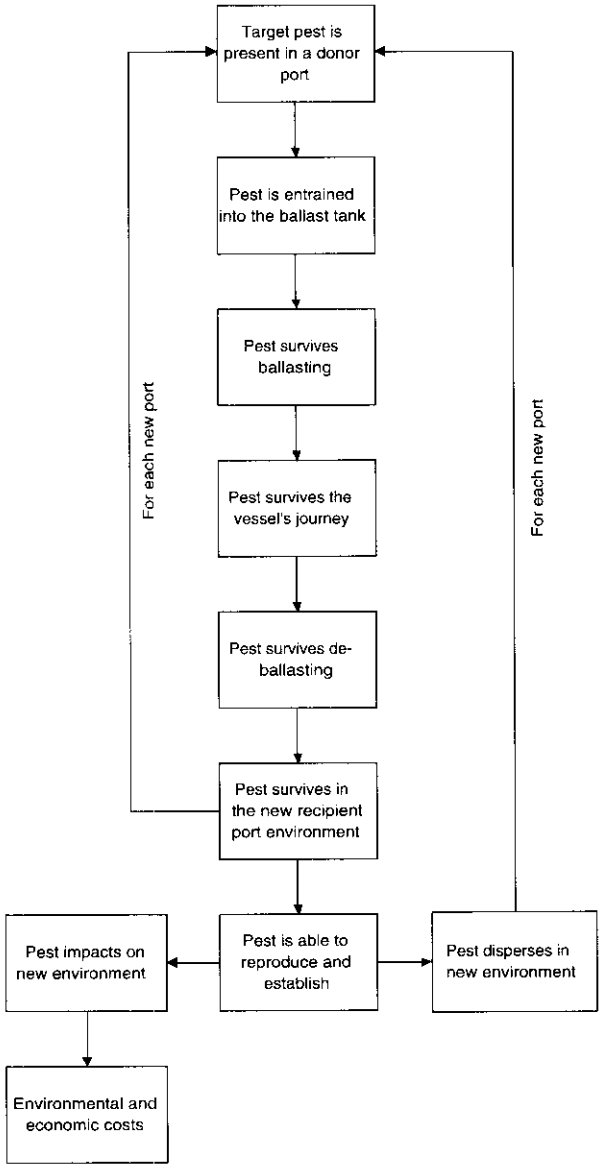

Figure 1. The ballast water invasion cycle conceptualized as a series of steps through which the invading species must pass. Once the species is introduced to, and becomes established in, a new port, that port becomes a possible donor port and the cycle is repeated.

founded upon a risk-assessment method capable of dealing with the complexities of the ballast water invasion cycle, has been adopted (Figure 1).

Approaches to the Ballast Water Problem

In 1991, the International Maritime Organization (IMO) introduced voluntary ballast management guidelines (Julian 1994), which recommend that all merchant vessels, *inter alia*, exchange their ballast water in mid-ocean, preferably in water depths of 2,000 m or more and beyond the 200-mi EEZ. Numerous nations are currently seeking a vehicle for international agreement, such as a new Annex to the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78), to make these guidelines mandatory.

In the meantime, individual nations will continue to be threatened by international and domestic (or regional) vessels that do not undertake ballast water management (*e.g.*, exchange). Vessels may not exchange their ballast water because of several possibilities:

- safety concerns–exchanging ballast imposes bending moments and shear stresses on the vessel. At sea, the combination of bending moments and shear stresses imposed on the vessel by wave action and exchange activities can compromise the structural integrity of the vessel. Thus, even for largest vessels (>160,000 dwt), ballast exchange is not recommended if the significant wave height is greater than or equal to 6 m (AQIS 1993).
- the journey is too short–it takes time to safely exchange large volumes of ballast. For example, three days are needed to exchange the ballast water in all the tanks of the MV *Iron Whyalla* (141,475 dwt), using a three-volume flow through method (Rigby and Hallegraeff 1994).
- the vessel does not have a ballast water management plan approved by a certification society.
- the vessel chooses not to comply with the guidelines or regulations.

A nation can adopt one of three approaches to the problem of ballast water invasions: (1) it can do nothing, (2) it can decide that no introduction is acceptable ("zero risk"), or, (3) it can try to minimize the risk of ballast water introductions by tailoring management strategies to meet specified objectives ("risk management").

The first approach costs nothing, but introductions will continue unabated or even at an increasing rate. A proportion of these species will result in populations of pest species becoming established in the nation's ports and along its coasts. The economic and environmental costs can be high: the zebra mussel *Dreissena polymorpha*, for example, is estimated to cost US $300 million a year by obstructing cooling water intakes, and perhaps another US $200 million a year in nuisance costs (Weathers and Reeves 1996).

In the modern global economy, "zero risk"

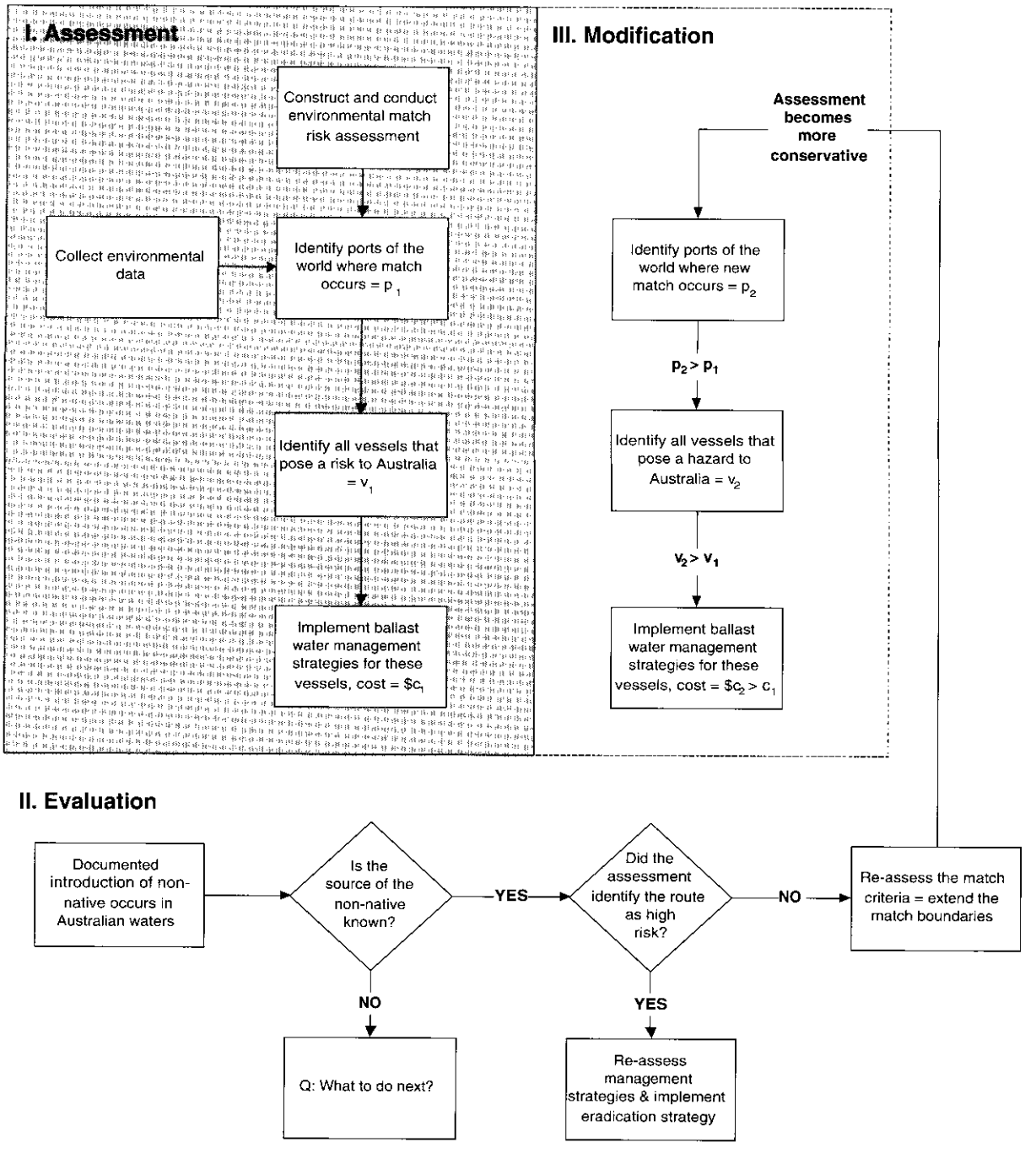

Figure 2. Risk assessments based on environmental matching (I. Assessment) face two problems when a new exotic species is discovered. First, if the origin of the new species is unknown then it is impossible to evaluate (II. Evaluation) the accuracy, or otherwise, of the risk assessment process. Second, if the origin of the species is known, but the assessment predicated the donor and recipient environments to be unmatched, then the assessment must be modified (III. Modification) so that these environments are matched. In effect, the assessment can only become increasingly conservative.

quarantine policies are untenable. Australia also views such policies as undesirable and has formally rejected them (Department of Primary Industries and Energy 1988). Furthermore, short of banning all shipping trade, one would have to devise a management strategy that is acceptable to all stakeholders and 100% effective in order to implement such a policy. No such strategy currently exists. The risk management approach, therefore, is a compromise between doing nothing and the zero risk approach.

Ballast Water Risk Assessment

At least three different approaches to ballast water risk assessment have been proposed in Australia and elsewhere: (1) environmental matching, (2) qualitative, species-specific; and, (3) quantitative, species-specific.

Environmental Matching

Environmental-matching seeks to measure ballast water risk by evaluating the degree of environmental similarity between donor and recipient ports. This approach assumes that if a non-native species is repeatedly transferred to a foreign port, the likelihood that it will establish a viable long-term population is heavily dependant on the similarities between the biophysical conditions of the recipient port and donor ports (Hilliard and Raaymakers 1997). As a hazard analysis this is a very useful first step, but as a risk assessment it has flaws.

Ballast water management, via risk assessment or any other quarantine management system, acts only as a filter, not as a barrier (Carlton *et al.* 1995). Ballast water risk assessment based on environmental matching, however, is a far less effective filter for four reasons:

1. Environmental-match assessments will identify similarities or differences between port environments, but will not incorporate species-specific environmental tolerance ranges. Consequently, potentially hazardous routes that lie well within the tolerance range of an individual species, but are between two ports that differ in environmental parameters, will not be identified.
2. Temporal and spatial scales of the environmental information from the ports will define the similarity/dissimilarity match. For example, two ports may match at the start of a Northern Hemisphere spring/Southern Hemisphere autumn, but be otherwise unmatched. Alternately, the microenvironments in ports could confound match predictions. For example, the discharge into a port of power-station cooling water could raise the water temperature at a site, making it capable of supporting species that could not survive elsewhere in the port (Carlton 1992).
3. Carry-over of ballast water between ports can make an environmental match between the recipient port and a vessel's last port of call (LPOC) immaterial to the true risks posed by the ballast water. In a study of American shipping patterns, Carlton *et al.* (1995) found that a vessel's LPOC was a poor predictor of ballast source: 53% of all vessels (and 66% of container vessels) contained ballast water that did not originate from the LPOC. Furthermore, a small proportion of a vessel's ballast is "unpumpable" because the pump pipe ends some distance from the bottom of the tank. For example, each vessel entering the Great Lakes, on average, carries 158 mt of residual unpumpable ballast (Locke *et al.* 1993). Thus, sediments at the bottom of a tank and some ballasted water are likely to be much older than the LPOC. Risk assessments based only on environmental similarity for all traded ports could become extremely conservative if they addressed this issue without estimates of how long organisms can survive in ballast water (see for example Murphy 1997; Gollasch *et al.* 1995; Wonham *et al.* 1996).
4. It is difficult to improve the risk assessment when a new introduction appears, for two reasons. If the origin of the species is unknown, then it is impossible to comment on the accuracy of the risk assessment in light of the new discovery. On the other hand, if the origin of the species is known, but the assessment predicted the source as unmatched, then the source must logically be reclassified as matched, which means that all vessels on that route must be regarded as hazardous. In effect, the assessment can only become increasingly conservative with increasing costs to the shipping industry (see Figure 2).

Despite these problems, the environmental-match approach has some merit. It can provide a useful measure of ballast water *hazard*, particularly if combined with a species-specific assessment. Together the two approaches can improve the risk assessment by helping to offset their individual limitations. The risk assessment developed by the Centre for Research on Introduced Marine Pests (CRIMP) advocates a combined approach (see below).

Qualitative, Species-specific

A qualitative, species-specific approach requires the analyst to qualitatively score the probability of a species successfully passing through each step of the ballast water invasion cycle. For example, the pest risk assessment developed by the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (Orr 1993, 1995) scores a species' presence in a vector, its entry

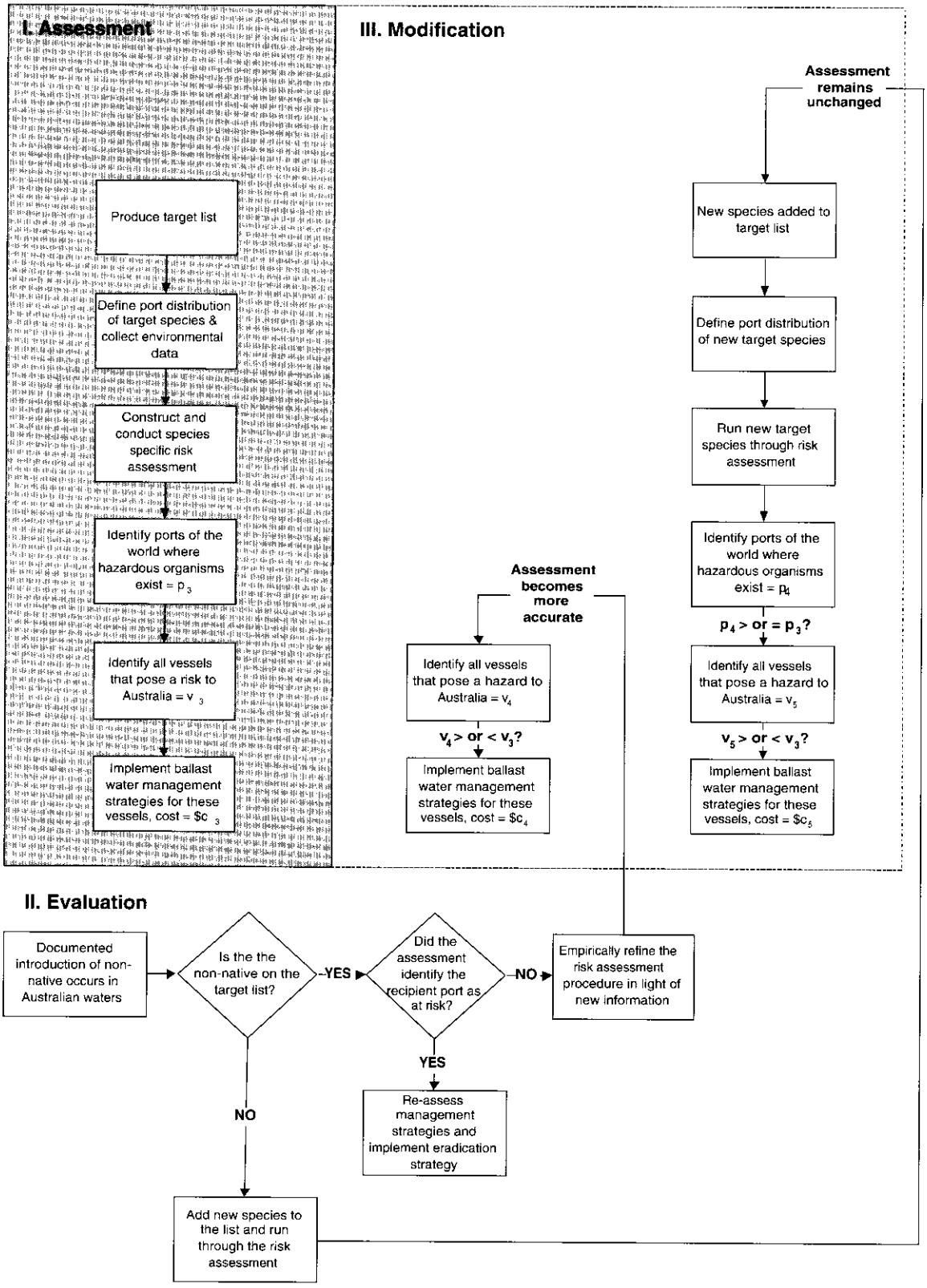

Figure 3. Species-specific risk assessments (I. Assessment) can be evaluated (II. Evaluation) and improved (III. Modification) when a new exotic species is discovered. If the species is not on the current target list, it should be added immediately and the assessment run to protect those areas that are not infected. If the species is already on the target list, the risk assessment is examined to see if the calculations can be improved in light of the new information.

potential, colonization potential, spread potential, economic impact potential, environmental impact, and perceived impact. It also allocates an uncertainty code to each of these scores to reflect the assessor's certainty in this respect. New Zealand's National Institute of Water and Atmospheric Research (Bradford-Grieve *et al.* 1998) advocate this assessment style for ballast water introductions.

In the short to medium term, this approach will provide only qualitative or mixed quantitative/qualitative outputs based on quantitative estimates of the early steps in the ballast water invasion cycle (*e.g.*, presence and entry potential). Ecological science is many years away from being able to provide reliable quantitative measures of establishment (colonization potential) and impact (economic or environmental) for all species without extensive tests. Qualitative (or mixed output) risk assessments are well suited to examination by expert panels, who can discuss the evidence and reach a consensus on the risk, but are unsuited to rapid or real-time evaluations, because (1) the risk assessment process is difficult to automate, and (2) the overall vector risk (due to the presence of two or more species in a single vector) cannot be calculated.

The process is difficult to automate because there are (usually) too many risk permutations –overall risk estimates that are unique for a single species. For example, the APHIS pest risk assessment process scores seven steps in the invasion cycle as high, medium, or low, and allocates one of five uncertainty codes to each of these scores. The total number of risk permutations for any one species is therefore $(3 \times 5)^7 = 1.7 \times 10^8$–clearly a difficult procedure to automate.

If the probability of success at each step in the ballast water invasion cycle is independent (in a statistical sense) from species to species, then the overall vector risk for two or more species is the sum of the individual species risk–or more correctly, the multiple of the invasion probability complement. But how does one sum qualitative or mixed output expressions of species risk in a meaningful way? How many "negligible risks" sum to a "moderate risk"? This question simply cannot be answered in a qualitative fashion.

Quantitative, Species-specific

A more rigorous quantitative approach to the ballast water invasion cycle also requires a species-specific approach; however, extant risk assessment models do not adequately address the steps of the invasion cycle. Quantitative Risk Assessment (QRA) models, initially developed for the chemical and nuclear industries, however, provide a general approach to the development of a Quantitative Biological Risk Assessment for species introductions. The QRA model is broken into five steps: hazard identification (what are the undesired events), likelihood of the undesired events, consequences of these events, risk calculation, and significance and uncertainty analysis.

Hazard identification is the key component of any risk assessment process. Hazards that are not identified in the early stages of the assessment will not be taken into account at later stages, leading ultimately to an underestimate of risk. Numerous methods for identifying hazards are available, including the collation of expert "heuristics", the use of fault tree analyses, and the use of hazard and operability analyses.

These methods are well suited to vector-based hazard identification; however, in order to develop a species-specific analysis it is assumed that criteria for identifying target species (the species hazard) are accepted and defined in advance. A well-established argument in quarantine risk assessment (see for example Orr 1993) is that mitigating measures developed for one species can be just as effective for other species with similar characteristics. The protection afforded by a species-specific assessment can therefore be improved by selecting target species that are representative of broad guilds or that are among the most robust relative to the determinants of ballast water transport: for example, species with wide environmental tolerances, pelagic larvae, or resistant, resting life-stages. Ballast water strategies that are effective against these species will protect recipient ports from a much wider group of species.

The assessment procedure can be easily designed so that it is identical for any species and therefore any species can be assessed, assuming the data requirements are met. The important question remains, however, as to which species are pre-selected for assessment. Once an initial "target list" is developed, new or suspected marine pests discovered anywhere in the world could be added to this list. A quantitative species-specific risk assessment could then estimate the probability that this species would

also be introduced by ballast water once key biological information was collected.

If an existing target species is detected in a recipient port, but the site was not marked as at risk, then the assessment can be empirically modified to account for the error. Thus, the risk assessment can improve with time in an iterative learning fashion (see Figure 3). This might lead to more vessels being identified as hazardous but, unlike the environmental-match approach, this outcome is not inevitable.

Calculating the likelihood and consequences of undesired events is the most difficult component of a quantitative ballast water risk assessment. The undesired events in question usually relate to the adverse economic and environmental impacts associated with the establishment of exotic species. To express these quantitatively, the analyst must model each step in the ballast water invasion cycle and ultimately link the probability that the species will be introduced, survive, and establish in the recipient region, with the consequences of this expressed in terms of, for example, loss of a commercial species or environmental impairment. As noted above, however, ecological science is many years away from being able to provide reliable quantitative measures of establishment and impact (economic or environmental) for invasive species without extensive tests. For example, toxic dinoflagellate introductions are estimated to have social costs (tourism, public health, and aquaculture–excluding broader ecosystem effects) in the region of $350–2000 million (AQIS 1994). The upper end of this range is almost certainly an underestimate because of environmental costs that are notoriously difficult to estimate.

Having said this, it would be unduly pessimistic to believe that qualitative risk estimates are the best that ballast water risk analysts can hope to achieve in the near term. The ballast water invasion cycle involves several steps–vector infection, survival, establishment and/or dispersal, and impacts. The uncertainty associated with each step increases from left to right, *i.e.*, from infection to impacts. Quantitative probabilistic techniques become increasingly inappropriate as one moves from low to high uncertainty. It may not be necessary, however, to quantify all of the steps in the invasion sequence. For species which are *a priori* pests, with a well-documented impact history, quantified estimates of inoculation (*i.e.*, all those steps up to and including survival in the recipient area), which are relatively certain, may be sufficient from a risk manager's perspective.

The advantages of more certain, quantitative risk measures are enormous–risk estimates relative to acceptance criteria become meaningful, risk management strategies can be compared, and risk-benefit analyses conducted. To achieve this, however, an earlier endpoint in the ballast water invasion cycle (such as inoculation survival) must be used in the assessment. Note also that this remains a suitable platform to address the probability of establishment and subsequent economic/environmental impact when these components can be estimated with reasonable accuracy and scientific rigor.

The analyst can calculate the invasion risk associated with an individual species by multiplying the probability that the species successfully negotiates each step in the ballast water cycle by the attendant economic and environmental costs of establishment and spread. Currently, however, no quantitative species-specific approach has been developed that can readily be applied to the invasion process via ballast water, largely because of the difficulty of calculating the probability and cost of establishment.

Alternatively, if invasion risk is expressed purely in terms of inoculation, multiplying the probability that the species negotiates each step up to and including survival in the recipient region allows a much simpler risk calculation. The overall vector risk for one or more species is calculated by multiplying the complement of the risk of each individual species such that

$$\text{Risk}_{\text{vector}} = 1-\prod_{i=1}^{n}(1-p_i), \qquad [1]$$

where p_i is the invasion risk for species i.

The CRIMP Risk Assessment Framework

The CRIMP risk assessment approaches the ballast water problem from the perspective of the invading species. It is therefore species-specific and will, in the first instance, be applied to a target list of selected species (Table 1) ratified by ABWMAC. These species have been designated as marine pests in their native or introduced ranges through a qualitative assessment by a panel of experts. The CRIMP assessment assumes that each of these species will represent a high risk if it were to be inoculated and survive in an Australian port.

Table 1. The current Australian Ballast Water Management Advisory Council target species list.

Species	Common name	Life-stage category[a]
Sabella (=Spirographis) spallanzanii	Sabellid fan worm, European fan worm	Larvae/gametes = meroplanktonic[b]; juvenile/adult = benthic (hard); possibly tychoplanktonic or floating detachable
Carcinus maenas	European shore crab, green crab, N. Atlantic edible shore crab	Larvae/gametes = meroplanktonic[b]; juvenile/adults = benthic (hard and soft); possibly tychoplanktonic as juveniles
Asterias amurensis	N. Pacific seastar, Japanese seastar	Larvae/gametes = meroplanktonic[b]; juvenile/adult = benthic (hard and soft); possibly tychoplanktonic as juveniles
Undaria pinnatifida	wakame	Gametophytes/sporophytes = benthic (primarily hard, some soft, associated with seagrasses and shells) and tychoplanktonic; some indication of formation gametophyte ball, which may become suspended in water column; possibly floating detachable due to settlement of other algae
Alexandrium catenella *A. minutum* *A. tamarense* *Gymnodinium catenatum*	Toxic cyst-forming dinoflagellates	Adults = holoplanktonic[b]; cysts = tychoplankton
Musculista senhousia	Asian mussel, Bag or Senhouse's mussel	Larvae/gametes = meroplanktonicb; juvenile/adults = benthic (hard and soft); possibly floating detachable and tychoplanktonic due to settlement on seagrass and algae.
Corbula gibba		Larvae/gametes = meroplanktonic[b]; juvenile/adults = benthic (soft and some nestling); possibly tychoplanktonic
Crassostrea gigas	Japanese oyster, Pacific (king or rock) oyster	Larvae/gametes = meroplanktonic[b]; juvenile/adults = benthic (primarily hard but can settle on soft to form oyster beds); possibly tychoplankton as juveniles due to settlement on seagrass and algae
Potamocorbula amurensis[c]	Chinese clam, Asian bivalve	Larvae/gametes = meroplanktonic[b]; juvenile/adults = benthic (soft and some nestling); possibly tychoplanktonic
Mnemiopsis leidyi[c]	Comb jelly	Adults/larvae = holoplanktonic[b]

[a] after Carlton *et al.* (1995), [b] possible vertical migrators, [c] not currently known from Australian waters

The risk assessment aims to provide a predictive, quantitative estimate of ballast water risk to:

- screen and prioritize vessels for sampling (on arrival) by field officers;
- allow a risk-benefit analysis for different strategies of ballast water management;
- encourage the shipping industry to change its operations in order to minimize the risk of introducing (or spreading) exotic marine species in Australian waters, while simultaneously decreasing its management costs; and
- compare the threat from ballast water to other vectors.

Endpoint Selection and Ballast Water Risk

The purpose of a predictive risk assessment is to prevent or minimize harm, which is expressed through the assessment endpoint. Appropriate endpoint selection is critical to the success of any ecological risk assessment (Suter 1993). The endpoint selected for the CRIMP ballast water risk assessment is the survival of a non-native organism in the recipient port at the time of inoculation.

It has been suggested that limiting the risk assessment to a survival endpoint can be justified only when the probabilities and consequences of establishment are indistinguishable between targeted species (Bradford-Grieve *et al.* 1998). This is not implied; rather, the survival endpoint was chosen for three reasons

- it is appropriate for a quarantine, barrier-control, risk assessment;
- it provides a suitable basis for risk-benefit analysis for species that are *a priori* classified as marine

Table 2. The universal set in a donor port can be characterized according to the habitat and behavior of the species concerned.

Category	Member examples
HOLOPLANKTONIC organisms that spend most or all of their life cycle in the water	PHYTOPLANKTON: diatoms, dinoflagellates, blue-green algae, nanoplankton, autotrophic picoplankton, and other groups. ZOOPLANKTON: comb jellies, jellyfish, hydrozoans (siphonophores), polychaete worms, rotifers, gastrotrichs, planktonic gastropods (snails: the pteropods and heteropods), copepods, hyperiid amphipods, isopods, mysids, ostracods, cladocerans, pelagic shrimps, krill (euphausiids), arrow worms (chaetognaths), pelagic tunicates (including salps, doliolids and larvaceans). FISH
NEUSTONIC organisms that swim or float at or near the air-sea interface	Larvae and juveniles of the by-the-wind-sailor siphonophore *Velella*, the blue button *Porpita*, nauplii and cyprids of the barnacle *Lepas*, and the sea strider *Halobates*
MEROPLANKTONIC Temporary plankton — organisms that spend a portion (usually the shorter) of their life cycle in the plankton.	PHYTOPLANKTON: the dispersal propagules of benthic plants. ZOOPLANKTON: the larvae of many benthic invertebrates including sponges, sea anemones, corals, hydroids, molluscs (snails including sea slugs or nudibranchs), chitons, mussels, clams, oysters, scallops, crustaceans (barnacles, shrimp, lobsters, crabs, hermit crabs), nemerteans (ribbon worms), sipunculans, polychaete worms, bryozoans, phoronids, echinoderms (seastars, brittle stars, sea urchins, sea cucumbers), hemichordates, tunicates (sea squirts) FISH: eggs and larvae
MIGRATORY organisms, including DEMERSAL organisms that migrate vertically towards the surface	These organisms include a variety of small crustaceans (including gammarid amphipods, isopods, mysids, cumaceans, crangonid and other shrimp, and benthic harpacticoid copepods), some fish species, and polychaete worms. Other examples are the wood-boring gribble *Limnoria*, a tiny isopod crustacean that migrates at night by swimming between wood habitats
TYCHOPLANKTON (organisms that can be removed from their previous habitat by tidal currents, waves, and ship's propellers, etc., including benthic organisms that could be brought into the vessel with bottom sediment)	Foraminiferans, flatworms, polychaetes, crustaceans (copepods, amphipods, isopods and tanaids), hydroids, benthic copepods, insect larvae and adults, mites, nematodes, leeches, oligochaete worms
FLOATING DETACHED biota including EPIPHYTIC organisms on the blades of floating plants	Seaweeds (algae), seagrasses (eelgrass, *Sargassum*, turtle grass), marsh plants, spirorbid tubeworms, bryozoans, sea squirts, sponges, molluscs, crustaceans
DISEASES, PATHOGENS, and PARASITES	Marine diseases, pathogens, and parasites, including mariculture and aquaculture diseases

pests, or potential pests; and
- it provides a clear (and verifiable) risk measure that avoids the more complex expression of the likelihood of establishment and of adverse environmental or economic impact.

By limiting the endpoint to survival, the CRIMP assessment is able to provide decision-makers with a quantified measure of invasion risk with reasonable bounds of uncertainty. Furthermore, with an inoculation survival endpoint, ballast water risk on a per vessel/per voyage basis can be calculated with the following elements for each target species:
- the probability $p(\omega)$ that each port from which a vessel derives ballast (donor port) is contaminated with the target species;
- the probability $p(\phi)$ that the vessel is subsequently infected with any of the life-stages of the target species;
- the probability $p(\psi)$ that the inoculum survives the journey; and
- the probability $p(\upsilon)$ that the life-stage at the end of the journey will survive if discharged into the recipient (Australian) port.

Ballast water risk can be calculated by assigning probability to each of these steps, such that:

$$\text{Risk}_{\text{species}} = p(\omega).p(\phi).p(\psi).p(\upsilon) \qquad [2]$$

Hazard Identification–Vessel Infection Scenarios

Two hazard-identification procedures are used in the CRIMP risk assessment framework:

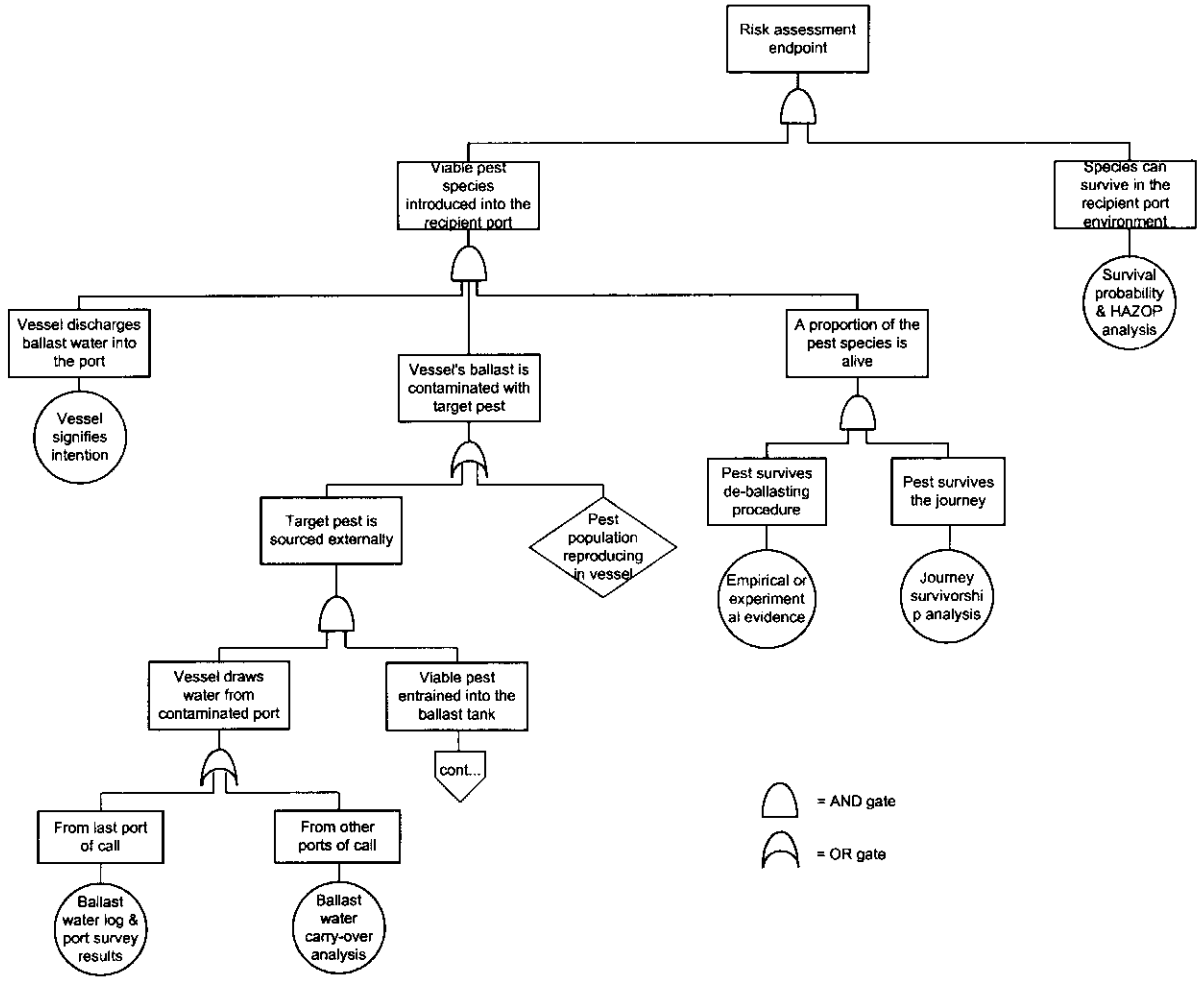

Figure 4. The first page of the ballast water introduction fault-tree analysis developed for the CRIMP ballast water risk assessment framework (note continuation to additional fault trees; for the complete analysis see Hayes and Hewitt 1998)

1. Fault-tree analysis to identify vessel-infection scenarios—the circumstances in which a vessel's ballast water becomes contaminated with target pests; and
2. Hazard and Operability (HAZOP) analysis to test for deviations from the "normal" environmental conditions in recipient ports and port-based activities that might confound the predictive algorithms in the assessment (refer to Hayes and Hewitt 2000 for further details).

Fault trees are hazard analysis tools that are used in quantitative risk assessment to identify the chain of events leading to a hazardous occurrence (Kletz 1986). They identify the logical combinations of events that are precursors of hazardous situations and, importantly, highlight the ways in which the event chain can be broken. If probability or frequency data can be generated for the basic events at the ends of each branch, then Boolean algebra can be used to estimate the overall frequency of the hazardous occurrence. This is not, however, the objective of the fault trees developed for this framework. Rather, their purpose is to identify all the potential vessel-infection scenarios in contaminated donor ports (for example see Figure 4).

The fault tree analysis provides a rigorous, explicit, and systematic description of the taxonomic hazard in donor ports and identifies a number of subtle (and less tractable) hazards within the ballast water introduction cycle. The "taxonomic hazard" is the set of species that can be introduced via ballast water. The universal set is defined as the complete floral and faunal assemblage in the donor port. This set can be categorized according to the life-stage characteristics of the species concerned (Table 2).

The fault-tree analysis helped define 10 vessel-

Table 3. Vessel-infection scenarios identified in the CRIMP risk assessment by a fault tree analysis.

	Planktonic	Tycho-planktonic	Neustonic	Vertical Migrator	Floating Detached
Water column					
Soft					
Hard					
Epiphyte					

Table 4. Hazard and risk assessment levels in the CRIMP risk assessment framework.

Level	Assessment (cumulative)	Principal data needs (additional)
0	Tests for donor-port infection and species tolerance of recipient port. Provides qualitative hazard rank.	Ballast source, donor port/bio-region infection status, recipient port temp/salinity maxima, species temp/salinity tolerance.
1	Tests life-stage entry into ballast tank, journey duration against life-stage duration, and simple entrainment analysis. Provides qualitative hazard rank.	Life-stage size and duration maxima, ballast sieve size, date since sieve was last serviced, life-stage habitat and characteristics, ballast event date and time.
2	Models journey survival. Provides quantitative estimate of risk (assuming probability of survival in recipient port =1).	Journey survival model and requisite parameters, ballast method.
3	Models life-stage survival in recipient port. Includes environmental subunit definition (recipient port) and environmental HAZOP analysis. Provides quantitative estimate of risk.	Recipient berth details, temperature and salinity extreme values, density estimate of extremes, EV distribution and requisite parameters.

infection scenarios (Table 3) based on the life-stage characteristics and the life-stage habitat–soft substrate, hard substrate, water column, or epiphyte. The likelihood of vessels becoming infected in each of these scenarios, for each life stage, will be modeled within the framework (see Module III below). The large number of infection scenarios underlines the complexity of the ballast water problem, and emphasizes the importance of using systematic hazard identification tools. Indeed, the success of the fault trees in this context suggests that they could be usefully employed in other complex biological systems.

The fault-tree analysis also helped to identify the roles of less obvious ballast water hazards, namely

- ballast tank populations–species that reside and reproduce within the ballast tank. This could be a very hazardous scenario if "fresh" (*i.e.*, not subject to the rigors of the journey) inoculations of larvae occur when the vessel de-ballasts.
- ballast water carry-over caused by multiple ballast sources in tank, or because of unpumpable ballast.
- crevicolous species–species which actively seek crevices and holes. The risk assessment assumes that entrainment into the ballast tank is essentially a passive process, and the infection probability is estimated accordingly. However, the probability of infection could be much higher for species that seek sea chests for shelter.
- third-party infection scenarios–the infection of vessels in ports that are themselves uncontaminated, but may have infected vessels in port that are discharging contaminated ballast water next to vessels which are taking on ballast water. This scenario is unlikely but not impossible.

With a species-specific approach, all of these hazards are amenable to analysis. For example, if the journey duration exceeds the duration of the larval stages of a fouling species, then these vessels can be flagged as potentially having juveniles settled on the inside of their ballast tanks. Hazards associated with unpumpable ballast can be assessed in relation to the age of the water and the likelihood of the species surviving in the ballast tank. Finally, the probability of vessel infection can be increased to allow for the behavior of crevicolous species whenever they are present in a donor port. Third-party infection scenarios, however, could probably be addressed only by a very sophisticated analysis (such as that envisaged at level 5 of the risk assessment framework--see below). For this reason, the probability of a vessel becoming infected is set at an arbitrary minimum value (0.05).

Levels of Hazard and Risk Assessment

The CRIMP risk assessment is designed to provide an increasingly accurate estimate of risk as more information is made available to the analysis. This has been achieved by writing six levels or tiers of analysis (0–5) into the framework. In the first instance (level 0), two qualitative hazard assessments are made based on simple environmental comparisons (between donor and recipient ports) and the

known distribution of target pests. The data requirements of this level are minimal: donor-port infection status, monthly temperature and salinity extremes of the donor and recipient ports, and the temperature and salinity tolerances of the target pest.

Table 4 summarizes the additional analyses and data requirements of the framework up to and including a level 3 analysis. At level 3, the assessment tests infection scenarios, models the survival of a pest during the vessel's journey, and estimates the probability of a pest surviving in the recipient port. Data requirements are increased, but the estimate of ballast water risk becomes more accurate. Additional levels of analysis (levels 4 and 5) are envisaged to include vessel-specific tychoplankton suspension analysis and port-circulation models (for further details see Hayes and Hewitt 1998, 2000).

In the absence of the requisite data, the assessment defaults to the highest level of analysis for which data are available. For example, the probability of survival in the recipient port is assumed to be 1.0 in a level 2 analysis. Similarly, a level 2 analysis stops and defaults to level 1 if there are insufficient data to run a model of journey survival. This ensures that the assessment maintains a conservative stance in the face of uncertainty, while also providing tangible benefits (in terms of risk reduction) for additional data costs.

The Risk Assessment Modules

The risk assessment framework is made up of five modules. The first (Module 0) collects data from the vessel on the origin of its ballast water and associated details. The remaining modules mimic the invasion process by dealing with individual steps in the ballast water introduction cycle up to the point of survival in the recipient port. The risk assessment modules and the role they play in the overall framework are illustrated in Figure 5.

Module 0–Data Collection

Module 0 collects information on the assessment date (expected arrival of the vessel in the recipient port), the name of the recipient port, the berth, the vessel's name, IMO number, and details of the ballast on board.

Ballast information is collected on a tank-by-tank basis, including date, volume, start/end time, method, and the vessel's draft at the start and end of ballasting. The module currently allows for three different ballast sources for each tank, in order to assess the risks of ballast water carry-over and the mixing of ballast water in and between tanks, but it is desirable to take into account all ballast origins for each tank.

Module I–Port Infection Status

The objective of Module I is to determine the probability of port infection. If a survey of a donor port detects a target species, then the probability of infection $p(\omega)$ is equal to 1.0. If the survey did not detect a target species, then the probability of infection is the product of the probability of a type II error (the species was present but not detected by the survey) and the probability that species can actually survive in the port. The probability of a type II error can be determined using geometric probability arguments and line intersect and transect sampling theory (see Hayes and Hewitt 2000 for details) and is in part alleviated by using a standardized sampling design and methodology (Hewitt and Martin 1996). Ultimately, the probability of port infection will also be linked to the time elapsed since the last survey.

If the port concerned has not been surveyed, then its infection status is inferred from that of the bioregion in which the port is located. A database detailing the bioregions of the world, their infection status, and the ports they encompass has been developed by CRIMP. This is largely based on the bioregional classification of Hayden *et al.* (1984) as adopted by the International Union for the Conservation of Nature (Kelleher *et al.* 1995), ports information from the Fairplay Ports of the World Guide (Fairplay 1998), and the published distributions of species on the target list.

Module II–Port Environment

Module II characterizes the port environment. It is used at level 0 to determine whether the species can tolerate the temperature and salinity maxima and minima of the recipient port. In level 3, the module uses empirical density or kernel density estimates (Silverman 1986) of daily temperature and salinity extremes or, given enough data, extreme-value theory, to estimate the probability $p(\upsilon)$ that the target species will survive in the recipient port. The same techniques are used to calculate the probability that the target species can survive in the donor port, in order to determine the probability of infection (see above).

From level 3 onwards, the geographical unit of

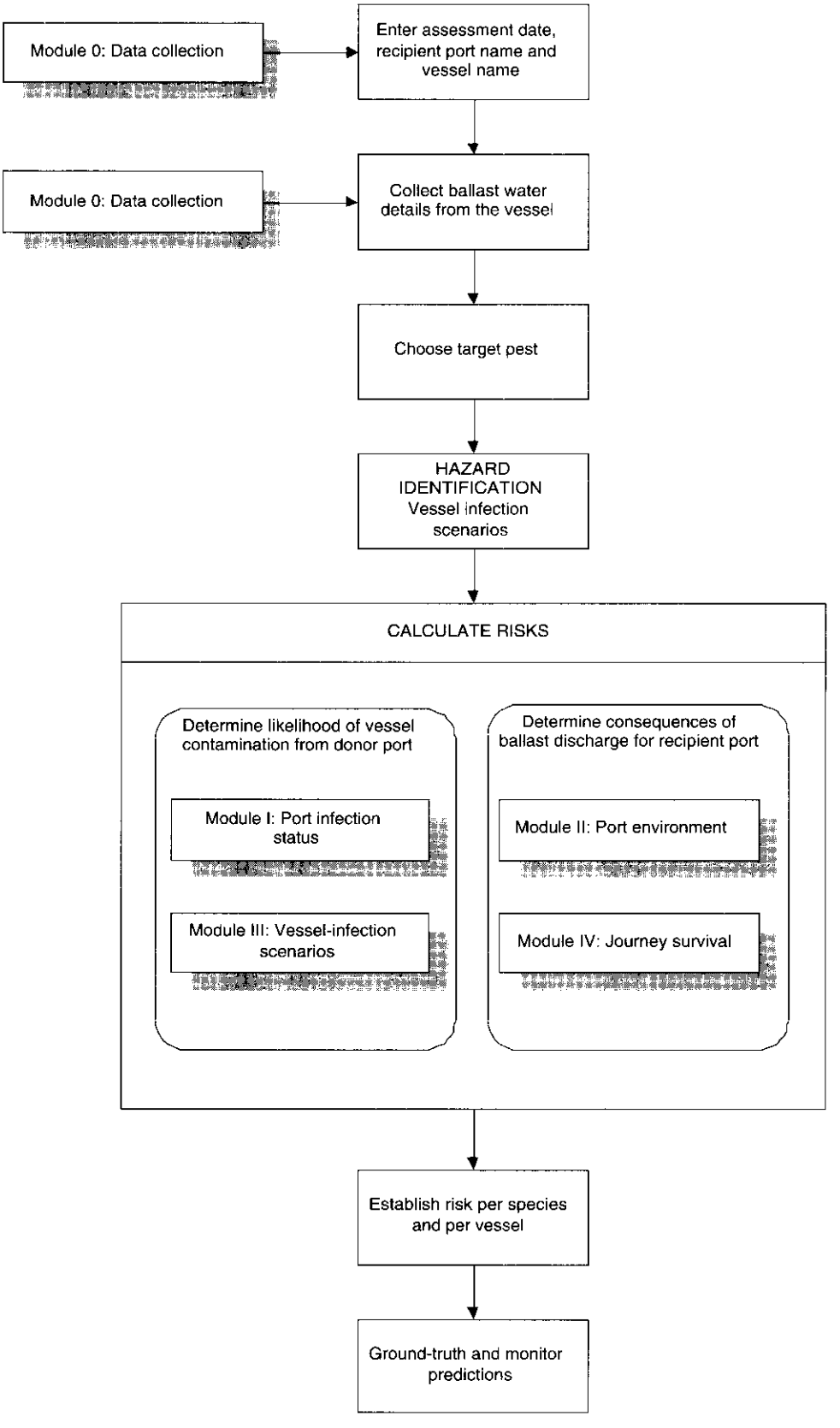

Figure 5. The CRIMP ballast water risk assessment framework illustrating the risk assessment modules used to collect data from the vessel and to calculate ballast water risk.

assessment is based on environmental subunits, to which all berths in the recipient port are allocated. The subunits are analogous to the delineation of eco-regions based largely on climatic similarity (Bailey 1983). Relatively small ports may comprise just one environmental subunit, while larger ports may comprise two or more subunits. In addition, ports with unique artificial environmental areas such as heated outflows will be identified. This approach emphasizes that the management boundaries of a port may not be the most appropriate for risk assessment purposes. At levels 4 and 5, module II may also be used to extend the survival analysis to include a wider set of environmental parameters such as dissolved oxygen, pH, or nutrients, depending on the availability of data.

Module III—Vessel-Infection Scenarios

Module III models the vessel-infection scenarios for life stages that are small enough to enter the ballast tank (as determined by the ballast sieve diameter, maximum life-stage size, and some allowance for corrosion of the sieve). For the life-stages of most species, vessel-infection scenarios will be mutually exclusive. The overall probability of vessel infection is defined as:

$$p(\phi) = 1-\prod_{r=1}^{m}\prod_{i=1}^{n}[1-p(\phi_{r,i})] \qquad [3]$$

for the life-stages (r = 1 to m) of particular target-species, under infection scenarios (i = 1 to n).

Infection analysis is not conducted at level 0; the probability of vessel infection is simply assumed to be 1.0. At level 1, the vessel-infection analysis is relatively simple. Water-column-sourced planktonic and neustonic infections occur ($p(\phi_{r,i}) = 1.00$) whenever life stages of the species are expected to be in the water column. Otherwise the life-stage(s) are assumed to be unavailable to the vessel ($p(\phi_{r,i}) = 0.05$), allowing for the unquantified third-party risk.

Asterias amurensis, for example has five life history stages: egg/gastrula, bipinnaria, brachiolaria, juvenile, and adult. Vessel-infection scenarios for each life stage are mutually exclusive. The larval life stages (egg/gastrula, bipinnaria and brachiolaria) can cause water-column sourced, planktonic infections (Table 3). Like many echinoderms, the larvae spend a relatively long time in the plankton. In the Derwent estuary, larvae are likely to be in the water column from July to January (Byrne *et al.* 1997; CSIRO unpublished data). In a level 1 analysis, vessels ballasting in Hobart during this period would be classified as infected $p(\phi_{r,i}) = 1.0$; where i = 1 [water-column/plankton], and r = 1 to 3 [three larval stages]).

Gymnodinium catenatum has two life history stages: vegetative cells and cysts. The vegetative cells can cause water-column-sourced planktonic infections whenever they are present in the water column, particularly during bloom events. The cysts, however, are associated with two vessel-infection scenarios that are not mutually exclusive: cyst production during blooms can lead to water-column sourced planktonic infections, and resuspension of cysts from contaminated sediments can lead to soft-substrate sourced tychoplankton infections (Hallegraeff 1998).

In a level 1 analysis, vessels ballasting in deep ports, outside of a bloom, would be classified as infected with vegetative cells ($p(\phi_{i,r}) = 1.0$; where i = 1 [water-column/plankton] and r = 1 [vegetative cells]). In shallow ports where sediment resuspension occurs due to natural processes, vessel-berthing activity, or other port-based activity during a bloom, vessels would be classified as infected ($p(\phi_{i,r}) = 1.0$, through three scenarios: i = 1, 2 [water-column/plankton and soft-sediment/tychoplankton], for r = 2 [cysts]; and i= 1 [water-column/plankton], for r = 1 [vegetative cells]). More sophisticated levels of analysis are envisaged at level 4, based on the Rouse equation and propeller-wash models, together with an analysis of the ballast-withdrawal envelope (see Hunter 1997; Hayes and Hewitt 2000). This analysis, however, requires extensive data input, including information on third-party vessel activity in the donor port. So while most of the theory for these models is well developed, they have not been incorporated into the lower levels of the framework because they are data intensive.

Module IV—Journey Survival

The objective of Module IV is to determine the probability that the life stage(s) entrained into the ballast tank survive the journey. At level 1, a simple competency analysis compares the journey's duration with the minimum time to settlement for those life stages that are small enough to enter the ballast tank. If the journey's duration exceeds this period, then a warning is issued about the potential for ballast-tank populations.

At level 2, module IV models journey survival based on journey duration, an appropriate statistical

model and its associated parameters (for example see Hayes 1998). Higher levels of analysis may also incorporate the effects of en-route ballast management strategies (*e.g.*, open-ocean ballast water exchange, heat treatment) and pump versus gravity ballasting in order to reflect the expected influence of the ballast pump on the species surviving the journey (see for example Gollasch *et al.* 1995; Murphy 1997 and Hallegraeff 1998).

Future Research Directions

The framework provides a blueprint for ballast water risk assessment. In the first instance, the risk/hazard assessments may be quite crude. The models and risk algorithms used in the framework will probably change as it develops and additional data are collected. The multi-level, modular approach, however, will remain, and with time the framework will provide more accurate estimates of risk. The future development of the framework is expected to include

1. increasing the knowledge base by gathering port environmental data (international and national) and biological data on the behavior and tolerance of target species
2. assessing port-infection status relative to the probability of a type II error during a survey, date of the last survey, and the availability of pest-monitoring strategies
3. developing journey-survival models for relevant life stages of species on the targeted list
4. modeling propeller wash and sediment resuspension. Initial studies in this area (Murphy 1998) indicate that the theory of propeller wash and sediment re-suspension/settlement is sufficiently advanced to implement vessel/berth specific models of tychoplankton infection from soft sediments. This is an important infection scenario for the cysts of dinoflagellate species outside their relatively short bloom windows (Hallegraeff and Bolch 1992)
5. examining the feasibility of using port circulation models in conjunction with models of the ballast withdrawal envelope to determine the probability of vessel infection and the size of the inoculum
6. developing ground-truthing procedures to test the predictions of the risk assessment and provide additional data to continually improve the assessment algorithms. These procedures could be developed in conjunction with Bayesian inference techniques to allow rapid updates of risk estimates, in accordance with the iterative improvement of quantitative risk assessment.

Discussion

Quantitative ballast water risk assessment, as with any other management strategy, will not eliminate ballast-mediated invasions. It does, however, offer at least two important advantages over our current state of uncertainty:

- it will help clarify the importance of ballast water in relation to other transport vectors; and
- it will provide a means to test the cost-effectiveness of ballast management strategies.

Currently, it is very difficult to investigate the cost-effectiveness of ballast management strategies. The economic costs are relatively easy to quantify: for example, the operating costs of ballast water exchange are estimated to be US $0.014–0.045 per mt ballast (Rigby and Taylor this volume), so the total operating cost of mandatory ballast water exchange, for foreign shipping bound for the United States would therefore lie in the region of US $112–362 million per annum (using Carlton's 1995 discharge estimate and assuming 100% compliance).

By contrast, the benefits of ballast water management are probable and extremely difficult to quantify. For example, ballast water exchange is thought to eliminate 67-86% of the zooplankton in a tank (Locke *et al.* 1993), but probably fewer of the organisms resident in ballast tank sediments, and may under some circumstances exacerbate the problem (Hay *et al.* 1997; Hallegraeff pers. comm.).

The benefits of exchange could be considerable–at least US 500 million per annum using Weathers and Reeves' (1996) estimate–assuming it could prevent the introduction of a species as noxious as the zebra mussel and that ballast was the responsible invasion vector in the first instance. Without a quantified risk assessment, however, it is impossible to quantify the importance of ballast water as a vector, to value the expected benefits of exchange, or to identify the circumstances when other management options might be more appropriate.

It seems very unlikely that any single management strategy will be the most cost-effective for all vessels on all domestic and international routes. A quantified ballast water risk assessment, using target or representative species or both, will allow managers and the shipping industry to maximize the impacts

of research and management activity. Ultimately, the assessment is expected to contribute to a wider Integrated Ballast Management strategy similar to that envisaged by Carlton *et al.* (1995), but allowing for a variety of management options, including instances where vessels cannot exchange their ballast water because the journey is too short or the weather is too bad. In this way, the risk assessment would allow flexibility in our response to the vessel, species, and site-specific factors that underlie ballast water invasions.

Acknowledgments

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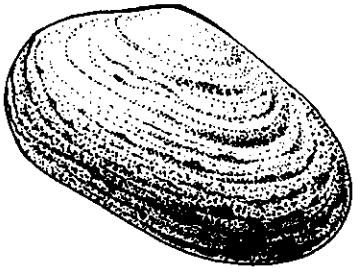
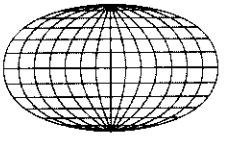
Outreach and Education



'An informed and educated public generates increased support for research, prevention, control, and outreach programs, helps slow the rate at which invasions occur or spread and increases the rate at which new invasions are discovered.'



Outreach and Education Overview

Linking Academia and the Public through Outreach and Education

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Since the discovery of zebra mussels, *Dreissena polymorpha*, in Lake St. Clair in 1998, Sea Grant outreach and education programs have played leading roles in facilitating the flow of information from the research community to the public about invasive nonindigenous species. An informed and educated public generates increased support for research, prevention, control, and outreach programs, helps slow the rate at which invasions occur or spread and increases the rate at which new invasions are discovered.

Outreach is one of the three mandated functions of Sea Grant, established in 1966 as a partnership between the National Sea Grant College Program of the National Oceanic and Atmospheric Administration (NOAA) and one or more universities in each of the coastal and Great Lakes states. Modeled after the Land Grant Colleges, it is a network of 30 programs that awards grants for marine and coastal research, serves a non-advocacy outreach function, and fosters public awareness of coastal and marine environments through educational programs.

Strong ties to both local constituencies and academic institutions enables Sea Grant outreach staff to react quickly to put together comprehensive programs addressing new issues at the local, regional and national levels. From international workshops to one-on-one meetings, fact sheets and technical reports to videos and radio spots, Sea Grant outreach builds bridges between good science and local constituencies who can use the information to make informed choices.

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Outreach and education programs for aquatic nuisance species (ANS) originated in the Great Lakes Sea Grant network, and much of their expertise and resources were drawn upon as the Northeast, Mid-Atlantic, Gulf and Western states began to address this global problem. A National Training Initiative was initiated to provide assistance and resources to non-Sea Grant states. Sharing of research results occurred via conferences, newsletters, peer-reviewed web sites, videos, and printed materials. And, as concerns about aquatic invaders extended to species other than zebra mussels and to other geographic areas, outreach programs shifted from primarily reactive modes to proactive modes as well.

The following three papers offer a glimpse of the breadth of Sea Grant outreach programs for aquatic nuisance species. Chuck O'Neill describes the improvements made to New York Sea Grant's National Aquatic Nuisance Species Clearinghouse that enables researchers worldwide to search a comprehensive database of published research and facilitates the timely sharing of information among all users. Christine Reilly, a former Massachusetts Institute of Technology student reports on a marine bioinvasions Internet web site, one of the first to address invasions occurring in our coastal waters. The site encourages visitors to take report sightings of a new invader (the Asiatic shore crab, *Hemigrapsus sanguineus*) to a researcher who is documenting its spread. Washington Sea Grant's Andrea Copping describes the aggressive approach undertaken in Washington state to raise public awareness of marine invaders like the green crab, *Carcinus maenas*. Her outreach program demonstrates the benefit of multi-

agency collaborations on issuing accurate, up-to-date information to the public. This helps communities to react quickly to new and potential invaders by working together to identify and disrupt introduction pathways and build community support for prevention and control programs. These are three examples of the important role that Sea Grant Programs play in serving as a liaison between scientists, coastal managers and policy makers and the public.

The First National Conference on Marine Bioinvasions is example of an excellent Sea Grant outreach program. In this case, the international participants have forged global ties and the work has just begun...

Using Public Outreach and Education as a Means of Prevention and Control of Nonindigenous Species Introductions

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Key words: public education, outreach, nonindigenous species, Pacific Northwest

INTRODUCTION

The introduction of nonindigenous species (NIS) into the coastal waters of the Pacific Northwest poses a serious economic and environmental threat (Copping *et al.* 1994; Chapman 1998; McMurray and Bailey 1998). Regulations and technology are needed to address a number of pathways by which NIS enter coastal and estuarine waters. However, there are a number of pathways by which NIS can be spread or introduced into a body of water that can only be addressed by outreach and education.

EDUCATION AND OUTREACH

Education is an effective tool for slowing or preventing the spread of marine invasive species, also called "aliens," "exotics," or nonindigenous species. A survey conducted by the Minnesota Sea Grant Program showed that the second most effective source of information in their region, behind newspaper and television, was signage placed at water accesses. Because of the results, natural resource management agencies, regional task forces, and other organizations in 27 states are now using signs at water accesses (D. Jensen, pers. comm.). Furthermore, there is growing evidence that boater education works to prevent or slow the spread of NIS (D. Jensen, pers. comm.).

The reason public education is effective is that it can be the only means to address certain vectors for the spread of NIS. For example, recreational boaters may have encrusting organisms on the boat hull, or plants entangled in the propeller. When the boats are launched in a new body of water, they can bring viable organisms with them. Recreational anglers using live bait often dump the bait buckets overboard. Bait species are frequently nonindigenous. Dumping them overboard can bring a new infestation. Aquarium hobbyists, who don't want to kill unwanted pets, decide to "set them free" by releasing them into a nearby body of water. In eastern Washington state, goldfish have become a nuisance species in a number of lakes as a result of this activity. And the nursery industry sells NIS species that can spread or are improperly disposed of into nearby waterways.

The concept of NIS species is still unknown to large sectors of the public. Greater awareness of the problem through public education, and of how individual actions play a role in the transmission of NIS, is essential to slowing or preventing introductions.

The primary goal of environmental education is to change behavior on the part of individuals. In this instance, the goal is to lead people to change behaviors that may introduce marine invasive species. Early environmental education efforts focused primarily on providing information, believing that providing information to the public would change their behavior, an assumption that was later undermined.

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A synthesis of environmental education research undertaken by Hungerford and Volk (1990) revealed that there were three essential components to environmental education to change behavior: entry-level variables, ownership variables, and empowerment variables. Entry-level variables include fostering environmental sensitivity through contact with nature or, as the early environmental education campaigns did, providing information. For example, bringing people out on a boat ride or to an aquarium helps to put them in touch with the environment or resource that needs protecting. Ownership variables establish a sense of place and a stake in the resource. The Puget Sound region, for example, is an area highly prized by the residents of Seattle and the surrounding towns and cities. People consider it "their" sound, part of their back yard, a feature of the region they are proud of and consider their own. Puget Sound helps define the place they live, and this feeling can be used to engender environmental stewardship. Finally, a component that was not often provided in the initial environmental education programs is empowerment variables. Empowerment variables allow an individual to feel that they can take action, that they can do something to protect the resource that they care about. Hungerford and Volk (1990) found that without this variable, behavior change is unlikely to occur.

With behavior change as the ultimate goal, developing an outreach and education campaign consists primarily of four steps: identifying target audiences, designing the message, determining the vehicle or delivery mechanism for the message, and evaluating the outcome. The planning of these four stages should be done prior to developing and distributing a product or program.

Identifying target audiences

The first task in developing outreach is to identify target audiences. The number and type of target audiences can vary greatly depending on the message behind the campaign. These groups or audiences will often require different messages and/or delivery vehicles. For example, Washington Sea Grant Program began working on marine invasive species outreach by identifying appropriate target audiences. The target audiences broke down into about five major groups, but even within those groups, it was necessary to identify subgroups. Short press briefings or fact sheets would be appropriate to reach decision-makers; hands-on training or a display at a fair is more effective for the general public. Once the target audiences are identified, the next step is to develop the appropriate message.

Designing the message

In developing simple and consistent messages regarding marine invasive species, the focus is on:

- impacts/relevance of the issues – why should the audience care?
- ways for the audience to identify new or existing infestations; and
- how the audience can prevent the spread of NIS – the empowerment variable identified by Hungerford and Volk (1990).

Each of the final products for nonindigenous species outreach developed by the Washington Sea Grant Program included these elements. In effect, each product tried to include all three of the variables identified by Hungerford and Volk (1990) for successful environmental education – entry-level, ownership, and empowerment variables.

Determining the delivery mechanism

There are a variety of delivery vehicles, and both budget and target audience will help determine the best mechanism for delivery. For example, although public service announcements may be an effective method to reach the general public, few organizations have the resources to produce one. However, there are other ways to reach target audiences. For example, the general public can be reached effectively with a traveling exhibit for fairs; a hands-on training workshop would reach a more specific audience such as a "beachwalkers" volunteer group; and plastic signage at boat ramps is an effective way to reach recreational boaters.

Washington Sea Grant Program has been developing a series of NIS educational products in collaboration with the State of Washington, the National Estuary Program, the U.S. Fish and Wildlife Service, and the Georgia Basin/Puget Sound International Task Force. The goal of these educational products is to increase awareness of NIS and their potential impacts on the environment and economy.

Among the educational products produced by the Washington Sea Grant Program are

- A 20 page booklet entitled "Bio-Invasions: Breaching Natural Barriers," which explains in simple, clear terms the threat of non-native aquatic

species and potential impacts on the economy and on the environment. Audience: anyone interested in a more than cursory explanation of what NIS are and why we should care.

- Fact sheets on common pathways for aquatic NIS introductions, the European green crab (*Carcinus maenas)*, and the Chinese mitten crab (*Eriocheir sinensis*). Audience: natural resource managers, volunteer monitoring groups, beachwalkers, tribes, general public, decisionmakers, and policy makers.
- A full-color identification card for the European green crab (*C. maenas)*, including features that distinguish them from similar-looking native crabs. Audience: research managers, volunteer monitoring groups and beachwalkers, aquaculture industry workers, and tribes.
- A training workshop on identification of the European green crab (*C. maenas*). Audience: volunteer groups, industry workers, tribes, and agencies.
- A poster and T-shirt with an eye-catching design introducing the concept of invading species and a simple explanation of what invading species are and how they can be transported. Audience: general public, state parks, zoos, and aquariums.
- A card for distribution at pet stores and other venues explaining why aquarium hobbyists should not release pets or plants into the wild and suggesting alternatives to dumping. Audience: general public.

The identification guide for the European green crab was developed with a specific concern in mind. Since an overly eager public might start smashing native crabs, mistaking them for the European green crab, the identification guide provides photos of *C. maenas* and four native species. On the flip side are scientific illustrations and descriptions with distinguishing features of the crabs highlighted, allowing individuals to carefully identify any crabs.

Evaluation

The final step of public outreach and education is evaluation. How do you know if the project is effective? The evaluation should be considered during the planning stages of the project so that all steps of the project will be focused on fulfilling the stated objectives. There are a large number of ways to evaluate a program, particularly if the staff and monetary resources are available for evaluating changes in behavior over time. However, it is sometimes necessary to do the next best thing. With limited resources, Washington Sea Grant Program, for example, frequently uses questionnaires and follow-up surveys. Another way to evaluate success is by the amount of media attention generated. Washington and Oregon Sea Grant Programs held a workshop on the European green crab in February 1998. Although no European green crabs were present in Washington or Oregon at the time, researchers felt certain that the crabs would soon arrive through natural dispersal and currents along the coast. The conference generated some press coverage, including a lengthy newspaper article. Responding with an opinion-editorial piece generated even more information. Five months later, when European green crabs were found on the outer coasts of Washington and Oregon, Washington Sea Grant Program became a major source for information on the green crab, and has sent out more than 3,500 identification guides on the European green crab and over 6,000 fact sheets on pathways and the green crab.

Summary

Marine invasive species do not respect state or national boundaries. They travel from one region to another through both natural dispersal, such as tides and currents, and human-mediated dispersal, such as ballast water. Consequently, regional efforts are required to slow the spread of NIS. This recognition spurred the formation of the Pacific Northwest Marine Invasive Species Team, or MIST, a joint program between Oregon and Washington Sea Grant Programs to coordinate efforts along the West Coast. MIST has also been working cooperatively with California, British Columbia, and Alaska.

The Chinese mitten crab (*E. sinensis*) is one of the latest exotics to threaten the western U.S. coast. The Chinese mitten crabs spawn and develop in estuaries and migrate up into the watershed until time to spawn, when they return to the estuaries. They are highly mobile and aggressive and have been causing major disruptions in northern California; it is likely to follow the path of the European green crab up the coast to the Pacific Northwest. MIST worked cooperatively with the California Department of Fish and Game to produce a fact sheet describing the mitten crab and the threat it poses to our region, and will probably follow up with further efforts.

Successful invasions by NIS species are increasing, sometimes with far-reaching consequences for ecological diversity and survival of endangered species. An informed and educated public is widely

recognized as the cornerstone of effective prevention and control of aquatic NIS species (Jensen 1998). To achieve this end, it is essential that information/education efforts convey up-to-date and accurate facts that are appropriately targeted and offer a consistent message.

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Source of Unpublished Material

Jensen, D., Exotic Species Information Center Coordinator, Minnesota Sea Grant College Program, 22 March 2000.

Reaching Out: Use of Web Sites to Increase Public Awareness of Marine Bioinvasions

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ABSTRACT: An Exotic Species Web Site was developed to inform and educate the public about marine bioinvasions. In order to determine what held the interest of visitors, a tracking program was used to monitor web site activity. From February 1998, when the site first became available to January 1999, visitations increased stepwise with listing the site on search engines and links from other sites. Analysis of traffic on the site indicates that users were primarily interested in obtaining information about marine invasive species and that maintaining fresh new material is important in keeping their interest.

Key words: outreach, monitoring web use

INTRODUCTION

Marine bioinvasions are increasing, but public awareness has lagged behind the efforts to prevent introductions of new species and to control and manage invasive species. The Massachusetts Institute of Technology Sea Grant (MITSG) College Program initiated an outreach project to increase public awareness and understanding of the issues related to marine bioinvasions, with a focus on species that are found in New England. Among the several components of the outreach activities was the development of a web site, http://massbay.mit.edu/exoticspecies/index.html, that included sound scientific information, highlighted specific invasive species, suggested contacts for more information, provided a list of selected references on marine invasions, and highlighted new activities and efforts. In addition, information about the First National Conference on Marine Bioinvasions was available on the web site, including online conference registration.

A group of advisors guided the MITSG outreach activities and provided advice on what to include on the web site. Three phases were identified to attract visitors, sustain their interest, and induce them to return: (1) creating an attractive, clever pull, (2) organizing the first page for easy accessibility and clarity of what information is available, and (3) keeping the web site dynamic. In addition, there was a commitment to have scientific and technical information be peer-reviewed. Although the review process delayed making pages available to the public, it ensured quality control of materials made available.

USE OF THE MITSG WEB SITE

The Exotics Species Web Site had approximately 2000 visits between June 1998 and January 1999, with the greatest number of visits shortly before the First National Marine Bioinvasions Conference, which was held January 24–27, 1999. Prior to June 1998, there was little activity; however, in June the site was listed with major web search engines. Activity on the web site tripled over the next several months with periodic peaks associated with the First National Marine Bioinvasions Conference. About a quarter of the visitors came from links on related sites, another quarter were repeat visitors, who came specifically for

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the Conference information, and over half of the visitors went to one or more pages at the site. Use of the web site is one measure of interest; another measure is the number of requests, for the additional materials that are listed on the web page, including scientific references, general information on a variety of topics, slides of invasive species, and use of the zebra mussel kit. The number of responses to requests for the questionnaire on sightings of *Hemigrapsus sanguineus,* the Asian shore crab, has been minimal and suggests that field observations will not necessarily be reported through the web site. This analysis of web site traffic showed that web site users were primarily interested in obtaining information about marine invasive species and that maintaining fresh new material is important in keeping their interest.

Information on use of the site and requests for materials have been incorporated into redesign of the site. These include identifying which pages have been peer reviewed, a "what is new" section, and increased links to other sites.

The National Aquatic Nuisance Species Clearinghouse Searchable Electronic Database: A Tool for Researchers Worldwide

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Key words: aquatic nuisance species, database, coastal U.S., Great Lakes, North American inland rivers, information

INTRODUCTION

The National Aquatic Nuisance Species Clearinghouse serves as a major link between the aquatic nuisance species research community and a wide array of end-user audiences, encouraging and facilitating the transfer of aquatic nuisance species research information and technology among universities, governmental agencies, industries, and special interests throughout North America and worldwide. The Clearinghouse also plays a high-profile role as a primary nexus for identifying completed, current, and proposed aquatic nuisance species research activities and for linking researchers with similar interests pertaining to important marine and freshwater aquatic nuisance species introduction, spread, research, and policy initiatives.

CLEARINGHOUSE

The Clearinghouse addresses marine and freshwater aquatic nuisance species throughout the Gulf of Maine, Northern Atlantic, Mid-Atlantic, Southern Atlantic, Gulf of Mexico, Central and Northern California, Pacific Northwest, and Great Lakes regions, as well as North American inland river systems, and is the home of North America's most extensive technical library of published research and other materials pertaining to zebra mussels (*Dreissena polymorpha*) and "quagga" mussels (*Dreissena bugensis*), and has libraries on the Atlantic green crab *(Carcinus maenas)*, the Amur River Corbula *(Potamocorbula amurensis)*, the Chinese mitten crab *(Eriocheir sinensis)*, gribbles (*Limnoria* spp.), the grass carp *(Ctenopharyngodon idella)*, the Suminoe oyster *(Crassostrea ariakensis)*, *Limnoperna fortunei*, shipworms *(Teredo navalis)*, the brown mussel *(Perna perna)*, the green lipped mussel (*Perna viridis*), the Asian clam (*Corbicula fluminea*), the blue mussel (*Mytilus edulis*), the dark false mussel (*Mytilopsis leucophaeata*), the Asiatic mussel (*Musculista senhousia*), the New Zealand mud snail (*Potamopygrus antipodarum*), the Eurasian ruffe (*Gymnocephalus cernuus*), the round goby (*Neogobius melanostomus*), the tube-nose goby (*Proterorhinus marmoratus*), the rudd (*Scardinius erythrophthalmus*), the spiny water flea (*Bythotrephes cedarstroemi*), the fish hook water flea (*Cercopagis pengoi*), and the blueback herring (*Alosa aestivalis*). All of the information in the Clearinghouse is accessible to any researcher, agency, industry, utility, student, or other individual or group having need of the information. Most publications in the library can be obtained in hardcopy on interlibrary loan directly from the Clearinghouse.

A new, searchable electronic database of the Clearinghouse's Technical Library Bibliography is now available on the Clearinghouse's World Wide Web home page. Citations include: author(s), title, document source and date, an annotation, whether the document is a journal article or other type of publication, document length, the language in which the document is written, whether the document is available on interlibrary loan from the Clearinghouse or direct from some other source, and the copying/mailing fee if the document is available from the Clearinghouse. The database is keyword searchable via a 170+ keyword, four level search outline. Documents which are available directly from the Clearinghouse on interlibrary loan can be ordered on-line by means of a convenient order form at the end of each search result report.

The World Wide Web address for the database is: http://cce.cornell.edu/seagrant/nansc/. The web site

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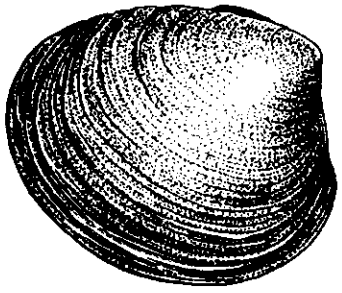
also contains a series of detailed maps charting the range expansion of the zebra mussel and the "quagga" mussel in North America since 1989, as well as information on a number of other informational and educational materials available from the Clearinghouse.

The poster presentation introduced attendees to the *new* National Aquatic Nuisance Species Clearinghouse Searchable World Wide Web Aquatic Nuisance Species Database. A laptop computer containing a working copy of the Clearinghouse's entire web site on CD will be utilized, linked to a data projector, to enable attendees to explore the Clearinghouse's web site, especially the searchable database, including being able to perform keyword and full text searches of the database.



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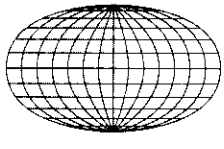

ABSTRACTS

Patterns of Invasions

Insights from a Toy Ocean: Invasion Dynamics in Lake Victoria and Implications for Marine Coastal Waters

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Key words: fish, species interaction, water quality, Lake Victoria

A series of deliberate and accidental introductions into the world's largest tropical lake can shed light on the dynamics of invasive species in semi-enclosed and isolated marine water bodies. We have examined the landscape dynamics and conservation genetics of the interaction between a rich indigenous fish fauna and three highly disruptive invaders in the context of chronic, progressive eutrophication in Lake Victoria, East Africa. The invading species are Nile perch (*Lates* cf. *niloticus*), Nile tilapia (*Oreochromis niloticus*) and water hyacinth (*Eichornia crassipes*). Following a series of rapid and catastrophic initial impacts including a mass extinction, a still-rich remnant indigenous fauna has exhibited an astonishing tenacity and resiliency. This is attributable mostly to the spatial and temporal complexity of refugia, the powerful shaping influence of water column conditions, and counterintuitive interactions between invaders and their indigenous relatives. Maintenance of indigenous taxa is partly dependent upon the amelioration of chronic water quality insults unrelated to the invasions themselves. Analogies are drawn to impacted and invaded coral reef systems.

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Rapana venosa in the Chesapeake Bay: Current Status and Prospects for Range Extension Based on Salinity Tolerance of Early Life History Stages

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Key words: *Rapana venosa*, gastropod, Chesapeake Bay, larva, salinity tolerance, range

The veined Rapa whelk, *Rapana venosa*, has recently been identified as present in the Hampton Roads region of the Chesapeake Bay. The species is native to the Sea of Japan, but was introduced to the Black Sea in the 1940s, and has since spread to the Aegean and Adriatic Seas. There is strong evidence that range extension is mediated by transport of early life history stages in ballast water. The current status of knowledge of distribution of *R. venosa* in the Chesapeake Bay is described. There is concern over the potential impact of *R. venosa* on local shellfish populations and the industry that they support. Egg cases of *R. venosa* have been collected from the field, and larval forms cultured in the laboratory. Estimates of the salinity tolerance of the larval stages of *R. venosa* are described as a precursor to estimating a potential range of distribution of the species within the Chesapeake Bay and its subestuaries. Such estimates are crucial to establishing which shellfish resources are potentially susceptible to predation by local *R. venosa* populations.

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Habitat and Prey Preferences of Veined Rapa Whelks (*Rapana venosa*) in the Chesapeake Bay: Direct and Indirect Trophic Consequences

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Key words: *Rapana venosa*, gastropod, Chesapeake Bay, habitat use, infauna, burrowing, hermit crab, *Clibanarius vittatus*

The recent discovery of veined Rapa whelks, *Rapana venosa* in the lower Chesapeake Bay has ecological consequences beyond the obvious potential for predation on commercially valuable shellfish prey species (*e.g.*, *Crassostrea virginica*, *Mercenaria mercenaria*). In the Black Sea and in their native Sea of Japan, *Rapana* have been reported primarily from hard bottom habitats. Adult Chesapeake Bay *Rapana* have been collected from both hard and soft bottom habitat. Laboratory observations indicate that adult *Rapana* prefer sand bottom and will burrow almost completely into the sand at water temperatures >20°C (*i.e.*, not overwintering behavior). Burrowing behavior by these large apex predators expands the potential suite of vulnerable prey items to include infaunal shellfish (*e.g.*, *Mya arenaria*, *Ensis directus*, *Cyrtopleura costata*). The presence of large (>100 mm) empty *Rapana* shells in Chesapeake Bay may enhance growth of the local hermit crab, *Clibanarius vittatus*. Recent collections of *C. vittatus* from the Hampton Roads area indicate that these animals use empty *Rapana* shells as shelters and are reaching previously unrecorded sizes. The implications of abnormally large crustacean scavengers on Chesapeake Bay benthic epifauna (*e.g.*, oyster spat) are discussed.

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Ecology and Ballast-Mediated Transfer of *Vibrio cholerae* O1 and O139

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Key words: *Vibrio cholerae*, transfer patterns, ballast water, pathogen, cholera

The large-scale dispersal of ballast water by commercial ships provides a significant vector for the transfer of pathogens. We are studying *Vibrio cholerae*, the etiological agent of human cholera, as a model system to measure ballast-mediated transfer of pathogens as well as possible ecological and human health consequences. We sampled the ballast water of 15 ships arriving to Chesapeake Bay from foreign ports, and all ballast water originated from coastal regions of the North Sea or Mediterranean Sea. Our analysis showed a high prevalence of two different serovars: O1 (100% of vessels) and the recently emerging O139 (93% of vessels). *Vibrio cholerae* O1 was found at greater densities than O139. Furthermore, in a comparison of whole water samples (a composite of plankton and bacteria >0.2 mm) and homogenized plankton samples (plankton >80 mm), *V. cholerae* densities were significantly greater in the whole water samples. Analysis of Chesapeake Bay water now indicates the presence of this novel serotype, 0139, previously unreported in U.S. waters. These data suggest that ship-mediated dispersal may play an important role in the emergence and epidemiology of some waterborne diseases.

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Importation of Organisms Associated with Bait Worms from Vietnam

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Key words: bait, microorganism, *Vibrio*

The importation of bait creates a potentially important pathway for invasions by nonindigenous species and their associated biota. Since at least 1994, a large nereid polychete worm has been imported from Vietnam and distributed to various places in California, Maryland, and Oregon for use as fishing bait. We measured the abundance of microorganisms, and especially *Vibrio* bacteria, associated with these bait worms and the material in which they were shipped. Twelve worms were obtained directly from various bait shops in the Maryland/Virginia area. Both the worms and packing materials were analyzed using a range of techniques. We measured the abundance of many taxa by direct counts under a compound microscope. We estimated the abundance of culturable *Vibrio* bacteria, using standard plating techniques on agar. We also measured the abundance of two serotypes of *Vibrio cholerae*, using monoclonal antibodies for direct detection. Our results indicate diatoms, ciliates, flagellates, nematodes, and at least seven different genera of amoebae were present in the packing materials. In addition, we found multiple species of *Vibrio*, including both serotypes of *V. cholerae*. Although the fate of these organisms upon release remains unknown, our data indicate an active pathway exists for invasion of many different microorganisms and possible pathogens.

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The Risk of Nonindigenous Species Introductions to Puget Sound, Washington through the Shipment of Live Bait

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Key words: marine worm, aquatic nuisance species, pathway, Puget Sound, Washington, risk assessment, vector

The shipment of marine bait worms from Maine for recreational purposes–a likely pathway for nonindigenous species (NIS) introductions in other Pacific coast estuaries (Carlton, 1989; Cohen *et al.*,1995)–is not presently regulated in Washington State. In order to assess the risk of NIS introductions to Puget Sound, Washington through this pathway, we surveyed users of the product and product suppliers. The surveys consisted of telephone interviews, visits to local live bait worm vendors and product inspection. We found no evidence that live marine bait worms were available through local vendors or of a local market for live marine bait worms. Therefore, we determined that the live bait worm trade does not appear to pose a threat to the Puget Sound region.

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Cryptogenic Seaweeds, Seagrasses, and Marine Lichens in Port Valdez, Alaska: Who Are They and How Did They Get There?

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Key words: seaweed, seagrass, marine lichen, alga, estuary, cryptogenic, dispersal, biogeography, Port Valdez, Alaska, distribution

During a recent field and literature survey of marine species introductions in Port Valdez, Alaska, a new checklist of the seaweeds, seagrasses and marine lichens was prepared and examined for the presence of non-indigenous species. Although no unequivocal introductions were discovered, over half of the 113 species were found to be widely distributed: 25 occurred circumboreally and 34 had ranges extending into the southern hemisphere. These widespread species, considered cryptogenic because of their obscure origins, are thought to have a high probability of being anthropogenically introduced or misidentified in some parts of their range. Many of these taxa have excellent natural dispersal capabilities. Of the 59 cryptogenic species reported for Port Valdez, 44 are known to survive unattached, and at least 26 have life histories that are ephemeral and frequently reproduce and/or fragment. Nearly all also occur as fouling organisms on the hulls of ships, and the propagules of many can survive in ballast water for the shorter times now required for ships to cross the North Pacific. It is likely that the broad ranges of these species have been created by both natural and anthropogenic means. Future studies that employ field, molecular and morphological techniques should help to resolve the cryptogenic nature of these species and determine their native vs. introduced status in the areas they inhabit.

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Shellfish Culture as a Vector for Biological Invasions

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Key words: polychaete, shellfish culture, oyster

The introduction of seed stocks of nonindigenous commercial shellfish has acted as a vector for the introduction of exotic marine invertebrates into the United States coastal waters. The most important consumable oysters in U.S. restaurants are not indigenous. On the Pacific coast, the Japanese oyster, *Crassostrea gigas* has been cultivated for more than 50 years. Formerly, seed stocks were imported from Japan and set out on tidal flats to grow and mature. On the Atlantic coast, the European oyster, *Ostrea edulis*, was imported in a similar manner. Although modern culture methods include rearing of larvae in local laboratories rather than importation of juveniles, there is considerable evidence that many species of polychaetes were probably imported with the oyster seed stocks. The distribution of polychaetes by this vector may account for the wide distribution of some species. Two types of polychaetes are capable of transportation with seed stocks: (1) shell borers that form tunnels or channels in the shell itself; and (2) soft-sediment worms that are transported in mud on and between the shells. Shell borers that appear to have been transported in this manner include: *Polydora websteri* and *P. brevipalpa*. Sediment dwellers include: *Polydora cornuta, Pseudopolydora kempi, P. paucibranchiata, Phyllodoce mucosa, Harmothoe imbricata*, and *Nereis succinea*. An additional mode of transportation is with the direct importation of marketable

products from a source country to a host country where the shellfish is sold in local markets. For example, in the early 1980s large specimens of a nonindigenous shell boring spionid, *Boccardia acus*, were found in a New Zealand mussel that was for sale at a fish market in Honolulu. This review suggests mechanisms of establishment for several nonindigenous species of marine polychaetes and recommends strong quality control measures intended to protect local shellfish from damage caused by exotic shell borers.

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Introduction of the Green Porcelain Crab, *Petrolisthes armatus* (Gibbes, 1850) into the South Atlantic Bight

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Key words: *Petrolisthes armatus*, oyster reef, rocky shore, South Atlantic Bight

The green porcelain crab, *Petrolisthes armatus* (Gibbes, 1850), is widely distributed in the eastern Pacific, from the Gulf of California to Peru. In the Atlantic, it is found in tropical western Africa, Ascension Island, Bermuda, the Bahamas, throughout the Gulf of Mexico, the West Indies and Caribbean, and South America to southern to Brazil. As early as the 1930s, the species was collected from the Florida Atlantic coast at Biscayne Bay and Miami Beach, and it was found on *Phragmatopoma* reefs south of Cape Canaveral in the early 1970s. Since that time, it has become well established in the Indian River system, however it was not reported north of Cape Canaveral until 1994, when faunal surveys on St. Catherines Island, GA revealed its presence there. After its initial discovery on St. Catherines Island, the species increased dramatically in abundance there, becoming the dominant decapod crustacean on rocky substrates and tidal creek oyster bars by the following spring. In South Carolina, it was first observed in low densities in the spring of 1995 at various locations, becoming quite abundant by the fall. It is now well established on rocky rubble, oyster reefs and other shallow subtidal and intertidal habitats throughout Georgia and South Carolina, as far north as Pawley's Island, SC. Densities of *P. armatus* greater than 20,000 indiv./m^2 were observed on collectors deployed for one month in its preferred habitat, and larval stages were collected from the plankton of coastal inlets in Georgia during the past two years. Data on abundance, geographic distribution, length-frequency, sex ratio, and reproductive status of *P. armatus* have been collected to document its introduction into the South Atlantic Bight. There are many potential pathways for its introduction, both natural and anthropogenically assisted. Although we have no data to suggest which of these is the principal pathway, several possibilities have been considered, including transport in ballast water from foreign and domestic ports and among cultured mollusks transported from localities within its previously established range. Nothing is presently known about the ecological impact of this recently established filter-feeding crustacean in its newly exploited habitat; however, studies are underway to examine its recruitment and to evaluate its interactions with co-occurring species.

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Evaluations of Marine Encrusting Community Invasibility

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Key words: encrusting community, fouling, invasibility, resistance

The Eltonian concept of a native community-level resistance to the invasion of new species forms the foundations of modern invasion theory. This paradigm has received tacit support from mathematical models and recent work in terrestrial plant communities supports the corollary argument that disturbance surmounts the intrinsic native resistance. An empirical evaluation of invasibility in a marine encrusting community of Coos Bay, OR is presented in which a reciprocal community transplant was used to explicitly test these assumptions. Species-rich (marine derived) communities were hypothesized to be less invasible (*i.e.*, more resistant) than species-depauperate (estuarine) communities. In summary, native communities were susceptible to invasion in all instances, but to varying degrees. These differences in invasibility were primarily due to differences in native species cover at the outset of the experiment. These results are then compared with more recent information collected from a series of port surveys from around Australia.

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Northward Extension of the Geographic Range of *Hemigrapsus sanguineus* in Massachusetts, 1996-1998

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Key words: *Hemigrapsus sanguineus*, geographic distribution, crab, range expansion

Hemigrapsus sanguineus, a crab (family Grapsidae) native to the western North Pacific Ocean, was established on the east coast of the United States by 1990. Its abundance noticeably increased between 1993 and 1994 in Buzzards Bay, Massachusetts. In 1996, we received reports of the crab in Sandwich and Wellfleet on Cape Cod, and on Martha's Vineyard. Since 1996, we have been tracking the northward spread of the species and monitoring changes in newly-established populations in Cape Cod Bay, Nantucket Sound, and Narragansett Bay. In 1996, density estimates were made on populations in Sandwich (Cape Cod Bay) and Washburn Island, part of Waquoit Bay National Estuarine Research Reserve (Nantucket Sound). In 1997, the population on Washburn Island had increased more than ten-fold. *Hemigrapsus sanguineus* was also present in 1997 in Manomet (Plymouth, MA) and near Brant Rock (Marshfield, MA), along the shore of Cape Cod Bay. By 1998, *H. sanguineus* was found as far north as Scituate, MA, and populations in Plymouth and Marshfield had doubled in size. In addition, well-established populations occurred on Hope Island, in the middle reaches of Narragansett Bay.

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Early Life History of *Hemigrapsus sanguineus*, a Nonindigenous Crab in the Middle Atlantic Bight (USA)

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Key words: *Hemigrapsus sauguineus*, reproduction, salinity tolerance

The Asiatic shore crab, *Hemigrapsus sanguineus* (de Haan), was recently introduced to the northeast coast of the USA. The crab has established intertidal populations extending throughout the Middle Atlantic Bight. This study defines early-life-history characteristics that are germane to range extension in this species. Results of the investigation showed that the spawning season of *H. sanguineus* continues for at least 4 mo in the southern Middle Atlantic Bight. This is considerably longer than the spawning seasons of co-occurring native crabs. Eggs hatch about 14 da after extrusion, and females have the potential to produce several broods each year. Zoeal larvae are tolerant of a wide range of temperature/salinity combinations, and mean duration of zoeal development ranges from approximately 16 da at 25°C to 55 da at 15°C. At 25°C, zoeae are capable of development to the megalopa stage at salinities as low as 15 parts per thousand. At lower temperatures the zoeae require salinities above 20 ppt. The megalopa stage appears to have more stringent temperature/salinity requirements, which may restrict *H. sanguineus* to rocky shores of the coastal ocean and the adjacent high-salinity regions of the estuary. Under these conditions megalopae molt to the first juvenile stage in approximately 25 da post hatching. Newly metamorphosed crabs reach the fifth juvenile instar in 35 da. Dry-weight growth of zoeal larvae and early stage juveniles is exponential at respective rates of 23 and 8% of body weight per day.

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Occurrence of Nonindigenous Species in the Gulf of Mexico

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Key words: Gulf of Mexico, nonindigenous species, ballast water

The Gulf of Mexico is considered a large marine ecosystem because of its hydrography, geomorphology and the inter-relationship of its flora and fauna. It contains two zoogeographic provinces along with tropical, subtropical and temperate flora and fauna shared between three countries. A series of National Oceanic and Atmospheric Administration and U.S. Environmental Protection

Agency co-sponsored workshops on the introduction of nonindigenous species in the Gulf of Mexico was held from June 1997–September 1998. The workshops characterized the extent of nonindigenous species introduction phylogenetically and geographically, an overview of bioinvasion pathways, and a discussion of preventive recommendations and subsequent actions to be taken.

This phylogentic and geographical overview will address the introduction of primary shrimp viruses, zebra and brown mussels, coastal fishes, nutria and introduced flora. Pathways and unintentional distributions of species will be discussed with emphasis on the extent of shipping and potential ballast water exchanges in major Gulf of Mexico ports.

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The Incidence of *Hemigrapsus* Relative to Salinity Values in the Delaware Bay Estuary and the Inland Bays of Delaware

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Key words: *Hemigrapsus,* salinity tolerance, Delaware Bay

The Asiatic shore crab *Hemigrapsus sanguineus,* is an invasive species well established in the Delaware Bay Estuary (large estuary) but has not been documented in the Inland Bays of Delaware (small estuary). In this study *Hemigrapsus* crabs were collected at the highest salinity levels and along sampling stations toward lower salinity levels in both the Delaware Bay and the Delaware Inland Bays. Salinity and ambient water temperature values with the incidence of *Hemigrapsus* were compared between the two estuaries systems.

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Polydora cornuta Bosc, 1802 (Polychaeta: Spionidae): World Wide Invasion

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Key words: world wide invasion, polychaete, spionidae, *Polydora cornuta*

A spionid polychaete, *Polydora cornuta* Bosc, 1802 (formerly known as *Polydora ligni* Webster, 1897), is recorded for the first time from the western Pacific: Russia (the Sea of Japan) and Taiwan, and from Brazil in spite of numerous investigations of polychaetes in these countries. Two specimens of the species were found in 1994 in Peter the Great Bay, Sea of Japan, fouling shells of the scallop, *Mizuhopecten yessoensis* (Jay). No other specimens have been collected in the area since then. Whether one immigration event has taken place or if a continual invasion to the Sea of Japan is occurring is unknown. Numerous reproducing specimens of the species were found off western coast of Taiwan in 1996 and in Paranagu Bay, Brazil in 1998. In all the cases, the species was found near international seaports, in areas of reduced salinities. It is likely that the species, an oyster killer in North American fisheries, has been transported to new areas in the water-ballast tanks of cargo ships. Being previously reported from east, west and gulf coasts of North America, from Europe, Mexico, Argentina and Australia, *P. cornuta* becomes one of the world wide spread species. Ecology, reproductive biology, the characteristic features and morphological variability of *P. cornuta* are discussed. The high degree of polymorphism previously reported for the species is suggested to be due to confusion with other species rather than the individual variability of *P. cornuta.*

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The Introduction of Polychaetes *Hydroides elegans* (Haswell), *Polydora limicola* Annenkova, *Pseudopotamilla occelata* Moore into the North-Western Part of the Sea of Japan

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Key words: polychaete, Sea of Japan, thermal pollution

The polychaeta fauna of the benthos and fouling of the north-western part of the Sea of Japan was studied during the period of 1971-1998. Three introduced species of polychaetes: *Hydroides elegans* (Haswell), *Polydora limicola* Annenkova, *Pseudopotamilla occelata* Moore were found. *H. elegans* was discovered only on the artificial surfaces in Zolotoy Rog Bay (port Vladivostok), where this species may occur because of "thermal pollution" due to the discharge of warm waters of the water cooling system of Thermal-Electric Power Station-2 (TEPS-2) in Vladivostok, which has been in function since 1971. The abundant population *H. elegans* exists in the bay throughout the year and is capable of reproduction. The biomass of *H. elegans* may reach several kg/m^2 in August–September. *P. limicola* was

found at the same time in the fouling of hydrotechnical structures of Vladivostok, Nakhodka, Holmsk and Uglegorsk ports with a biomass of 1-3 kg/m^2. Slow introduction *P. limicola* occurs by coastal sail ships at present.

The invasion of *P. occelata* into the Peter the Great Bay may be an example of introduction and subsequent naturalization, which produced considerable changes in the structure of benthic communities. The three species of polychaetes are sessile organisms and their invasion occurred by ocean and coastal sea-going ships (unintentional transport vectors). *H. elegans* and *P. occelata* were most probably transported to the north-western part of the Sea of Japan from Japan, and *P. limicola* from the Kamchatka Peninsula.

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ABSTRACTS

Ecological and Evolutionary Consequences

Botryllid Ascidians: Few Invaders or Many?

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Key words: botryllid ascidian, species identification, genetic marker, life-history variation

Successful invasions of botryllid ascidians have been documented worldwide both recently and over the past 50-100 years. Ascidians, including botryllids, are now recognized as important invaders overtaking fouling communities and interfering with bivalve culture. What are the sources of these invasions, how many species are involved, and how might these invasions be controlled? These questions have not been easily answered due to problematic taxonomy for many ascidians including the botryllids. Molecular identification of problematic taxa, by comparing known voucher specimens from potential source populations with specimens from invading populations, is a promising technique for invasion tracking. Here, I show that genetic markers such as small ribosomal subunit and mitochondrial cytochrome oxidase DNA sequence data may be used to distinguish species of botryllids. Genetic information on North American botryllids on both coasts reveals more distinct species than previously understood from morphological characters. Behavioral and life-history variation between populations has raised the possibility of rapid differentiation or plasticity in introduced populations. I discuss multiple character approaches to distinguishing botryllid species including methods for collecting and preserving useful voucher specimens. Careful study of botryllid invasions should answer questions about mechanisms of invasion for a diversity of taxa with limited natural mechanisms of dispersal.

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Ecological and Evolutionary Consequences of Invasions: The Impacts of the European Green Crab on Multiple Trophic Levels in Central California

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Key words: European green crab, ecological impact, trophic level

Using long-term data and experiments in both field and laboratory, we quantitatively examine the ecological impacts of the nonindigenous European green crab in a soft-substrate food web in Bodega Harbor, CA. Our results show that significant direct effects of green crab predation—large reductions in green crab prey including bivalve

molluscs and crustaceans–have occurred within two years of the invasion. These prey populations have remained at very low abundances for four years since the introduction of the green crab. Also, significant indirect effects of green crab predation–increases in non-prey including polychaetes and tube-building crustaceans–have also occurred within two years of the direct effects. These direct and indirect responses to green crab predation are rapid and are nearly concurrent given the time scale of sampling. However, reductions in invertebrate prey populations have not resulted in changes at the higher trophic level occupied by shorebirds in the Bodega Harbor system. We have found no reductions in the abundances of wintering shorebirds over the four years since the green crab invasion. Therefore, the timing of changes in the abundances of native species, whether directly or indirectly affected by the invading green crab, may be more a function of life-history and recruitment rate than of position in the food web.

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Sources for Global Invasions by the Crab *Carcinus maenas* Using Sequence Variation in the Mitochondrial Cytochrome Oxidase Gene

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Key words: invasion, *Carcinus maenas*, phylogeography, mitochondrial DNA, population genetics

The European crab in the genus *Carcinus* has invaded five regions. Previous genetic analysis of the mitochondrial 16S ribosomal RNA gene in *Carcinus* allowed identification of Mediterranean or the Atlantic sources for introduced populations. However, more genetic variation partitioned geographically in Europe is needed to track sources for the invasions in a more refined way. We examined sequence data from mitochondrial cytochrome oxidase b (cyt-b) gene among introduced and native populations of *Carcinus*. A 450 bp portion of cyt-b was amplified using PCR for 125 individuals from various native populations and 237 individuals from introduced populations in Japan, Australia, South Africa, California and New England. Cyt-b sequences were much more variable than16S rRNA sequences, with 191 haplotypes in our sample. Sequences were aligned and geographic structure was inferred from genetic distances among populations. Despite high variability of cyt-b, identification of source regions was confounded by strong genetic bottlenecks associated with colonization events for most introduced populations.

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Where North Meets South: Invasion of Tasmania by the European Green Crab and Its Consequences for Native Crabs

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Key words: *Carcinus maenas*, green crab, nonindigenous species, predation

Some species have been remarkably successful at invading multiple global sites, but few studies have compared the effects of invasion among these sites. We have been studying the effects of the European green crab, *Carcinus maenas*, in multiple geographic regions to make such comparisons. Here, we describe effects of this invader on native crabs in Tasmania (Australia), invaded within the past decade, and briefly compare these to effects observed at other sites. During the austral summers of 1997 and 1998, we (1) conducted an intensive survey of decapod crustaceans in shallow water embayments of Tasmania, (2) measured mortality rates of native crabs as a function of *C. maenas* density, and (3) performed a series of field experiments to test for possible predation of *C. maenas* on native crabs. Our results indicate direct negative effects of *C. maenas* on the abundance of two native crabs: *Paragrapsus gaimardii* and *Philyra laevis*. Our survey data revealed that southern and southeastern coasts of Tasmania have not yet been colonized by *C. maenas*, and that native crabs are significantly more abundant here compared to within the invaded area. Mortality rates were also significantly higher for these native crabs in the presence of *C. maenas*, either in the field or manipulative cage experiments. Our data suggest *C. maenas* controls the abundance of both native crab species, and these results are similar to effects of *C. maenas* on a native crab in California.

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History and Impact of an Intertidal Invasion: Green Crabs (*Carcinus maenas* [L.]) in New England, 1900-1998

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Key words: *Carcinus, Littorina,* predator, climate change, Maine, sea surface temperature

The range of the green crab, *Carcinus maenas* (L.), in New England has changed with changing sea surface temperature (SST). In this paper I present (1) an historical review of changes in the distribution of *C. maenas* in New England since 1900 and (2) new data on expansion of *C. maenas* in Maine in the 1990s. I will also discuss the relationship between climate change and expansion of *C. maenas*, and the impact of green crab expansion on its periwinkle prey, *Littorina littorea.*

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Feasibility of Control by Trapping of the European Green Crab, *Carcinus maenas*, on Martha's Vineyard, MA (USA)

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Key words: *Carcinus maenas,* control, trapping

In response to the threat to publicly maricultured shellfish posed by the invasive European green crab, *Carcinus maenas*, in embayments on Martha's Vineyard, MA, several municipalities conduct trapping programs designed to reduce the abundance of this pest, thereby increasing shellfish survival. The presumed benefits of control of the long established green crabs has led to extensive trapping, although the effectiveness of this trapping remains to be tested. Despite large catches, municipal catch records of *C. maenas* do not suggest obvious decreases in catch per unit effort and/or changes in population structure. Given this experience, we attempt to determine if, in fact, trapping can be effective and, if so, what is the optimum level of trapping. Within each embayment, the adult *C. maenas* population appears to be relatively closed, although short-term movements within embayments will affect trapping strategy and success. Multi-year data on *C. maenas* abundance and population structure will be presented to identify seasonal fluctuations. Additionally, trap-independent snorkel surveys are used to evaluate short-term effects of trapping on *C. maenas* density. Lastly, we compare trapping success of two trap models. Broadly, given the increased need for control of exotic marine pests, the feasibility of trapping programs such as this need to be assessed.

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The Impact of *Carcinus maenas* on Patterns of *Mya arenaria* Survivorship

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Key words: *Mya arenaria, Carcinus maenas,* survivorship, size, burial depth, crab behavior

Studies conducted at the Wells National Estuarine Research Reserve in Wells, Maine evaluated the impact of the invasion of Atlantic green crabs, *Carcinus maenas*, on the population of native soft-shell clams, *Mya arenaria.* Field experiments conducted in the Little River estuary investigated differences in survivorship of clams depending upon clam size, clam density, site, and burial depth. The importance of clam size was tested using plastic flowerpots buried in the mud with each pot containing clams from one of four discrete size classes (5-15mm, 20-50mm, 55-60mm, 65-75mm). Half of the pots were covered with protective mesh (5 mm^2) to exclude crabs. Large clams (20-75mm) had high survivorship whether covered with protective mesh or not. Small clams (5-15mm) had low survivorship whether covered with protective mesh or not ($P<0.001$). High survivorship by large clams when protected and unprotected may be due to either clam size or burial depth making them less susceptible to crab predation. Low survivorship by small clams when protected and unprotected may be due to predation by crabs small enough to penetrate the mesh. Crabs small enough to penetrate the mesh were found in both protected and unprotected pots. Within the small size class of clams, survivorship was tested between different clam densities, but no significant difference in survivorship was found between high (50 clams/pot) and low (10 clams/pot) clam densities ($P=0.627$). Also within the small size class of clams, survivorship was tested between different sites within the estuary. The protective mesh was found to be effective at increasing survivorship only at the inlet site ($P<0.01$), which suggests there may be relatively more crabs small enough to penetrate the mesh at the upriver site. Separating clam size from burial depth tested the mechanism responsible for high survivorship by large clams. Large clams were found to have high survivorship in all cases except when not protected at shallow burial depths

($P<0.001$). Low survivorship at shallow depths suggests that deeper clam burial depth, not larger clam size, is the mechanism responsible for high survivorship of large clams. The patterns in *M. arenaria* survivorship indicate a need for further field work on size distributions and foraging strategies within *C. maenas* populations. The goal of restoring native clam populations in Maine requires identifying the mechanisms responsible for clam susceptibility through investigating the behavioral ecology of the invasive crabs.

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The Influence of Water Temperature on Induced Defensive Responses by an Intertidal Snail to a Introduced Crab Predator

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Key words: phenotypic plasticity, shell thickness, water temperature, *Carcinus maenas, Littorina obtusata,* Gulf of Maine

Two important goals of invasion ecology are to understand how populations respond to introduced predators and how such responses are modified by environmental conditions over the geographic range of prey. Crab-molluscan predator-prey relationships provide an outstanding system in which to address these questions. Recent transitions in shell form of two intertidal snail species to better defended morphologies have coincided with the invasion of a crab predator (*Carcinus maenas*) into the Gulf of Maine. Although these adaptive shifts have been attributed to rapid selection by *Carcinus*, recent experiments have shown that gastropods can alter shell form (*e.g.*, thicken shells) during ontogeny in response to crab effluent. The ability of gastropods to contend with an invading predator over a range of water temperatures, however, is uncertain, because calcium carbonate solubility increases as water temperature decreases. As a consequence, any predator-induced increase in shell thickness could be both physiologically difficult and costly for snails in colder waters. In the Gulf of Maine, water temperatures in the north average 6-8°C colder in spring and summer than those in the south, and previous surveys have shown that northern populations of the smooth periwinkle (*Littorina obtusata*) are thinner shelled and more vulnerable to crushing than southern populations. To test the influence of water temperature on the degree and cost of a predator-induced phenotypic response, *L. obtusata* individuals collected from a northern (Lubec, Maine) and southern (Manchester, Massachusetts) site were reciprocally transplanted and exposed to two predator treatments (*Carcinus* effluent, no *Carcinus* effluent) in field experiments. Regardless of source population or transplant location, snails raised in the presence of *Carcinus* produced significantly thicker shells than conspecifics raised in the absence of *Carcinus*. Surprisingly, our data indicate that predator effluent has effects on shell form that are comparable to those resulting from water temperature (geographic) differences. Predator-induced changes were accompanied by trade-offs. Snails raised with *Carcinus* grew more slowly in terms of shell size and also exhibited significant reductions in tissue mass and tissue growth. Trade-offs were greatest for the northern populations raised in their home site. Thus, while colder water snails maintain the ability to respond to a novel predator, the costs are potentially greater than for warmer water snails. These results will be useful in predicting responses by prey populations to anticipated changes in global climate and community composition. Warming trends evident in the Gulf of Maine and elsewhere will likely expand the species ranges of both prey and predator. This climatalogical phenomenon, in conjunction with increasing rates of biological invasions in marine systems, guarantees that prey populations will be exposed to novel predators.

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Geographic Differentiation of an Introduced Crab Species (*Hemigrapsus sanguineus*) on the Atlantic Coast of North America

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Key words: *Hemigrapsus sanguineus*, population genetics, invasion, crab

The shore crab, *Hemigrapsus sanguineus*, native to the western Pacific Ocean, was first discovered in the eastern United States in September, 1988 in Cape May County, New Jersey. Since then, *H. sanguineus* has been found in coastal areas from southeast Massachusetts to North Carolina. *H. sanguineus* was likely introduced via ballast

water from ships traveling from the western Pacific. Introduced species often have detrimental ecological effects on their new environments. Understanding the mechanisms of species introduction and their subsequent spread is very important. Restriction enzyme digest patterns of mitochondrial DNA obtained from individuals collected in Massachusetts, New Jersey, North Carolina, and one location in Japan are being compared. The hypothesis of multiple introductions predicts that the patterns obtained from crabs from at least two locations will be distinctly different. The degree of difference will be used to infer the degree of allelic variation within and between the populations. The presence of near-identical patterns from individuals along the East coast will support the hypothesis that either a single introduction of *H. sanguineus*, or multiple introductions from the same source, has occurred. Primers specific for the mitochondrial cytochrome c oxidase subunit I (COI) gene successfully amplified a 700 bp region of DNA from individuals from Massachusetts. Seven of twelve assayed restriction digests of this PCR product showed multiple bands and can be used for genetic comparison. Currently the study is being extended and DNA from 30-50 individuals from each sampling location will be amplified with COI primers, and will subsequently be restriction digested with the same 12 enzymes. Sequences of PCR product from a few individuals from each location will also be obtained to confirm that the region being amplified is COI, and to compare sequences to each other and to results from restriction enzyme digestions.

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Prey Preferences of the Recently-Introduced Western Pacific Shore Crab, *Hemigrapsus sanguineus*, Feeding on Molluscs and Macroalgae in Southeastern Massachusetts

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Key words: *Hemigrapsus sanguineus*, crab, mollusc, macroalga, prey preference, predation

The prey preferences of the recently-introduced western Pacific shore crab *Hemigrapsus sanguineus* were investigated to gain insight into the crab's potential to alter New England rocky intertidal ecosystems through predation. Laboratory experiments were conducted to determine prey preferences of the crab feeding on molluscs and macroalgae of the area. *H. sanguineus* were collected from the rocky intertidal zone of two southeastern Massachusetts sites from June to October 1998. Prey selection was examined in relation to mollusc prey of different size and species. Crabs of three size classes (12-18mm, 19-25mm, 26-31mm) were offered three mollusc species: the bivalves, *Mytilus edulis* and *Mercenaria mercenaria*, and the gastropod, *Littorina littorea*. Equal ratios of prey from three size classes were offered concurrently to indicate size preference. In another set of experiments, equal ratios of each species of the preferred size were offered simultaneously to determine species preference. When presented with a range of prey sizes, crabs selected small sizes, male crabs opening larger sizes than females. Crabs offered macroalgae in both multiple-choice and no-choice experiments readily consumed green algae in the laboratory. *Enteromorpha* spp., *Ulva lactuca*, *Codium fragile* ssp *tomentostoides*, *Chondrus crispus*, *Polysiphonia* spp., *Fucus* spp., and *Ascophyllum nodossum* were presented to individual crabs separately to determine consumption rates and together to ascertain species preference. Additional feeding trials will examine the crab's preference for animal or plant material by presenting individual crabs with both mollusc and macroalgae species found to be preferred by previous experiments.

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Potential Impact of the Recently Introduced Asian Shore Crab, *Hemigrapsus sanguineus*, on Rocky Intertidal Communities of the Northeastern U.S. Coast

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Key words: *Hemigrapsus sanguineus*, crustacea, distribution, ecology, rocky intertidal, feeding rate

The Asian shore crab, *Hemigrapsus sanguineus*, was introduced to the mid-Atlantic coast in the late 1980s. We have been studying population density and distribution on a cobble/boulder beach in central Long Island Sound. We have also done preliminary experiments on feeding activity. Average seasonal abundance on the cobble shore at Crane Neck Point during 1997-1998 ranged from 7-9 m^{-2}. During the summer, the crabs were fairly evenly distributed at different elevations in the intertidal zone; however, they appeared to move from high to low elevation during the winter. The crabs readily consumed all common species of macroalgae and invertebrates occurring at Crane Neck Point. Based on laboratory experiments, we estimate that approximately 49-162 juvenile (<10mm) *Littorina littorea* and 28-171 small (<20mm) *Mytillus galloprovincalias* could

be consumed daily per m^2. Reduced foraging efficiency in the field where more cover is available for prey items, may result in actual predation rates that are considerably lower than these estimates. However, based on high crab densities and feeding rates on the early stages of these keystone species, we predict that this recent invader will strongly impact the community structure of the rocky intertidal in the western north Atlantic. Ironically, much of the impact may occur through predation on *Littorina*, a previously introduced species which itself has had a major impact on these communities.

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Effects of the Invasive Seaweed *Sargassum muticum* on Native Marine Communities in Northern Puget Sound, Washington

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Key words: *Sargassum muticum*, epiphytic communities, invasive seaweed, species diversity

Sargassum muticum was introduced to the West Coast of the United States in the 1940s and has since established itself as a persistent member of coastal communities from British Columbia to California. The efficient dispersal methods and fast growth of *S. muticum* allow it to effectively compete for space and light with native seaweeds. *S. muticum* reaches up to 3.5 m, forming large "trees" due to its extensive branching and buoyancy provided by floats. This study investigated the epiphytic communities associated with *S. muticum* to determine if the seaweed's presence affects the composition of shallow subtidal communities in northern Puget Sound.

The community that *S. muticum* supports differs from that associated with the native seaweed *Laminaria saccharina*, the seaweed most often displaced by the invading *S. muticum*. The *S. muticum* species community composition is dominated by five snails, one polychaete, four crabs, four caprellids, several other amphipods, two isopods, and two shrimp. The *L. saccharina* species community composition is dominated by one bryozoan, one scaleworm, and a variety of larger snails. Several species of polychaetes, bryozoans, and nudibranches associated with *L. saccharina* were never found associated with *S. muticum*. Unlike the seasonally stable community associated with *L. saccharina*, the community associated with *S. muticum* changes throughout the growing season (May through October), and with the local habitat. Individual *S. muticum* thalli also support more epiphytic biomass per gram of algal tissue than do *L. saccharina* thalli. The concentration of detritus and diatoms on the extensive thallus is then available to browsers. *S. muticum* is also a primary food source for grazers, including the snails *Lacuna vincta* and *L. variegata*. The seaweed provides a refuge for several fish species and juveniles of both the red rock crab and the dungeness crab. In summary, the invasion of *S. muticum* changes the ecology of the coastal communities by allowing certain native species to dominate over others that are associated with the native *L. saccharina*.

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Food Web and Contaminant Flow Effects of an Exotic Bivalve In San Francisco Bay, California

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Key words: ecosystem, bivalve, contaminant, food web

The introduction of the Asian clam, *Potamocorbula amurensis*, into San Francisco Bay has resulted in changes to the benthic community structure, food web, and transfer of contaminants within the ecosystem. The large declines in phytoplankton biomass coincident with the introduction of *P. amurensis* is shown to be due to "over-grazing" by these filter-feeding bivalves. We estimate that the water column is passed through the northern bay clam populations in excess of twice a day which leads to an imbalance in phytoplankton growth rate (doubling less than once per day) and filter-feeder consumption rate. Other sources of particulate organic carbon (e.g. detritus, bacteria, and bacteria on particles) may also be declining due to grazing by *P. amurensis*. Estimates of the amount of food consumed by the *P. amurensis* populations in northern San Francisco Bay are considerably higher than the amount of food available from the phytoplankton. Because northern San Francisco Bay has never had high primary production, it is likely that detritus and bacteria have always been important food for secondary producers. It is unknown what percentage of this food source is consumed by *P. amurensis* and how this reduction might affect other levels of the food web. We have begun to see some food web responses to the reduction in primary producers and other carbon sources. Populations of the opossum shrimp (*Neomysis*) and several zooplankton species, all important food species for larval and adult fish, have declined coincident with the invasion of *P. amurensis*.

The increase in benthic biomass in the northern bay is also changing the flow of contaminants through the ecosystem. Because of the small benthic biomass in the northern bay prior to the arrival of *P. amurensis*, the food web was dominat-

ed by pelagic forms. Although pelagic predators (e.g. midwater fish, dabbling ducks) may have lost a primary food source, bottom feeding predators (e.g. sturgeon, diving ducks) now have an enhanced food source. However, because *P.amurensis* has been shown to be a bioaccumulator and biomagnifier of trace elements (e.g. Cd and Se) this shift in the food web may ultimately prove to be detrimental to the bottom feeding predators. Concentrations of some trace elements have begun to accumulate at harmful concentrations in the sturgeon and diving ducks since the appearance of *P. amurensis*.

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Marine Bioinvasions in the Rocky Subtidal Zone (Massachusetts 1977-1998)

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Key words: nudibranch, *Tritonia*, ascidian, *Botrylloides*, subtidal

Rocky subtidal communities in Northern Massachusetts, Gulf of Maine, have been studied bimonthly to quarterly for the past 20 years. Surveys by SCUBA divers include photography of marked quadrats, transects to determine predator abundance, and measurement of characteristics of the physical environment. During this time period, there has been an approximate doubling of sea urchin (*Strongylocentrotus droebachiensis*) population density, and we have observed and documented the appearance and community level effects of several nonindigenous species. The European nudibranch (*Tritonia plebeia*) and the colonial ascidian (*Botrylloides* sp.), of Pacific origin, have been particularly successful invading species, and have severely impacted indigenous assemblages. *Tritonia* predation destroyed populations of the octocoral (*Alcyonium siderium*), which have not recovered by 1998. Several other ascidians (*Botryllus schlosseri, Styela clava, Diplosoma* sp.) have appeared rarely in our photographic samples and do not appear to have developed stable local populations at our study sites, although some are abundant elsewhere along this coast.

Alcyonium removal by *Tritonia*, and a major increase in sea urchin population density (during 1984-1986), caused most other invertebrates to be cleared from vertical rock surfaces; there was also a local increase in areas covered by crustose coralline algae. Horizontal rock dominated by kelp and foliose red algae (1978-1988) were reduced to coralline-dominated urchin barrens after the urchins expanded laterally in 1989. *Botrylloides* sp. colonies are now common on rock walls and horizontal surfaces where urchin grazing is continuous; this ascidian may have chemical defenses that deter urchin feeding. The synergistic effects of increasing urchin population density and the appearance of several nonindigenous species, within one decade, caused a major alteration in invertebrate and algal assemblages at these sites and a general decrease in small-scale species diversity. The present high densities of urchins currently favor urchin-resistant spatial dominants, such as the indigenous coralline algae, sea anemones (*Metridium senile*) and sponges (*Isodictya* spp.), and certain nonindigenous species (*e.g.*, the ascidian *Botrylloides* sp.). Reduction in sea urchin population density, as has occurred along the coast of Nova Scotia following epidemic disease, could increase invertebrate and algal biodiversity, although the strong overgrowth competitive ability of *Botrylloides* may allow it to persist even without heavy urchin grazing.

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Effectiveness of Functional Feeding Modes of Invasive and Native Predators

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Key words: feeding mode, prey selection, whelk

A novel function may provide an invasive species with advantages relative to natives. In particular, invasive predators might have an advantage if they introduce a novel functional feeding mode to native prey. Models of coevolution can both support and refute this assertion. A lack of coevolution may provide invasive predators with an advantage, because native prey have ineffective or inappropriate anti-predatory defenses. Conversely, invasive predators may be outcompeted by native predators for native prey. The recent expansion of Kellet's whelk, *Kelletia kelletii*, from south of Point Conception northwards to Monterey Bay, California introduced a novel feeding mode to the guild of invertebrate predators preying on trochid snails in central California. Before the appearance of *Kelletia* in 1980, sea stars (an ecological equivalent of whelks) were the primary invertebrate predators of trochids in central California. Stars feed using an eversible stomach while *Kelletia* feeds with a prehensile proboscis.

I used native and non-native sea stars and whelks as predators and allopatric *Tegula* spp. as prey in a series of non-choice and choice experiments to: 1) compare consumption rates between different functional feeding modes; and 2) assess prey anti-predatory defenses within the genus *Tegula*. I used the southern rainbow star,

Astrometis sertulifera, which feeds on trochids but occurs only in southern California to represent a non-coevolved predator in central California, and the giant spined star, *Pisaster giganteus*, which occurs in both southern and central California to represent a coevolved predator. Three subtidal macroherbivores in the genus *Tegula* were used as prey, one from central California (*T. brunnea*) and two from southern California (*T. aureotincta*, *T. eiseni*). In a non-choice experiment, I compared *T. brunnea* and *T. aureotincta*, both of which are poorly defended relative to sympatric congeners. Consumption rates were always highest for *T. aureotincta*. In a second non-choice experiment, I compared *T. eiseni*, which is well-defended, to *T. brunnea*. *Astrometis* consumed both prey species at equal rates, while both *Pisaster* and *Kelletia* ate significantly more *T. brunnea* than *T. eiseni*. Deep withdrawal by *T. brunnea* was a partially effective defense against sea stars, but it was less effective against the novel feeding mode of *Kelletia*. Escape frequency and consumption time were greater for *T. eiseni*. Among predators, *Kelletia* ate a significantly higher proportion of *T. brunnea* than either *Pisaster* or *Astrometis*. In a binary choice experiment using only southern California prey, all predators consumed *T. aureotincta* almost exclusively. Combining these results, *T. aureotincta* was most preferred, followed by *T. brunnea*, and *T. eiseni* was consumed when other prey were unavailable. The high performance of the whelk relative to the native and non-native sea stars indicates that its novel functional feeding mode is advantageous.

For *Pisaster* and *Kelletia*, performance was not influenced by geographic origin; individuals from populations separated by 500 km consumed prey at nearly identical rates. This similarity between widely separated populations does not fit models of coevolution that predict local adaptation and thus differences between populations. Predator performance was enhanced by novelty of function, and not by novelty as a species.

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Geographic Variation in the Freezing Tolerance of the Ribbed Mussel, *Geukensia demissa*

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Key words: freezing tolerance, *Geukensia demissa*, introduced species, supercooling

The ribbed mussel *Geukensia demissa*, native to tidal marshes on the American Atlantic coast, was accidentally introduced to the American Pacific (hereafter, Pacific) coast over 100 years ago. Mussels native to the northeast American Atlantic (hereafter, Atlantic) coast experience wide variation in seasonal temperature, including subfreezing winter conditions. To combat potentially lethal winter freezing temperatures, Atlantic mussels use a variety of seasonally induced biochemical and physiological adjustments to tolerate ice in their extracellular fluids. Pacific coast mussels, however, are not exposed to freezing temperatures experienced by their Atlantic counterparts. It was hypothesized that Pacific coast mussels may have lost the adaptive ability to withstand these freezing conditions. I examined the geographic and seasonal variations in the response of *Geukensia* to subfreezing conditions. Mussels from Connecticut and southern California were exposed to a variety of subfreezing temperatures. Survival, supercooling point, and final temperature were used as indices of response to freezing. For all indices measured, in a given season there were no significant differences found between California and Connecticut mussels in freezing response. However, a significant difference existed between summer- and winter-acclimated mussels: both California and Connecticut winter-acclimated mussels had significantly different supercooling points ($df=1$, $F=6.37$, $P<0.05$) and final temperatures ($df=1$, $F=24.6$, $P<0.01$). These results support previous literature on changes in seasonal cold tolerance. The fact that mussels from California did undergo seasonal acclimation, despite the lack of strong seasonal temperature cues, lends itself to the role of other environmental cues in triggering winter acclimation. Introduction to the Pacific coast has not resulted in a loss of freezing tolerance of *G. demissa*.

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Marine Bioinvasion Research at the Cawthron Institute

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Key words: Cawthron Institute, risk assessment, invasive species

The Cawthron Institute is an aquatic science organization based in Nelson, New Zealand. We have carried out research on marine bioinvasion since the mid 1980s. The aims of recent work are to provide the shipping industry and coastal managers with procedures for reducing invasion rates, and to improve our understanding and management of invasive species already established in New Zealand. The unifying theme of the program is a risk

assessment model in which both a species-specific and port by port approach are being taken. The transport, establishment, impact and management components of our conceptual model identify the topic areas around which our present marine bioinvasion research is based.

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The Ecology of the Japanese Shore Crab (*Hemigrapsus sanguineus* De Haan) and its Niche Relationship to the Green Crab (*Carcinus maenas*) Along the Coast of Connecticut, USA

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Key words: Asiatic shore crab, green crab, interspecific competition, primary prey species, niche habitat, sediment selectivity

The Asiatic shore crab (*Hemigrapsus sanguineus* de Haan) was first introduced to Atlantic waters through ballast water on September 24, 1988 in Cape May, New Jersey. Since then, *H. sanguineus* has spread at an alarming rate and has become well established on the Atlantic coast. *H. sanguineus* is now extremely abundant on the Connecticut coastline. *H. sanguineus* is thought to exploit the different but overlapping habitats on cobble and boulder shores in rocky intertidal habitats (Fukui 1988). In areas where *Carcinus maenas* (the green crab), used to be abundant, *H. sanguineus* is the dominant species and few *C. maenas* are found. This study focuses on the relative abundance, distribution, and feeding habits of *H. sanguineus*. Principle prey species for each size class of *H. sanguineus* was evaluated through stomach content analysis, and competition between these crabs and other indigenous crab species for niche habitat through sediment selectivity was evaluated. The introduction of nonindigenous species and their effects on native habitats is an area of growing concern as commerce and shipping increases. Little is known about this topic especially for marine habitats. This study will provide important base-line data and possible management strategies for future studies concerning *H. sanguineus*.

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ABSTRACTS

Ballast Water

Inventory of Microbes in Ballast Water of Ships Arriving in Chesapeake Bay

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Key words: microbe, Chesapeake Bay, ballast water

Perhaps the least-studied aspect of marine bioinvasions is the transfer of nonindigenous microbes. Given the high densities of naturally occurring bacteria, viruses, and phytoplankton in coastal waters, it seems inevitable that high numbers of microorganisms are transported globally via ballast water. As a first step in evaluating the rate and extent of this transport, we boarded vessels, primarily colliers, arriving in Norfolk, VA, and Baltimore, MD, to quantify the microbes present in their ballast water tanks. We used epifluorescence microscopy to measure abundances of bacteria and virus-like particles and incorporation of DNA precursors to determine bacterial production rates. We also measured the ballast-waters' temperature, salinity, and concentration of chlorophyll *a*. Direct counts of bacteria and virus-like particles ranged over two orders of magnitude, from 10^7–10^9 cells per liter and 10^8–10^{10} cells per liter, respectively. Similarly, bacterial production rates, expressed as incorporation of tritiated thymidine into DNA, varied broadly, from 0.40 - 13 picomoles per liter per hour. Chlorophyll *a* values ranged from 0.005 - 0.04 micrograms per liter. Relationships between hydrographic characteristics of ballast waters and microbial signatures will be discussed.

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Characterization of Bacterial Assemblages in Ships' Ballast Water

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Key words: microbial ecology, Biolog, Chesapeake Bay, ballast water

Microbiological studies of ballast water will help us understand the transfer, dynamics, and invasion potential of microorganisms carried by ships (see related abstracts by Rawlings *et al.* 1999 and Drake *et al.* 1999). We have begun to characterize the ballast water bacteria in samples collected from ships arriving in the lower Chesapeake Bay. Such characterization is based on interpreting substrate-utilization patterns. We inoculate ballast-water samples into Biolog microtiter plates (Biolog, Inc., Hayward, CA), which contain 95 different carbon substrates in separate wells. These wells, and a control well, also contain a tetrazolium salt that indicates (via color development) microbial utilization of a substrate. The rate and extent of a substrate's utilization is determined through time-series meas-

urements of a well's optical density. There has been a wide range of response among the ships (n=13) sampled to date; clearly the bacterial assemblages of ballast waters vary considerably. In more than half the cases, however, there are commonalities in response that may be influenced by duration of voyage, source water, and exchange history. In addition to its furthering our understanding of ballast-water ecology, this technique potentially has uses for routine monitoring of arriving ships and evaluating the efficiency of water exchange or filtration.

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Implications of the Transport of Viable Phytoplankton in the Ballast Water of Ships

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Key words: ballast water, phytoplankton, biogeography, exotic species, invasion, North Carolina, range

The spread of exotic species is cited repeatedly as one of the greatest threats to marine ecosystems today. One of the chief transport vectors of non-native aquatic organisms is ballast water discharged from ships. The principal objective of this study was to determine the taxonomic composition of viable phytoplankton species in ballast water collected from ships berthed in the Port of Morehead City, North Carolina, USA. During 1997, a ten-liter volume of unfiltered ballast water was collected from each of nine ships. These ships originated from ports in Japan, Spain, Dominican Republic/Florida, Belgium, Louisiana and North Carolina. Each sample was filter concentrated, and the live phytoplankton organisms were cultured for 21 days with a range of media and light intensities to simulate different receiving waters. Over 150 genera of viable freshwater and marine phytoplankton were identified from the cultures. Additionally, over 450 more live species morphologies were observed (predominantly protists and unidentified microalgae). These results provide evidence that numerous species of both freshwater and marine phytoplankton are not only viable when discharged in the ballast water of ships, but may also establish successfully reproducing populations. The biogeography of marine phytoplankton species is imperfectly understood; many phytoplankton species are considered globally ubiquitous. Yet, some phytoplankton species are known to have limited distributions. It may be possible that earlier ballast water transfers contributed to the current cosmopolitan distribution of some phytoplankton species. In an effort to reduce the probability of future marine biological invasions, further research needs to focus on the present, as well as historical, capacity of ballast water to homogenize the distributions of phytoplankton species globally.

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Four Centuries of Biological Invasions in Chesapeake Bay

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Key words: Chesapeake Bay, invasion, historical survey, cryptogenic species, ballast water, ecological impact

Chesapeake Bay has been subject to biological invasions since the start of European colonization in 1609. In a comprehensive analysis of the historical patterns of invasion, we identified 160 nonindigenous species that now occur in tidal waters of the Chesapeake. These species represent 17 different phyla, including vascular plants and invertebrates to vertebrates and single-celled protistan pathogens. Invasions are documented from all regions and habitats of Chesapeake Bay. Source regions and transfer mechanisms have varied greatly over time and by taxa, and the rate of invasions has increased in recent decades. In addition to known invaders, the Chesapeake has a high proportion of cryptogenic species (*i.e.*, those of unknown origin). Historical surveys and first records of many taxa are relatively recent, occurring well after possible transfer by European colonization. Of 800 species of benthic inver-

tebrates and macroalgae in Chesapeake Bay that we have examined, 30% (237 species) also occur in Europe, suggesting that many unsuspected introductions exist. Today, multiple pathways for new invasions are still active in Chesapeake Bay. Shipping is presently the largest pathway for the transfer and release of organisms to the region. Two recent ballast-mediated invasions underscore the continued importance of this vector. Although invasions have had significant ecological and economic impacts in the Chesapeake, the consequences of most marine invasions remain unmeasured here and elsewhere.

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The Great Lakes Ballast Technology Demonstration Project Filtration Mechanical Test Program

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Key words: ballast water filtration, nonindigenous species, filtration efficiency

The Great Lakes Ballast Technology Demonstration Project has investigated the effectiveness of automatic backwash screen filtration of ballast water during its uptake as a means of minimizing the potential for the introduction of nonindigenous species. The Great Lakes Protection Fund, the State of Minnesota (on the recommendation of the Legislative Commission for Minnesota Resources), the Lake Carriers' Association, Northeast-Midwest Institute, Federal agencies, and universities have supported this project. Initial testing during the 1997 operating season involved testing onboard the Seaway-sized bulk carrier *M/V Algonorth* during operations between the Gulf of St. Lawrence and Great Lakes ports. This testing provided important information on system design, operations, and filtration biological effectiveness, but the mechanical test results were of limited value as benchmark experiments. During the summer of 1998, the modular system was installed on a barge located in the Duluth, Minnesota, harbor to permit more controlled, intensive mechanical and biological testing.

The overall design of the shipboard and the barge ballast water filtration test installations are briefly summarized. The barge mechanical test program involved extended testing with 25-µ-, 50-µ-, and 100-µ-filter screens at a nominal 1,500 U.S. gpm using an operating profile that approximates normal ship ballast operations. The Great Lakes Ballast Technology Project filter mechanical test program yielded the following conclusions:

The barge installation and test program in Duluth/Superior harbor provided an effective model of shipboard operations and permitted a much more controllable and efficient test effort (84 hours of filter operation in 16 calendar days) than possible onboard an operating vessel.

Duluth/Superior harbor was representative of general harbor conditions, but much higher sediment loads can be found throughout the world. The peak turbidity experienced (5.4 NTU) is just over the 5.0 NTU typically considered the upper limit for water treatment plant intake. Patches of filamentous green algae were also observed during the test period.

The use of a 5-mm prescreen upstream of the filter was successful in protecting the finer screens and demonstrating that two filters in series, as initially used in the *M/V Algonorth* testing, should not be necessary in ballast filtration applications.

The 25-µm filter, with an overall count efficiency (based upon the total count of all particles above the nominal filter rating) of about 85%, and the 50-µm filter, with an overall count efficiency of about 90%, performed as should be expected. This is strong performance for a real world test with pliable and variable shaped particles.

The principal lessons learned in the design, installation, and mechanical testing of the ballast water filters include the following:

Adequate system hydraulic stiffness or a pressure-sustaining valve downstream of the filters will be needed to provide a pressure differential of 2.4 kPa (35 psid) required between the discharge chamber and the backwash chamber to drive the backwash process.

Careful manufacture and quality control will be needed in the construction of filter screen elements. The project experienced split screens early during the 1997 *M/V Algonorth* testing and the 100-µm screen tested in 1998 may have had an internal screen failure.

The backwash timing stated by the vendor had to be extended significantly to achieve effective cleaning in the shipboard application. The backwash flow rate and effectiveness requires careful attention in future designs.

Future designs, particularly those of the much larger sizes needed for bulk vessels, must have improved features to facilitate the alignment and handling of the heavy filter screen elements. Vertical, rather than horizontal, orientation would allow the use of hoists making the crew's task of changing elements more reasonable.

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The Great Lakes Ballast Technology Demonstration Project

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Key words: ballast water, filtration, nonindigenous species introduction, plankton, pathogen

Ballast water is the leading known vector of unintentional transfers of aquatic organisms. Unites States law and international guidelines direct ships to undertake ballast water management to reduce these transfers. The only method currently available to ships to reduce transfers of organisms, high seas ballast water exchange (BWE), is limited by safety and applicability. Domestic law provides for and encourages technological alternatives to BWE that are environmentally sound and equally or more effective at preventing organism transfers. Research on possible onboard treatment systems is accelerating; however, there is no standard approach for gauging biological effectiveness. This study (1) evaluates the biological effectiveness of a leading ballast treatment alternative–automatic backwash screen filtration and (2) presents a method for assessing biological effectiveness of ballast treatment systems, generally. Initial testing during the 1997 operating season took place aboard the Seaway-sized dry-bulk carrier, the M/V *Algonorth,* during operations between the Gulf of St. Lawrence and Great Lakes ports. This testing provided important information on system design, operation, and biological effectiveness but was limited by operational constraints of the vessel. During the summer of 1998, the modular system was installed aboard a stationary barge in the Seaway Port Authority of Duluth, in Lake Superior, for more controlled, intensive biological and mechanical testing. The barge also better simulated shipboard use because it was at water level and within a harbor. A variety of screen sizes (25 μm, 50 μm, 100 μm, and 150 μm) were evaluated aboard the experimental platforms at flow rates of 1200-1500 gal/min. The shipboard experimental platform consisted of two filter units in series and a pump mounted on the deck with piping to matched control and test upper wing ballast tanks. The stationary barge platform consisted of a single filter unit and pump with piping to three identical catchment tanks of 175 gal each with bottom outlets. Biological effectiveness was measured through comparing the concentrations of major taxonomic groupings of zooplankton, phytoplankton, and microbes with and without treatment. Biological effectiveness results were highly consistent on both experimental platforms, and differed in nature and complexity from particle count findings. In particular, organism morphology, rigidity, and colonial structure combined with size to influence filter effectiveness. The two smallest filters tested achieved 95-99% removal of macrozooplankton, and 70-80% removal of microzooplankton and phytoplankton. The results indicate that the filters do not have the undesirable effect of breaking up colonial or filamentous algae to yield more numerous propagules. Bacteria, which attach to other organisms and matter, were significantly reduced by the smaller filter sizes, but total bacteria counts were unaffected by filtration. The two filter series yielded no improvements in biological effectiveness over the single filter system. These results suggest that (1) filtration could significantly reduce the risk of introductions of harmful organisms by ships, but there would be a need for secondary treatment to achieve complete sterilization of the ballast water, and (2) evaluation of treatment effectiveness with respect to several major taxonomic groupings is necessary to accurately compare treatment alternatives and assess secondary treatment needs.

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Measuring the Efficacy of Ballast Water Exchange

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Key words: ballast water exchange, invasion rate

Ballast water exchange is being promoted nationally and internationally as a management strategy to (1) decrease the abundance of coastal organisms within ships' ballast water and thereby (2) reduce the risk of future invasions by

nonindigenous species. We are measuring the efficacy of two distinct exchange methods (Flow-Through and Empty-Refill) to remove organisms from ballast tanks across multiple ship types. Physio-chemical and biological tracers were used to estimate the effects of exchange on different components of ballasted communities. Preliminary results, based on salinity and Rhodamine dye tracers, indicate significant water mass exchange (>80%) for both Flow-Through and Empty-Refill methods, with the latter being more efficient. Further analysis of biological samples will test for variation in effects among taxa. Despite the apparent reduction in coastal organisms through exchange, a small percentage (but sometimes still a large number) of residual organisms can remain in exchanged tanks. The overall effect of ballast water exchange in reducing invasions therefore depends not only upon (a) the percent of vessels that exchange their ballast water and (b) the methods and results of exchange per vessel, but also (c) the relationship between supply and invasion rates. Although reduced inoculation densities should result in reduced invasion rates, the shape of this relationship is unknown. Thus, efficacy of exchange, or other management strategies, must be measured according to both rate of organism transfer and rate of invasion.

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Influence of Vessel Transit Patterns on Ballast Water Treatment Options for Exotic Aquatic Organisms

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Key words: vessel transit, ballast water, nonindigenous species

Ballast water discharge has been identified as an important means of introducing exotic aquatic organisms to waters well beyond their natural geographic range. Preventative measures such as offshore exchange have been used with some success by vessels carrying exchangeable ballast. Efforts to develop active treatment measures have so far not been successful because of a large number of variables. Major factors that can influence a treatment program include the target organisms which can range from microbes to fish, matrices that include water and sediment, volume of ballast treated, and its application based on onboard equipment or requiring an external source. The treatment itself may have a chemical, physical or mechanical basis. Vessel transit patterns can influence treatment methods, and time of application should be compatible with vessel operational procedures.

Vessel transit patterns will differ among ports because of traffic volumes and transit routes. This factor was examined by tabulating the frequency of visits by overseas and nonregional vessels to Canadian maritime ports at Halifax, Vancouver and Prince Rupert, and freshwater ports on the Great Lakes. There were about 420 vessels from overseas or distant ports of departures that visited the Port of Halifax between 1992 and 1997; 2400 vessels to the Port of Vancouver between 1995 and 1997; 1170 vessels to Port of Prince Rupert between 1992 and 1997; and 880 vessels to Great Lakes ports between 1989 and 1997. Tabulation on the basis of the number of visits indicated that 46 to 71% of the vessels visited a port once during the 3 to 9 year periods reviewed. Furthermore, 68 to 96% of the vessels averaged 2 or less visits per year over the same period. The relatively high level of single visits and low frequency of repeat visits to these ports could deter the adoption of possible regulations that would require the installation of onboard ballast water treatment equipment on vessels before port entry, unless this requirement is applied at the international level.

Voluntary or mandatory exchange is used by some ports as a preventative measure, but its effectiveness is limited to vessels carrying exchangeable ballast. Offshore ballast exchange could be effective at the Ports of Vancouver and Prince Rupert because over 90% of the total cargo handled is exported, therefore most of the inbound vessels arrive in ballast. In contrast, ballast water exchange may not be an effective preventative measure on the Great Lakes because about 50% of the total cargo handled is exported, and over 80% of the overseas vessels enter with no exchangeable ballast on board (NOBOB).

A vessel monitoring program may be required to assess the level of compliance of a ballast water treatment program. Annual visits by nonregional vessels averaged about 400, 1820, 310 and 470 for the Ports of Halifax, Vancouver, Prince Rupert and ports on the Great Lakes. The frequency of these visits may not be a limiting factor in their assessment because arrivals of 1 to 3 vessels per day were the most frequent at the Ports of Halifax and Prince Rupert, and ports on the Great Lakes, but could be a factor at the Port of Vancouver where 5 to 9 vessels arrive per day. Access to vessels arriving at the three maritime ports for inspection and possible treatment could be a problem. Access to vessels entering the Great Lakes is greatly facilitated by the need for all vessels to pass through a series of seven locks between the city of Montreal and Lake Ontario.

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Pulse Generator for Biofouling Prevention

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Key words: *Dreissenia polymorpha,* pulse power, control, Mississippi River, mollusc

Zebra mussels *Dreissernia polymorpha* are vigorous mollusks thought to originate in the Black Sea. Given a foothold the zebra mussels will colonize the area, killing off native species, and populate structures and pipes which can reduce water flow in critical cooling and irrigation operations tounsatisfactory levels.

The literature suggests that pulse power can be an effective and potentially economic method as a primary or as a complement to a mix of chemical and/or mechanical prevention/control technologies currently in use. The pulsed power method stuns or kills the velligers in the pipe entrance and has no effect on animals upstream of the entrance, nor downstream from the system discharge.

An experimental 20Kv pulse will be described which has been used to suppress zebra mussel settlement by more than 80% in a power plant cooled by Mississippi River water. Methods for improving the affectivity and cost will be described. Finally, application of this technique to control other marine species will be touched upon.

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Pathways and Management of Aquatic Nonindigenous Species in Delaware

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Key words: management, Delaware, vector

The introduction of aquatic nonindigenous species has the potential to cause significant environmental and economic harm in Delaware waters, as it has throughout the world. To date, the extent to which Delaware waters are invaded by nonindigenous species has not been assessed. Likewise, an examination as to effectiveness of Delaware's current management framework to prevent and control nonindigenous species has yet to be conducted.

Through literature searches and interviews with Delaware natural resource officials, approximately forty (40) nonindigenous species were found in Delaware's fresh, brackish, and marine waters. Six of these were considered nuisance species, costing the State a significant amount in control efforts. Of the five principal pathways of introduction highlighted in the literature (stocking programs, shipping, recreational boaters and anglers, aquarium and ornamental releases, and aquaculture), all five were likely responsible for some introductions into Delaware waters. Ballast water discharges and recreational boating and angling pose the greatest potential risk for introducing nonindigenous species into Delaware, although no one pathway appeared to be especially dominant. This rough assessment was based on the number of known harmful species likely being introduced *(e.g.,* the number of ships discharging ballast water potentially containing harmful nonindigenous species into Delaware Bay).

Interviews were conducted with personnel in six state-level nonindigenous species management programs (Chesapeake Bay, Lake Champlain, New York, Michigan, Minnesota, and Puget Sound). Lessons drawn from these current efforts might prove helpful in devising a management strategy for Delaware. The majority of programs focused exclusively on aquatic species (rather than an all encompassing terrestrial and aquatic species approach) and utilized education as the primary management tool. Stakeholder inclusion was a key component of the development process. Many policies became stymied by low recognition of the issue, leading to deficiencies in staff and funding, which in turn adversely affected implementation.

Effective state-level nonindigenous species management is hampered by five shortcomings: inadequate baseline information, insufficient authority, delays in response time, lack of an institutional framework, and low levels of support for action. I addressed these deficiencies through five corresponding policy prescriptions. The proposed measures set forth to close loopholes in the current regulations, establish a nonindigenous species monitoring program, create a mechanism to coordinate management actions, and call for education targeted at the responsible parties.

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Considerations in the Development of New Risk Assessment Techniques for Aquatic Nuisance Species: the Role of Transport Vectors in Risk Assessment

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Key words: risk assessment, introduction, vector

Aquatic nuisance species (ANS) are a growing concern to natural resource and environmental managers. The alarming rate at which nonindigenous species are being

both introduced and established is a driving force behind increasing awareness of and proposed actions aimed at ANS issues. However, it is not feasible to address every invasive species concern, thus, these issues must be prioritized. One conventional method by which land managers have ranked invasive species is via risk assessment. Before managers dealing with ANS rush to adopt these methodologies, we suggest that it is imperative that these tools be evaluated with special attention paid to their applicability in the aquatic realm. Do these risk assessment protocols produce answers that are important to ANS scenarios? That is, are we interested in the same questions that land managers need answers to or do ANS differ fundamentally from terrestrial invaders? Most terrestrial risk models for invaders follow a species oriented predictive risk assessment methodology. We suggest that in the aquatic realm the pathways or transport vectors by which ANS are introduced represent an important step in the invasion process, and one that is neglected in conventional risk assessment. We review risk assessment methodologies currently in use and demonstrate how pathways can be incorporated into these procedures to the benefit of managers concerned about both specific invasive species or general ANS issues.

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Distribution and Abundance of Ctenophores and their Zooplankton Food in the Black Sea. II. *Mnemiopsis leidyi*

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Key words: Black Sea, ctenophora, *Mnemiopsis*, range, biometry, stomach content

The distribution of *Mnemiopsis leidyi*, Agassiz 1865, in the Black Sea was determined using plankton samples collected above the anoxic zone (maximum depth 200 m) in the summer, winter, and spring from 1991-1995. Distribution was patchy. Average biomasses of 15 to 500 gm^{-2} were measured and abundances varied from 10 to 180 individuals m^{-2}. Biomass and abundance peaked in winter and there was a secondary peak in the summer. The distribution of *M. leidyi* was correlated with hydrographic features in the Black Sea with higher concentrations in anticyclonic gyres. The centers of the two main cyclonic gyres generally had a low biomass of *M. leidyi*. From July 1992 to March 1995, the populations were largely offshore. *M. leidyi* were confined to the upper part of the mixed layer both day and night. Some individuals displayed a negative taxis to daylight and, localized below the thermocline at night. Smaller *M. leidyi* (1.5-2 cm) were present in the winter and individuals reached maximum size in the summer. Although reproduction was continuous throughout the year, there were two distinct peaks: the larger peak in the summer and the smaller peak in the winter. Microscopic analysis of stomach contents showed that copepods and molluscs form their main diet.

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The Invasion of the Chinese Mitten Crab and Its Effects on Fish Protection Facilities

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Key words: Chinese mitten crab, estuary, economic impact

The Chinese mitten crab (*Eriocher sinesis*) is native to the coastal rivers and estuaries of the Yellow Sea. It was first observed in San Francisco Bay in 1992. Subsequent observations in 1994-1996 showed the crab had spread rapidly through the Sacramento-San Joaquin estuary. The mitten crab has undergone an explosive growth in numbers over the past several years with unknown impacts to the Bay-Delta ecosystem. Fall migrating adult crabs are now interfering with fish salvage operations at State of California and Federal fish collection facilities associated with large water diversion projects in the Sacramento River Delta. The mitten crab invasion has negatively impacted these facilities causing high mortality of fish in collection and transport apparatuses. The mitten crab is catadromous, *i.e.*, adults reproduce in salt water and the young migrate to fresh water to rear and develop (2-3 years). At maturity, adult crabs make a major spawning migration towards the ocean in fall. In 1998 adult crabs began appearing in early August with number reaching 25,000 -28,000 per day by mid-September. Through January and February of 1998, juveniles crabs (upstream migrants) were observed for the first time also. We expect mitten crabs to occur in even greater densities in 1999, and this species will probably remain an important component of the Delta ecosystem, perhaps forever. The Sacramento Delta fish facilities must find a way to cope with high numbers of crabs each fall with minimal harm to the fish. During the next year we plan to test various methods for crab exclusion and isolation both in laboratory hydraulic models and under field conditions.

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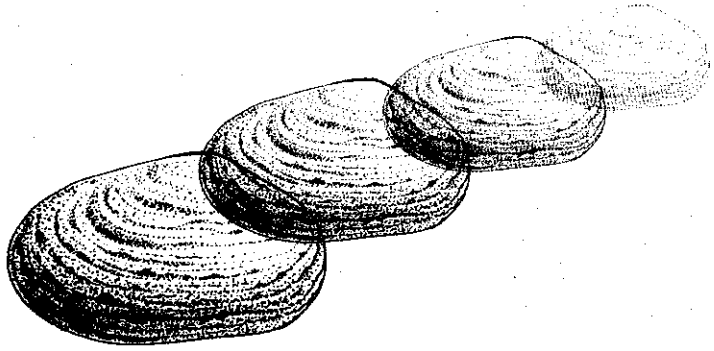

The study of biological invasions is a relatively new branch of ecology that is gaining in importance. *Marine Bioinvasions: Proceedings of the First National Conference* reflects the current research directions and management goals.

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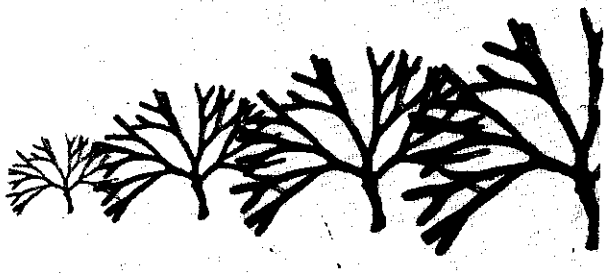

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