OER_2009_

NOAA OFFICE OF OCEAN EXPLORATION
FY09 PROJECT SOLICITATION COVER SHEET

IF A SECTION IS NOT APPLICABLE, DO NOT FILL IT OUT.

TITLE OF PROPOSED PROJECT:

THEMATIC CATEGORY

- ○ MARINE ARCHAEOLOGY
- ○ EDUCATION
- ● OCEAN EXPLORATION

CONTACT INFORMATION

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Significant Participant Name	Institution
1) Dr. Charles Messing	NOVA Southeastern University
2) Dr. Steven Haddock	Monterey Bay Aquarium Research Institute

BUDGET SUMMARY

FY09 OE FUNDING REQUEST ($):	424807	TOTAL OE FUNDING REQUEST ($):	424807	# YEARS:	1

COST-SHARE

COLLABORATING INSTITUTION	NATURE OF THE CONTRIBUTION	$ AMOUNT
1)		$0,000.00
2)		$0,000.00
3)		$0,000.00
	TOTAL COST SHARE DOLLARS:	$0,000.00

FIELD OPERATIONS AREA

EST. FIELD DATES (MM/YY - MM/YY): 7/27 - 10/09 | # TOTAL FIELD DAYS: 10 | MAX DEPTH: 1000 | APPROX. LAT/LONG: 14°32.643S, 168°10.663W

GENERAL REGION OF FIELD OPERATIONS

	WESTERN SOUTH ATLANTIC	[X] EASTERN SOUTH PACIFIC	CARIBBEAN SEA
EASTERN NORTH ATLANTIC	SOUTHERN MID-ATLANTIC	WESTERN SOUTH PACIFIC	GULF OF MEXICO
WESTERN NORTH ATLANTIC	EASTERN NORTH PACIFIC	SOUTHERN MID-PACIFIC	MEDITERRANEAN SEA
NORTHERN MID-ATLANTIC	WESTERN NORTH PACIFIC	INDIAN OCEAN	BERING SEA
EASTERN SOUTH ATLANTIC	NORTHERN MID-PACIFIC	ARCTIC OCEAN	GREAT LAKES

DESCRIPTION GEOGRAPHIC OF OPERATIONS AREA (EX. THUNDER BAY NMS, PUERTO RICO TRENCH)

Rose Atoll, South Pacific, east end of American Samoa

EXPLORE

FY09 PROJECT SOLICITATION COVER SHEET
NOAA OFFICE OF OCEAN EXPLORATION

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VESSEL INFORMATION

OPTION 1		OPTION 2	
If you have access to a vessel, and you do not require OE funding for the vessel.		If you have access to a vessel, and you require OE funding for the vessel.	
VESSEL NAME:		VESSEL NAME:	RV Seward Johnson + JSL Submersible
INSTITUTION:		INSTITUTION:	Harbor Branch Oceanographic
COST PER DAY:	$0,000.00	COST PER DAY:	31200
TOTAL COST:	$0,000.00	TOTAL COST:	312000
*(Note: Cost should **not** be included in Funding Request)*		(Note: Be sure to include this as a line item in the Budget)	

SUBMERSIBLE INFORMATION

SUBMERSIBLE TYPE	SUBMERSIBLE NAME	INSTITUTION	# DAYS REQUIRED	MAX DEPTH REQ
[X] MANNED SUBMERSIBLE	Johnson-Sea-Link	HBOI	10	1000 m
[ ] ROV				
[ ] AUV				
[ ] CAMERA SLED				
[ ] OTHER:				

PROJECT ACTIVITIES: CHECK ANY ACTIVITIES THAT YOU ANTICIPATE CONDUCTING DURING FIELD OPERATIONS

- [X] VIDEO & PHOTO DOCUMENTATION
- [X] VISUAL OBSERVATIONS
- [X] BIOLOGICAL SAMPLING
- [ ] BATHYMETRIC SURVEY
- [ ] GEOLOGICAL SAMPLING
- [ ] OTHER:

SYSTEMS: CHECK THE PRIMARY SYSTEMS YOU ANTICIPATE USING DURING FIELD OPERATIONS

- [ ] MULITBEAM
- [ ] MAGNETOMETER
- [ ] AUV
- [X] SCUBA
- [ ] CAMERA SLED
- [X] SIDE-SCAN
- [ ] ROV
- [X] SUBMERSIBLE
- [ ] REBREATHER
- [ ] OTHER:

EDUCATION & OUTREACH: CHECK ANY BOXES THAT YOU ARE WILLING TO PARTICIPATE/CONTRIBUTE TOWARD

- [X] BERTHING FOR EDUCATOR-AT-SEA
- [X] OE WEBSITE CONTRIBUTIONS
- [X] TEACHER PROFESSIONAL DEVELOPMENT INSTITUTES
- [X] PORT CALL EVENTS
- [X] MEDIA INTERVIEWS
- [X] OTHER: HBOI and MBARI webpages

PRODUCTS & OUTCOMES: CHECK ANY PRODUCTS THAT YOU ANTICIPATE DEVELOPING

- [ ] SIDESCAN SONAR DATA
- [ ] RELATIONAL DATABASE
- [X] SAMPLES/SPECIMENS
- [ ] MAPS
- [ ] BATHYMETRIC DATA
- [X] STILL IMAGE ARCHIVE
- [X] PEER-REVIEWED ARTICLE
- [ ] OTHER: 1)
- [ ] BACKSCATTER IMAGERY
- [X] VIDEO ARCHIVE
- [X] WEBSITE
- 2)

POST-FIELD WORK REQUIREMENTS FOR CONSIDERATION: WHICH OF THE FOLLOWING WILL BE REQUIRED

- [X] DATA ANALYSIS & PROCESSING
- [ ] FUTURE EXPLORATION
- [ ] GENERATION OF MANAGEMENT REQUIREMENTS/STRATEGIES
- [ ] OTHER:

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Bioluminescence in the Deep-sea Benthos.

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Funding Request: $112,807 + $312,000 ship/submersible time

We propose to explore bioluminescence in the deep-sea benthos, about which very little is known, as dredges and benthic trawls kill most of the collected organisms, and submersible/ROV lamps overpower any locally produced light. Based on the few but phylogenetically varied deep-sea sessile organisms that are known to be bioluminescent (octocorals, gorgonians, pennatulids, crinoids, ophiuroids and one holothurian), and the very large eyes of some of the motile predators, it is likely that benthic bioluminescence is abundant and plays a significant role in animal interactions. In addition, data obtained on previous OE-funded expeditions indicate that deep-sea benthos may produce novel, short wavelength bioluminescence. Two of 7 species of deep-sea benthic crustaceans studied to date (anomuran crabs *Eumunida picta* and *Gastroptychus spinifer*) possess an ultraviolet (UV)-sensitive visual pigment in addition to a blue-sensitive one. In their dim light environment, sacrificing photoreceptor space for a UV visual pigment suggests that UV sensitivity plays an important role in their ecology. Just as unusual UV sensitivity in several deep-sea pelagic species has been linked to bioluminescence, we suspect that UV sensitivity may also function to see as-yet undiscovered short wavelength bioluminescence from benthic organisms. UV bioluminescence in the benthos may be a novel private channel of communication, enabling motile animals to find their preferred niche. In addition, the eyes of the deep-sea isopod *Booralana tricarinata* have such a long integration time that they cannot track moving prey. As detritivores, they might cue in on bioluminescence produced by bacteria on detritus, which has been hypothesized but not yet observed due to the lack of opportunity to make such observations. We propose to look for deep-sea benthic bioluminescence and examine interactions between vision and bioluminescence utilizing A) thermally insulated, light tight Bio-Boxes to bring organisms to the surface alive, in cold water, and still dark-adapted, B) submersible-based low-light cameras to observe and photograph stimulated bioluminescence of living organisms *in situ* as well as background bioluminescence of decaying material or bacterial mats, C) the Eye-in-the-Sea camera system, to unobtrusively examine non-stimulated bioluminescence, as well as animal reactions to artificial bioluminescent prey, D) continuing studies on the photosensitivity of deep-sea organisms, E) spectral measurements of bioluminescence, and F) molecular studies to look for novel photoproteins involved in producing short-wavelength bioluminescence.

PROJECT DESCRIPTION

Background

Bioluminescence is a fascinating phenomenon that is relatively rare on land but is common in all the world's oceans. At least 12 animal phyla, as well as Eubacteria and Protista, have marine bioluminescent representatives (Herring 2002). Although bioluminescence has been extensively studied in the pelagic zone (water column), most information on its occurrence in the deep-sea (>200 m) benthos is fragmentary and anecdotal, a result of the difficulty in retrieving intact benthic organisms from trawls and dredges. Most specimens arrive at the surface dead or moribund, or are not examined for their bioluminescence potential before being preserved. While the ability to bioluminesce can be inferred from the presence of photophores or weak bioluminesce in moribund organisms, the true potential of bioluminescent systems must be examined on living, undamaged animals.

Unlike most deep pelagic fish, most deep bottom-associated (benthopelagic) fishes lack elaborate light organ systems such as lures or ventrally directed photophores. Over half the ~250 slope-dwelling rattails (Macrouridae) and a variety of deep-sea codlings (Moridae) bear a simple mid-ventral or peri-anal light organ (Marshall 1965, 1973; Marshall & Cohen 1973), but their function(s) remains unknown, and the bioluminescence from most of them has never been seen (Herring and Morin, 1978). Other known sources of deep-sea benthic bioluminescence include octocorals, pennatulids, crustaceans and members of all five echinoderm classes (reviewed in Marshall 1979, Herring 1991). An example of unique bioluminescence that portends what remains to be discovered on the seafloor is found in the deep-sea Atlantic sea pen, *Umbellula huxleyi*. In a freshly caught specimen, the bioluminescence was described as being bright green along most of the stalk, with bright blue light emanating from the distal stalk tip and bases of the polyps (Herring 1983). Spectral measurements made on a Pacific species *(U. magniflora* – EA Widder et al. 1983) indicate a similar anatomical difference, with most of the stalk and polyps emitting a blue-green luminescence (490-500 nm), and the distal stalk tip producing a more pure blue (470 nm). However, even when luminescence can be elicited in the laboratory, it is difficult to determine whether the flash kinetics observed are a result of excision or reflect what occurs *in situ* (Herring 1991). Observations of gorgonian bioluminescence are few, and the only *in situ* account from a deep-sea species, *Lepidisis olapa*, describes multiple waves of light traveling up and down the colony upon mechanical stimulation (Musik 1978), which Herring (1991) found considerably more dramatic and complex than what he saw in his laboratory studies of other deep-sea species.

Only recently have there been more attempts to observe bioluminescence *in situ* in the deep-sea. Herring et al. (2001) first reported aggregations of luminescent organisms on artificial food falls with an ISIT video camera on a lander. More recent studies by Gillabrand (2006) and Heger et al. (2007) found "bioluminescent hotspots" in two widely separated sites in the NE Atlantic (Porcupine Seabight and Rockall Bank), suggesting that benthic bioluminescence is more common in the NE temperate Atlantic than previously thought. In both cases, highly motile ostracods produced the only bioluminescence observed, leading Heger et al. (2007) to suggest that benthic bioluminescence is not present in cold-water corals or their associated fauna. However, as the lander could not mechanically stimulate any of the slow moving fauna that rarely moved into the camera's field of view, and because unstimulated bioluminescence remains

rarely observed, their conclusion cannot be verified. In addition, the only camera on their lander sensitive enough to record bioluminescence (ISIT camera) was focused on the bait on the seafloor, not on surrounding corals so it is not surprising that bioluminescence among the corals was not observed.

Because so little work has been done on deep-sea benthic bioluminescence, there are, in all probability, many unknown sources of benthic bioluminescence yet to be discovered. For example, in the pennatulids alone, 450 years of published observations have resulted in 80 published accounts of bioluminescence, but these encompass only 19 out of 186 valid species that have been described (Williams 2002). As luminescence likely serves a defensive purpose in sessile cnidarians (Morin 1974), and the deep-sea taxa live in perpetual darkness, Williams (2002) speculates that the actual number of bioluminescent sea pens may be much higher than is presently documented.

An intriguing, and as yet, unverified idea is that marine snow and detritus that accumulate on the ocean floor might contain bioluminescent bacteria (Lampitt et al. 2001). *Photobacterium* spp. occur throughout the marine environment (Ast and Dunlap 2005), luminous bacteria are known to colonize crustacean and fish carcasses (Wada et al. 1995), and fecal pellets in sediment traps luminesce (Andrews et al. 1984). Nishida et al (2002) suggested that the resulting background glow may be used as a cue by deep-sea scavengers. The possibility that this occurs is supported by a recent study on the visual physiology of the isopod *Booralana tricarinata*, collected at 600 m depth off Little San Salvador, Bahamas. Their maximum flicker fusion frequency (the highest stimulus rate at which the eye can produce electrical responses that remain in phase with a flickering light) is 4 Hz, the lowest ever measured in the animal kingdom (Frank 2008 and in prep). The flicker fusion frequency roughly reflects the eye's integration time, and can be thought of in terms of the shutter on a camera – the lower the flicker fusion frequency, the longer the shutter would remain open. The integration time of this isopod's eye is ~250 msec, far greater than the 80 msec measured in a deep-sea benthic crab and 42 msec measured in a deep-sea benthic benthic shrimp (Frank, in prep.). This extremely long integration time in the isopod eye indicates that this animal probably cannot track moving prey, as moving objects, particularly those emitting luminescent flashes, would be blurred temporally. As deep-sea isopods are thought to be scavengers (rev. in Barradas-Ortiz et al. 2003), an eye with a very long integration time might be able to visualize dimly glowing detritus, aiding the animal in finding a food target.

In addition, due to the demonstrated relationship between bioluminescence and an unusual red-sensitive visual pigment in some deep-sea pelagic fish (O'Day and Fernandez 1975, Partridge and Douglas 1995), and an unusual UV-sensitive visual pigment in several deep-sea pelagic crustaceans (Frank and Case 1988; Cronin and Frank 1996), bioluminescence might also be linked to a similar anomaly recently discovered in some deep-sea benthic crabs. Two of seven species studied to date possess a UV-sensitive visual pigment in addition to a blue-sensitive one. The isopod *Booralana tricarinata*, the caridean shrimp *Eugonatonotus crassus*, the brachyuran crab *Bathynectes longipes*, and the anomuran galatheid crabs *Munidopsis tridentata* and *M. erinacea* all possess a single blue visual pigment, while the anomuran chirostylid crabs *Gastroptychus spinifer* and *Eumunida picta* possess a UV visual pigment in addition to the blue visual pigment (Frank 2006, 2008 and in prep). Sacrificing photoreceptor space for a UV visual pigment suggests that UV sensitivity plays an important role in their visual ecology, and at these depths (400 – 600 m), UV sensitivity has to be related to bioluminescence as solar UV does not penetrate this far. These species are not bioluminescent, which raises the

possibility that the function of UV sensitivity is to see as-yet undiscovered short wavelength bioluminescence from their preferred niche, or perhaps a prey item. In the Gulf of Mexico, *Gastroptychus spinifer* was frequently observed associated with a gorgonian (Frank, Widder, Johnsen, pers. obs.), which was not collected, so neither the species nor bioluminescence potential are known. However, short wavelength luminescence from gorgonians is not unknown, as the spectra emission from *Thourella* initially peaks in the near UV (405 nm), with a secondary peak in the blue (475 nm) (Herring 1983).

Using our combined expertise in bioluminescence, taxonomy, visual ecology, imaging and molecular biology, we propose to use the unique capabilities of the Johnson-Sea-Link submersible to explore the deep-sea benthic environment and look for new sources of bioluminescence. We will use submersible-based low-light cameras to observe and photograph stimulated bioluminescence of living organisms *in situ*. In addition, we will attempt to image background bioluminescence of decaying material or bacterial mats. Utilizing thermally insulated, light-tight BioBoxes, we will be able to collect benthic specimens and bring them to the surface alive, in cold water, and still dark-adapted, enabling us to identify the organisms that produced bioluminescence *in situ*, conduct spectral measurements of their luminescence, initiate molecular studies to look for novel photoproteins involved in producing short-wavelength bioluminescence, and continue studies of the visual physiology of those specimens with photoreceptors. The Eye-in-the-Sea camera system will be deployed for 24 hour time intervals, allow us to unobtrusively examine non-stimulated bioluminescence, as well as animal reactions to artificial bioluminescent prey.

Thematic priorities addressed:

- ***Survey unknown, or poorly known, regions and sea floor features***
- ***Discover and describe new species and new communities of organisms***

Location for expedition: Rose Atoll is located approximately 130 nautical miles east-southeast of Pago Pago Harbor, American Samoa, (14º32.643S, 168º10.663W).

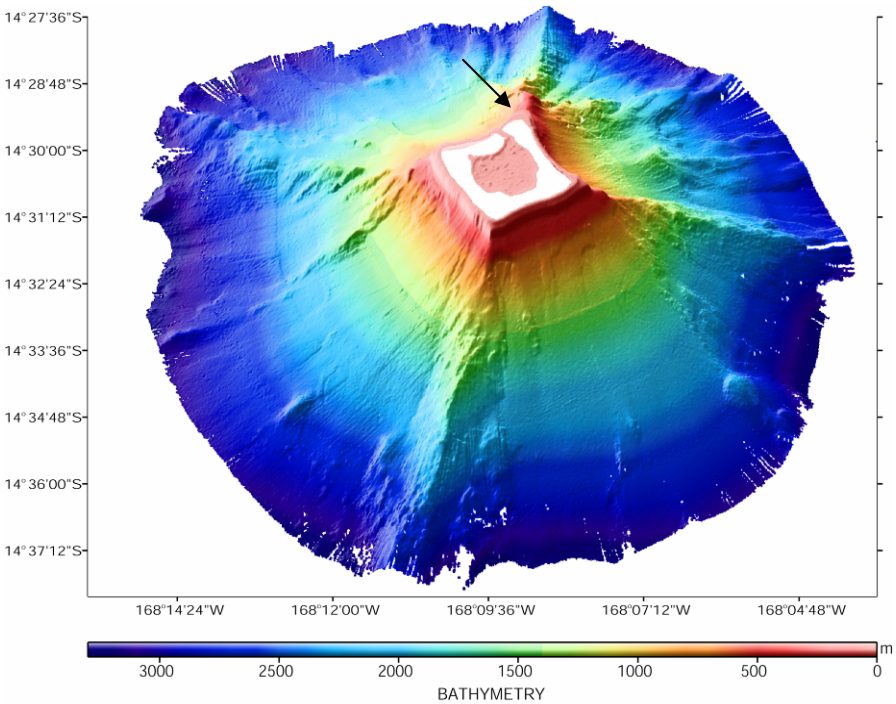

Figure 1. 3D visualization of the bathymetry around Rose Atoll (PIBHMC dataset, SOEST). Multibeam mapping shows fairly steep slopes around most of the atoll. The location for the proposed exploration is on the north-northeast end (arrow), where gently sloping terraces (300 – 1000 m) can be found that are within the dive depths of the Johnsen-Sea-Link submersible. (http://www.soest.hawaii.edu/pibhmc.pibhmc_amsamoa_rose.htm)

Extensive towed-diver survey observations of shallow areas show widespread coral cover and high benthic habitat complexity (reviewed in Brainard et al. 2008). While there have been a number of multibeam mapping surveys together with backscatter imagery of the deeper regions, there has been only two dives to waters deeper than 200 m, both with the Pisces Submersible in 2005. The submersible went to a maximum depth of 946 m (HURL, Dive P5-651), and on these two dives, in spite of the fact that most of the dives were devoted to searching for shipwreck debris, high levels of biodiversity were found, and scientists identified "as many as 60 new or rare species of marine life" (Wiltshire 2005).

Objective: Quantification of deep-sea benthic fauna in unexplored areas

A) Conduct visual surveys of the deep benthic fauna off Rose Atoll

Quantitative video transects to characterize benthic assemblages will be carried out by maintaining a relatively constant speed over the bottom while keeping the submersible's external video camera as close to perpendicular to the substrate as possible. Parallel lasers projected onto the substrate will provide scale, permitting calculation of frame area. Start and end points of each transect will be recorded and their locations used for geo-referencing. The camera will also record and display continuous date, time, depth, temperature and salinity readings. A project scientist will simultaneously record narration. Transect frames will be extracted from videotapes using Windows Movie Maker software, saved as high quality AVI format, and labeled with dive and transect number. Coral Point Count with Excel extensions (CPCe) version 3.4 software (Kohler and Gill 2006) will be used to calculate frame area and analyze percent cover of substrate categories (e.g., living coral, continuous hardbottom, rubble, sediment). Mobile megafanua (crustaceans, fish) will be quantified directly from dive narrations correlated with post-dive counts from video recordings.

We will collect small, discrete samples of benthic fauna using the hydraulic manipulators and maintain them in the BioBoxes (see below) until they reach the deck for processing. Organisms will be photographed *in situ* (at long range and close-up), then sampled and cataloged according to museum standards. As many species as possible will be identified on site; others will be distributed to recognized taxonomic experts for identification and ultimate deposit in major museum collections.

- ***<u>Description of poorly-known or discovery and description of unknown communities of organisms displaying novel relationships with their environment</u>***

Objective: Discover fundamental new information about the bioluminescence potential of benthic organisms

A) In situ observations of bioluminescence

There are essentially no images of the deep-sea floor under natural light. Instead, pictures are taken under full-spectrum floodlights. Not only do these lights disturb behavior, chase away and even permanently blind some animals, they give us a false view of the environment. Modeling based on imagery and methods from previous OE-funded cruises

shows that the actual visual world of deep-sea animals is quite different from what submersible video shows us (Figure 2).

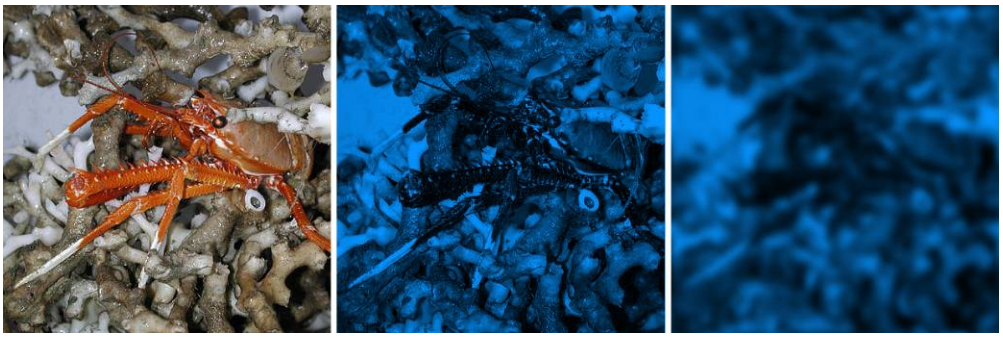

Figure 2: The deep-sea chirostylid anomuran crab *Eumunida picta*. Left panel shows view under usual submersible lights. Middle panel is corrected for the color of light in the deep-sea. Right panel is corrected for color and also for the typical (relatively poor) visual acuity of a deep-sea crab. Note how well the red and white coloration of the crab disrupts its outline and effectively camouflages it.

Models are a poor substitute for actual images, however, particularly for this expedition where constant benthic bioluminescence could dramatically affect how the deep-sea world appears. Unfortunately, because the intensity of this bioluminescence is at the limits of human vision, direct imagery has not been considered. However, we feel that recent developments in camera technology now make this possible.

We will be using two methods to image benthic bioluminescence. One uses an extremely sensitive video camera that images only in black and white, while the other is a color camera which we will use to take images under natural light conditions (dim remaining downwelling light/dim bioluminescent bacterial glow/stimulated animal bioluminescence)

1) **Videography**: A Kongsberg Simrad Zoom U/W ISIT video camera will be mounted on HBOI's camera boom system. This video camera is an extremely sensitive underwater camera system (5 X 10^{-6} lux) and is easily able to detect even dim sources of bioluminescence. The hydraulic boom system allows a wide range of camera positions without moving the entire submersible. This system consists of a three function hydraulically controlled boom that provides an 8-foot reach, and a horizontally synchronized platform with a camera pan and tilt mechanism. Possible sources of bioluminescence will be explored by setting the JSL on the bottom, positioning the robotic arm in front of the ISIT and then probing potential sources (e.g. soft corals, sea cucumbers, crinoids etc.) with the lights out. We will also position the claw between the camera and the bottom and take extended time exposures with the ISIT to see if there is any dim background glow from the seabed. Post-processing image analysis (Widder et al. 1989) will be carried out on the video data to see if frame averaging reveals the outline of the arm against a background glow. Specimens identified as bioluminescent will be collected in the BioBoxes for taxonomic identifications as well as laboratory investigations, including imaging and measurements of emission spectra.

2) **Still Photography:** Low-light photography has always been hampered by something known as reciprocity failure. This characteristic of all film leads to exposures lasting hours for anything much dimmer than twilight. However, digital cameras do not have

this problem and have now advanced to the point where photography at extremely low light levels is possible. Two cameras in particular, the Nikon D3 and Canon 5D mkII – both introduced in the last year - have film speeds of 25,600. Also, because their CCD sensors are the largest for any commercial camera, images at these speeds are far less noisy than has ever been possible. Although we haven't had access to these particular cameras yet, Figure 3 shows what is possible with even the Nikon D200, which is 1/8th as sensitive as the D3 and has a much smaller sensor area (~1/2) than this new camera. Even under starlight alone, the limit of human vision, the

Figure 3: Photograph taken outdoors in the dark on a moonless night (light levels equal to that found in rural areas without artificial lights). Despite being at the limits of human vision, the quality is quite high. Image was taken with a Nikon D200 fitted with a 50 mm f1.8 lens. Exposure was 30 seconds at ISO 3200. Dark frames (photos of pure dark) were taken and subtracted from the images to reduce noise. (Photo credit: S. Johnsen)

camera produced high quality color images with a relatively short exposure. Using a relatively insensitive Sony A100 with an f2.8 lens, we were also able to take the first ever images of the extremely dim luminescence from a cookie cutter shark (*Isistius*

Figure 4. Dim ventral luminescence from a cookie cutter shark (*Isistius brasieliensis*). Image on left is a grab from a video camera, filmed under room right. Image on right is very dim bioluminescence emitted from the entire ventral surface with the exception of the dark collar near the head. The luminescence was at the limit of human sensitivity. Image was a 30 sec exposure, taken with a Sony A100, f2.8, ISO 1600, in RAW mode, with contrast and brightness adjusted in Adobe Photoshop. (Photo Credit: T. Frank)

brasieliensis – Fig. 4*)*. Using a D3 or 5D fitted with an f1.4 lens, the images (with even lower noise) could be taken with an exposure of only 2 seconds.

The bioluminescent glow produced by bacteria (which may be present on the deep-sea floor) is on par with the light levels shown in Figures 3 and 4. In addition, stimulated bioluminescence from some benthic animals is considerably brighter, but of much shorter duration (Widder *et al*. 1983). With the sensitivity of the Nikon D3 or the Canon 5D, we are confident that we will be able to photograph both dim bacterial bioluminescence at longer exposures, as well as discrete flashes with a much faster shutter speed.

Because successful photography at these light levels requires flexibility, we propose to use cameras from within the passenger sphere of the *Johnson-Sea-Link*. Images will be taken with a Nikon D3 fitted with a 50 mm f1.4 lens. While f1.0 lenses do exist, the extra doubling of sensitivity is more than offset by the increase in aberrations and the extremely shallow depth of field, so the f1.4 lens is preferable. The camera will be mounted to a small tripod, monopod, or snake tripod and the submersible will be parked on the sea floor to minimize vibration during exposures. All lights on and within the submersible will of course be turned off. Dark frames will be taken and subtracted from images to reduce noise. Images will also be taken in 14 bit/channel "camera raw" format, so that is that one can brighten a dim image in Photoshop without losing tone gradation (a similar process done on a typical 8 bit/channel jpg image can result in very coarse jumps in tone).

3) **Long-term (24-48 hours) *in situ* observations**: *In situ* observations will also be made with the Eye-in-the-Sea camera system. This unique deep-sea observatory is an autonomous, battery-powered, video-capture and illumination system that uses far red light (680 nm light-emitting diodes with a high-pass filter for 700 nm and above) in combination with a highly sensitive camera that can record bioluminescence and compensate for the attenuation losses associated with using long wavelength illumination (Widder et al. 2005, Widder 2007). The system can be programmed to record in time-lapse and/or triggered mode. In triggered mode, a bioluminescent flash, detected by a PMT, activates the system. Illumination is set such that bioluminescence is clearly recorded, while still providing enough red-light illumination to reveal the organisms. Deployments will be made using bait and/or an electronic jellyfish lure capable of imitating five different luminescent displays, including highly conspicuous "burglar alarm" displays. All parameters are fully programmable by the user and EITS can be operated in a variety of modes during each deployment. EITS will provide video data, *in situ* measurements of marine activity and sounds, record spontaneous bioluminescence activity, carryout photo-behavior experiments, and, most exciting of all, offer the potential to reveal animals, behaviors and forms of bioluminescence <u>never</u> seen before. Another great advantage of the system is that it can be left to collect data for days while other mission-related operations are carried out and then collected when the schedule permits.

B) Laboratory studies of bioluminescence

Very little is known about the true bioluminescence potential of benthic organisms, because it is so difficult to collect living healthy specimens. Dredges and benthic nets are not an option due to 1) the tremendous damage they inflict on the environment and 2) the significant temperature gradients that tropical/subtropical animals are subjected to when the net or trawl is brought to the surface are generally lethal. Rather, we propose to collect these species utilizing the unique capabilities of the Johnson-Sea-Link submersible.

1) Collection of live specimens*:* For marine animals without air-filled spaces (i.e. any animal without a swim bladder or air-filled float), pressure changes are generally not lethal if the animals live shallower than 1000 m, so the major parameter than needs to

be controlled is temperature. Therefore, collections will be made into specially designed BioBoxes, constructed of 5/8 to 3/4 inch Plexiglas for temperature insulation. These BioBoxes can be closed at depth, and O-ring seals around the lids ensure that no exchange with warmer surface water will occur on the trip to the surface. A pressure relief valve releases excess pressure during the ascent. A single 36" long BioBox composed of clear Plexiglas will be used for the collection of larger non-motile benthic fauna, such as corals and crinoids. For animals with photoreceptors that need to be protected from light exposure, collections will be made under dim red light into black light-tight BioBoxes (see below). Any animals that show a bioluminescent response to light will be collected in a dark BioBox on subsequent dives even if visible photoreceptors are not present (eg, animals like pelagic pyrosomes, which has distinct light-activated bioluminescence, but no apparent photoreceptors) for tests of light-stimulated bioluminescence.

2) **Kinetics and flash patterns:** Using an integrating sphere and low-light video recordings, we will measure flash kinetics, patterns, and intensities of bioluminescent light emission from organisms. Even in well-known species, their bioluminescence capabilities are rarely known and by using specimens collected in good condition, there is a high potential for new discoveries (e.g., Haddock and Case, 1995)

3) **Emission spectra:** An OceanOptics QE65000 spectrometer, which uses a cooled back-illuminated CCD, has been shown to be sensitive enough to measure luminescence spectra at sea. Through spectral measurements in particular, we anticipate revealing ecological patterns of expression in organisms living in eternal twilight or darkness. Luminescent pigments will be extracted in a range of polar solvents and their absorbance and fluorescence spectra measured using NanoDrop instruments. These properties can improve modeling of the optical environment, and provide a mechanism for predicting luminescence related functions of coloration.

4) **Identification of novel bioluminescent photoproteins:** Cross-reactions with peroxides, coelenterazine, and Ca^{++} ions will be conducted at sea to determine basic photochemical processes and to identify potentially interesting luminescent chemistries. The photophores of specimens of interest will be dissected into RNAlater to preserve their mRNA. Expression screening of subsequent cDNA libraries (Schnitzler, et al. 2008) can be conducted back at shore on these enriched tissues.

Objective: Discovery of fundamental new knowledge about the visual systems of benthic organisms and their relationship to bioluminescence

Collections without exposure to light are imperative, as a number of studies, starting with Loew (1976) have demonstrated that even minimal exposure to bright light (1 minute of daylight or 5 minutes of moonlight) or submersible lights (Gaten et al. 1998) can cause irreparable damage to photoreceptors adapted for dim-light. Therefore, all collections of animals for these studies will be made with all the lights on the JSL turned off, with the exception of the DeepSea Power and Light HMI lights on the front frame. These lights will be fitted with orange and red cut-off filters (minimum cut-off wavelength 580 nm), as

previous work has shown that deep-sea species are very insensitive to wavelengths above 570 nm (Frank and Case, 1988, Frank and Widder, 1999).

1) **Animal Collections**: Animals collected with the suction sampler, fitted with a perforated grid at the collection orifice so that animals are gently held against the grid and not suctioned into the tube, will be deposited into one of 3 BioBoxes, constructed of 5/8" black Plexiglas (Fig. 5) installed in the front collecting basket of the JSL. The

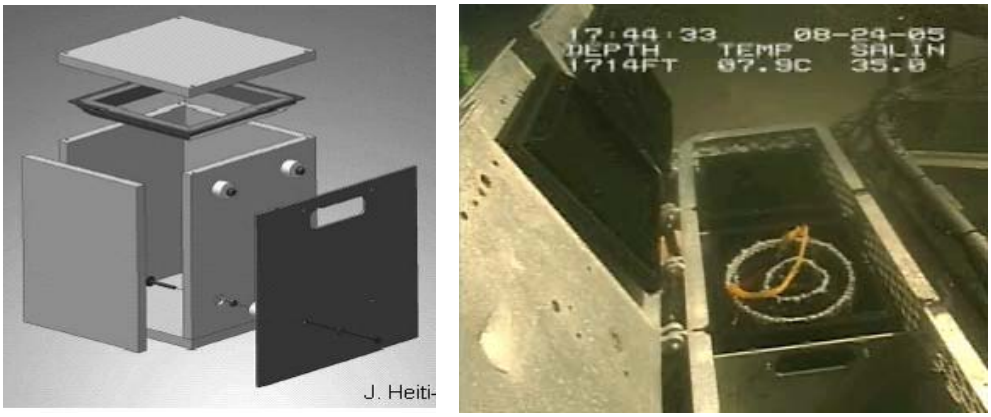

Figure 5: Black, light-tight, temperature insulated BioBox, showing construction with O-ring seal on left and position in front collecting chamber of JSL on right, with lids attached to hydraulically activated lids on collection basket

lids of the BioBoxes are attached to the hydraulically activated lids on the collection basket, so the lids can be closed at depth. The lids have O-rings seals, ensuring a water- and light-tight seal when the lids are in place. Pressure relief valves release excess pressure on the trip to the surface. Once at the surface, the BioBox lids can be disengaged from the collection basket lids without opening the BioBoxes, allowing for their removal for transport to a light tight room, where animals can be sorted and prepared for experiments under dim red light.

In addition to collections with the suction sampler, small baited traps that can fit into the black BioBoxes will also be deployed. Pingers attached to the traps will enable the submersible to pinpoint their location, and once the traps are visually sighted, all lights except for the red lights will be extinguished, and the traps recovered into the BioBoxes. Species associations will be determined during light transects, and the relationship between bioluminescence and photoreceptor sensitivity of organisms associated with bioluminescent fauna will be determined.

2) **Physiological Studies**: electroretinograms (ERGS) will be recorded from photoreceptors on shipboard using techniques used successfully on a number of research expeditions (Frank et al 2008, Frank in prep). These studies will allow us to determine the spectral sensitivity of the animals, as well as their temporal resolution, which are important indicators of how well these animals can track flashing or glowing prey. In addition, photoreceptors will be fixed using standard histological techniques for structural/ultrastructural studies of spatial resolution and optical adaptations to dim light environments, as well as quick frozen so that their visual pigment complement can be determined via MSP (Tom Cronin) and opsin sequence analysis (M Porter).

Collaborators and Expertise:

- Dr. Tamara Frank, Harbor Branch Oceanographic Institute at Florida Atlantic University – bioluminescence, visual ecology of deep-sea animals, low-light imaging
- Dr. Edith Widder, Ocean Research & Conservation Association – bioluminescence, low light imaging, in situ imaging and spectrometry
- Dr. Sönke Johnsen, Duke University- bioluminescence, spectrometry, modeling visual perception of deep-sea animals, low light imaging
- Dr. Charles Messing, NOVA Southeastern Univeristy – invertebrate biology, with particular emphasis in crinoids, deep-sea benthos community structure, benthic habitat mapping
- Dr. Steven Haddock, Monterey Bay Aquarium Research Institute – bioluminescence, spectrometry, molecular studies of luminescent photoproteins

Auxiliary Funding and Time Constraints

The Waitt Institute of Discovery is funding the transit of the Seward Johnson and the JSL submersible to Samoa, with Harbor Branch Oceanographic Institute providing some cost share. The current proposal is requesting funds for an exploration expedition off Rose Atoll while the ship is already in Samoa, after the 4th and final leg of the Waitt funded research expedition, currently set to end in American Samoa on July 22nd, 2009.

Anticipated Outputs/Benefits of Proposed Exploration

1) Quantification of benthic fauna in unexplored regions of Rose Atoll
2) Discovery of new or rare species inhabiting this region
3) First ever *in situ* photos of deep-sea benthic bioluminescence
4) Discovery of new forms of bioluminescence, including propagation patterns and possible UV output
5) Discovery of novel relationships between deep-sea crustaceans and specific bioluminescent organisms
6) Modeling of photosensitivity of benthic crustaceans to *in situ* light sources – spectral emission and intensity correlated with photosensitivity – can they see the light?

In addition to data presented in scientific papers and at scientific meetings, all of our institutions give us the opportunity to present lectures to the general public, which are announced via local newspapers and radio stations. Bioluminescence is a topic that has tremendous public appeal, and the exotic locale for the proposed research, as well as the inevitable discoveries that will result from the exploration will undoubtedly generate considerable media coverage. In addition to this commercial media coverage, we have actively participated in webpages that provide near real-time coverage of our oceanographic missions, either as contributors of background information and mission logs on the webpages maintained by NOAA Ocean Exploration when our expeditions have been signature expeditions (http://oceanexplorer.noaa.gov/explorations/04deepscope/welcome.html; http://oceanexplorer.noaa.gov/explorations/05deepscope/welcome.html; http://oceanexplorer.noaa.gov/explorations/05deepcorals/welcome.html) or by maintaining our own webpages on the @sea webpage at Harbor Branch Oceanographic Institute at FAU (http://www.at-sea.org/missions/migrationmystery/; http://www.at-

sea.org/missions/migrationmystery2/; http://at-sea.org/missions/deepscope3/preview.html). The Monterey Bay Aquarium Research Institute also has public expedition web pages which publicize ongoing work though at-sea blog entries (http://www.mbari.org/expeditions), as well as links to content on Google Earth, whereby browsers of the globe can be linked to online content describing the research and discoveries that are charted on their globe. These webpages generate tremendous public interest that will heighten public awareness of the amazing discoveries still to be made.

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SUMMARY OF RESULTS FROM PAST OCEAN EXPLORATION SUPPORT

1) Publications and Meetings

Brooke SD, **Messing CG**, Reed JK, Gilmore RG (2006) Exploration of deep-sea coral ecosystems along the east coast of Florida. 11th International Deep-Sea Biology Symposium, Southampton, UK, 10-14 July, Abstracts pp 35-36.

Cohen JH, **Frank TM** (2007). Vision in the hyperiid amphipod Scina crassicornis. *J Mar Biol Assoc UK 87***:** 1202 - 1206

Frank T (2008) Vision in the Deep. Committee's Choice Presentation. 2008 Ocean Sciences Meeting, Orlando, Florida

Frank T (2006) Ocean Sciences 2006, Hawaii – Ocean Exploration Symposium OS44L-06 – UV photosensitivity in a deep-sea benthic crab

Johnsen S (2005) Visual Ecology on the High Seas. Physico-chemical ecology of organisms conference. Roscoff, France.

Johnsen S (2005) Visual ecology on the high seas. *Mar. Ecol. Prog. Ser. 287*: 281-285.

Johnsen S (2005) The red and the black: Bioluminescence and the color of animals in the deepsea. *Integr Comp Biol* 45: 234-246.

Johnsen S (2005) The red and the black: bioluminescence and the color of animals in the deep sea. SICB Annual Meeting, 2005.

Johnsen S (2007). Does new technology inspire new directions? Examples drawn from pelagic visual ecology. *Int Com Biol 47:* 799-807.

Matz MV, **Frank TM**, Marshall NJ, **Widder EA**, **Johnsen S** (in press) Giant deep-sea protist produces bilaterian-like traces. *Current Biology*.

Messing CG, Brooke SD, Reed JK, Gilmore RG (2008) A possible role for agglutinating foraminiferans in the growth of deep-water coral bioherms. American Geophysical Union Annual Meeting, Fort Lauderdale, FL 27-30 May.

Messing CG, Reed JK, Brooke SD, Ross SW (2008) Deep-water coral reefs of the United States. Pp. 767-791 *In*: Riegl B & Dodge RE (eds), *Coral Reefs of the USA.* Springer-Verlag.

Messing CG, Reed JK, Walker BK, Brooke SD, Dodge RE (submitted) Remotely-Operated Vehicle surveys of benthic macrofauna across the outer southeastern Florida Shelf. *Bull. Mar. Sci.*

Marshall NJ, Cronin TC, Shashar N, **Johnsen S, Widder EA** (2004) Polarisation vision in terrestrial and aquatic environments. Insect Sensors and Robotics Conference. Brisbane.

Raymond EL, **Widder EA** (2005) Eye-In-The-Sea: A platform for unobtrusive in-situ observations of deep-sea nekton. Eos Trans. Amer. Geophys. Union.

Raymond EL, **Widder EA** (2006) Unobtrusive Observations of Deep-Sea Megafauna in the Gulf of Mexico. Eos Trans. Amer. Geophys. Union. OS24I-05

Reed JK, Pomponi SA, **Messing CG**, Brooke SD (2008) Habitat, Fauna, and Conservation of Florida's Deep-Water Coral Reefs. American Geophysical Union Annual Meeting, Fort Lauderdale, FL 27-30 May.

Shirur K, **Messing CG**, Rodriguez C, Feingold J, Reed JK, Brooke SD (2008) Quantitative Habitat Characterization and Benthic Assemblage Structure of Deep-water Scleractinian Reefs off Eastern Florida. 11th Intl. Coral Reef Sympos., Ft. Lauderdale, FL.

Sweeney A, Haddock SHD, **Johnsen S (2007**) Comparative visual acuity of coleoid cephalopods. *Int Comp Biol 47: 8*08-814.

Widder EA (2005) Ocean literacy: A scientist's perspective. Eos Trans. Amer. Geophys. Union.

Widder EA (2006) Reading the Ocean by the Light of its Inhabitants. (Plenary). Eos Trans. Amer. Geophys. Union. OS21A-01

Widder EA (2007) Sly eye for the shy guy: Peering into the depths with new sensors *Oceanography.* 20(4): 46-51 (invited review)

Widder, E.A. and B.H. Robison. (2004) Eye-In-The-Sea: A deep sea observatory for unobtrusive observations of animal behavior. ASLO/TOS Ocean Research Conference 2004:173

Widder, E.A., B.H. Robison, K.R. Reisenbichler, and S.H.D. Haddock (2005) Using red light for in situ observations of deep-sea fishes. *Deep Sea Res*. 52: 2077-2085.

2) Additional Education and Outreach Activities

Contributions to background articles and expedition logs – TM Frank, S Johnsen, CG Messing, EA Widder

- http://oceanexplorer.noaa.gov/explorations/04deepscope/welcome.html
- http://oceanexplorer.noaa.gov/explorations/05deepscope/welcome.html
- http://oceanexplorer.noaa.gov/explorations/05deepcorals/welcome.html
- http://at-sea.org/missions/deepscope3/preview.html

NOAA Office of Ocean Exploration: *Hidden Oases: Florida's Deep-Sea Reefs*. CG Messing, writer, producer. 48-min educational video and teacher's guide.

Teacher Training/Professional Development workshops utilizing data/images from OE expeditions

- NOAA Office of Ocean Exploration Professional Development Workshops
  - Ft. Pierce, FL, 2005 – EA Widder, TM Frank
  - St. Petersburg, FL, Nov. 2007 – EA Widder, TM Frank
  - Charleston, SC, Feb. 2008 – TM Frank
  - St. Petersburg, FL, Sept. 2008 – TM Frank

- AGU Geophysical Information for Teachers (GIFT) Workshop, Honolulu, HI Feb 2006 - EA Widder

- EARTH teacher training workshop, Tuckerton, NJ Jul 2006 – EA Widder

- COSEE Teacher Training Workshops
  - Cedar Key, FL – 2004, 2008 – TM Frank
  - USC, April 2008 – EA Widder

- *The Ocean Planet*, Indian River State College, Ft. Pierce, FL, May 2008 – EA Widder

- *Hands on Ocean Science*, Florida Association of Teacher Educators, 2008 Annual Conference Plenary lecture. Sep 26, 2008 – EA Widder

CURRENT AND PENDING SUPPORT

TM Frank – Harbor Branch Oceanographic Institute at Florida Atlantic University

Title	Source	Status	Months/ Year	Total Award	Period
*Oceanographic Expe*rience for Undergraduates	Florida Inst. Oceanography	Current	.25	$32,800	3/1/09 – 3/31/09
Deep Downunder: Designing a Deep Sea Exploration and Discovery Capability for Australia	Australian Research Council	Current	0	$1.4 million AUD	9/07 – 8/10
Operation Deep-Scope 2007: Characterization of Cliff Ecosystems Using New Technologies	NOAA-OE	Current	0	$352,140	07/07 – 12/08
Bioluminescence in the Deep-sea Benthos	NOAA-OE	Pending	.75	$430,365	5/1/09 – 4/30/10
Bringing Ocean Literacy to Land-Locked States	NOAA-OE	Pending	1.5	$72998	4/1/09 – 3/31/10
CI: Eastern U.S. Continental Shelf Frontier Exploration, Research and Technology	NOAA	Pending	1	$21.5 million	1/09 – 12/14

EA Widder – Ocean Research & Conservation Association

Title	Source	Status	Months/ Year	Total Award	Period
MacArthur Fellows Award	MacArthur Foundation	Current	0	$500,000	1/1/07 – 12/31/11
Coastal Marine Ecosystems at Risk: An Opportunity to Partner with ORCA to Solve America's Dirty Water Dilemma	Claneil Foundation	Current	0	$1 million	7/1/08 – 6/30/11
Bioluminescence Truth Data Measurement, Phase 5	ONR	Current	2.4	$706,912	1/1/06 - 2/23/09
Unobtrusive Undersea Observatory @ Monterey Canyon	NSF	Current	.25	$535,000	9/23/05 – 12/31/09
Monitoring Bloom Dynamics of a Common Coastal Bioluminescent Ctenophore	ONR	Current	.4	$288,800	1/16/07 – 12/31/10
Data Handling for EITS on MBARI's Monterey Accelerated Research System	MBARI	Current	1	$41,850	1/1/07 – 12/31/08
Operation Deep-Scope 2007: Characterization of Cliff Ecosystems Using New	NOAA-OE	Current	0	$352,140	07/07 – 12/08

Technologies					
Deep Downunder: Designing a Deep Sea Exploration and Discovery Capability for Australia	Australian Research Council	Current	0	$1.4 million AUD	9/07 – 8/10
Bioluminescence in the Deep-sea Benthos	NOAA-OE	Pending	.50	$430,365	5/1/09 – 4/30/10
Measuring and Tracking Dissolved Organic Matter In Situ	NSF	Pending	1	$292,219	3/1/09 – 2/28/11

S Johnsen – Duke University

Title	**Source**	**Status**	**Months/ Year**	**Total Award**	**Period**
Deep Downunder: Designing a Deep Sea Exploration and Discovery Capability for Australia	Australian Research Council	Current	0	$500,000	9/07 – 9/10
Collaborative Research: Transparency: Ultrastructural and Biochemical Modification in Muscular and Ocular Tissues	NSF	Current	2	$410,000	5/05 – 3/09
Operation Deep-Scope 2007: Characterization of Cliff Ecosystems Using New Technologies	NOAA-OE	Current	0	$352,140	07/07 – 12/08
Bioluminescence in the Deep-sea Benthos	NOAA-OE	Pending	.50	$430,365	5/1/09 – 4/30/10
Collaborative Research: Midwater Animal Models: Optical Measurements of Metabolic Transitions in Transparent Pelagic Biota	NSF	Pending	1.5	$383,353	1/09 – 12/10

CG Messing – Nova Southeastern University

Title	**Source**	**Status**	**Months/ Year**	**Total Award**	**Period**
Florida's Deep-water Oases: Exploration and Characterization of Deep Reef Ecosystems. Period	NOAA-OE	Current	1	$143,929	6/07 – 5/09
Bioluminescence in the Deep-sea Benthos	NOAA-OE	Pending	.50	$430,365	5/1/09 – 4/30/10

SHD Haddock – Monterey Bay Aquarium Research Institute

Title	**Source**	**Status**	**Months/ Year**	**Total Award**	**Period**
Beyond GFP and Aequorin: an Ocean-wide Study of Fluorescent and Luminous Proteins	NIH	Current	1	$187,177	9/1/06 - 8/31/09
Bioluminescence in the Deep-sea Benthos	NOAA-OE	Pending	.5	$430,365	5/1/09 – 4/30/10
Genetically encoded luminescence for in vivo imaging	NIH	Pending	3	$1,737,241	4/1/09-3/31/13

BIOGRAPHICAL SKETCH- Tamara M. Frank

Professional Preparation

California State University, Long Beach	Marine Biology	B.S.	1980
University of California, Santa Barbara	Aquatic Biology	M.A.	1985
University of California, Santa Barbara	Aquatic Biology	Ph.D.	1987

Academic Appointments

2008-pres	Associate Research Professor, Harbor Branch Oceanographic Institute@Florida Atlantic Univ.
2004-pres	Faculty of the Doctoral Program in Integrative Biology, Florida Atlantic University
2007-pres	Board Member, WhaleTimes,Inc.
2006-pres	Adjunct Scientist, Ocean Research and Conservation Assoc., Florida
2001-2007	Director, Department of Visual Ecology, Harbor Branch Oceanographic Institution
1998-2007	Associate Scientist, Harbor Branch Oceanographic Institution
1994-1997	Assistant Scientist, Harbor Branch Oceanographic Institution
1991 - 1993	Postdoctoral Fellowship, Harbor Branch Oceanographic Institution,
1989 - 1991	NIH Postdoctoral Traineeship, Hatfield Marine Science Center, Oregon
1988 - 1989	NIH Postdoctoral Fellowship, University of Connecticut Health Center

5 recent publications

Vision in the deep – a crab's eye view. (in prep for submission to *Marine* Biology)

Wagner, H.-J., Douglas, R.H., Frank, T.M., Roberts, N.W. and J.C. Partridge Wagner (accepted with revision). *Dolichopteryx longipes*, a deep-sea fish with a bipartite eye using both refractive and reflective optics. *Current Biology*

Whitehill, E.A.G., T.M. Frank, and M.K. Olds (accepted with revision) The structure and sensitivity of the eye of different life history stages of the ontogenetic migratory. *Gnathophausia ingens*. *Marine Biology*

Matz, M.V., T.M. Frank, N.J. Marshall, E.A.Widder, S. Johnsen. (in press). Giant deep-sea protist produces bilaterian-like traces. *Current Biology*

Frank TM, Porter M, Cronin TW (in press) Spectral sensitivity, visual pigments and screening pigments in two life history stages of the ontogenetic migrator *Gnathophausia ingens*. Journal of the Marine Biological Association of the United Kingdom.

Cohen, J.H., and T. M. Frank (2007). Vision in the hyperiid amphipod *Scina crassicornis*. Journal of the Marine Biological Association of the United Kingdom **87:** 1202 – 1206

5 recent conference abstracts

Frank TM (2008) Vision in the Deep. Committee's Choice Presentation. 2008 Ocean Sciences Meeting, Orlando, Florida

Frank TM (2007) Are there physiological differences between the photoreceptors of juvenile and adult ontogenetic migrators? SICB Annual Meeting, Phoenix, Arizona

Whitehill EAG, Frank TM (2007) Visual structural adaptations of life history stages of ontogenetically migrating crustaceans. Society of Int. and Comp. Annual Biology Meeting, Phoenix, Arizona – best student poster award, Crustacean Society

Frank TM (2006) UV photosensitivity in a deep-sea benthic crab. 2006 Ocean Sciences Meeting, Honolulu Hawaii

Frank TM (2006) Do ontogenetic migrators adapt to different light environments during their life histories? 11th International Deep-Sea Biology Symposium, Southampton, UK

Oceanographic Cruise Experience

1980–2007: 80 cruises, 1-6 weeks duration

- Chief scientist on 46 cruises on RVs *Cape Hatteras, Edwin Link/Seward Johnson II, Seward Johnson I with Johnson-Sea-Link submersible, New Horizon, Pelican, Sea Diver, Wecoma*

Selected Education Activities

- COSEE Summer Institute in Ocean Science for Middle School Teachers
  - Science Advisor – Cedar Key, FL, June 2004, June 2008
- NOAA Ocean Exploration Program Teacher Training Workshops
  - Sept. 2005, Nov. 2007, Feb. 2008, Sept. 2008
- National Ocean Science Bowl – Technical Advisory Panel – 2003, 2005, 2008
- Educator at Sea – provide opportunities for high school science teachers to participate on research cruises
  - 2005: Steve Shotola, St. Lucie West Centennial High School
  - 2006: Gordon Phail, Lincoln Park Academy
    Kelly Mazako, Ft. Pierce Central High School
    Chris Depasquale, Oak Park River Forest High School, IL
  - 2007: Chris Depasquale, now at Nazarene Academy, IL
    Hollis Hoier, Sebastian River High School
- Consultant for
  - "Into the Deep exhibition", curator Claire Nouvian, Paris Museum of Natural History, currently going around the world
  - Discovery Channel/BBC *Blue Planet: The Deep* (Penny Allen), 1999-2002
  - Discovery Channel *Cuba: Forbidden Depths* (Al Giddings, Jim Lipscomb), 1999

Funding (last 5 years)

- NOAA Ocean Exploration; $352,140 (includes 12 days of ship/sub time), 2007 – 2008
- Florida Institute of Oceanography
- NOAA Ocean Exploration; $328,905 (includes 14 days of ship/sub time), 2005 – 2006
- NSF Integrative Biology; $286,136 + 50 days ship time; 2004 - 2007

BIOGRAPHICAL SKETCH – EDITH A. WIDDER

a. Professional Preparation:

Tufts University, Medford, Mass.	Biology B.S. - *Magna cum laude* 1973
Univ. of California, Santa Barbara	Biochemistry M.S. 1977
Univ. of California, Santa Barbara	Neurobiology Ph.D. 1982
UCSB Postdoctoral Associate	Bioluminescence/spectroscopy/radiometry 1982-89

b. Academic appointments:

President and senior scientist, **Ocean Research & Conservation Assoc.** (2005-present)
Senior scientist and principal investigator, **Harbor Branch Oceanogr. Inst.** (1993-2005)
Adjunct Research Prof., Earth and Planetary Sciences Dept, **Johns Hopkins Univ**. (2000-present)
Distinguished Scientist Adjunct, **Monterey Bay Aquarium Research Institute** (1998-present)
Adjunct Professor of biological Sciences, **Florida Institute of Technology** (1991-present)
Adjunct Professor, Dept of Biological Science, **Florida Atlantic University** (2000-present)
Acting Division Director, **Harbor Branch Oceanogr. Inst.** (1993-1994)
Associate scientist and principal investigator, **Harbor Branch Oceanogr. Inst.** (1991-93)
Assistant scientist and principal investigator, **Harbor Branch Oceanogr. Inst.** (1989-91)
Scientific consultant for **Dynamics Technology**, Inc., Los Angeles (1987-89)
Assistant research biologist and co-principal investigator, **UC Santa Barbara**, Marine Science Inst. and Neuroscience Research Inst. (1985-89)
Postdoctoral research biologist, ONR bioluminescence program. **UC Santa Barbara** (1983-85)
Associate researcher, ONR bioluminescence program, **UC Santa Barbara** (1977-83)
Res. assoc. NIH resource lab in electron probe microanalysis. **Harvard Medical School.** (1973-75)
Lab. Assist in phycology. Field Station of Environmental Sciences of the **Univ. of Mass.** (1968-69)

c. 5 Related Publications:

Widder, E.A. (2007) Sly eye for the shy guy: Peering into the depths with new sensors **Oceanography**. 20(4): 46-51 *Invited review*.

Widder, E.A., B.H. Robison, K.R. Reisenbichler, and S.H.D. Haddock (2005) Using red light for *in situ* observations of deep-sea fishes. **Deep-Sea Res.** 52:2077-2085

Widder, E.A., C.L. Frey and J.R. Bowers (2005) Improved bioluminescence measurement instrument. **Sea Technology** 46(2): 10-16

Widder, E.A. (2002) Bioluminescence and the pelagic visual environment. **Mar. Fresh. Behav. Physiol.** 35 (1-2): 1-26. *Invited review*.

Widder, E.A. and T.M. Frank (2001) The Speed of an Isolume: A Shrimp's Eye View. **Mar. Biol.** 138:669-677.

5 Other Significant Publications (from more than 75 peer reviewed publications):

Herring, P.J. and E.A. Widder (2001) Bioluminescence in plankton and nekton. In; Steele, J.H., Thorpe, S.A. and Turekian, K.K. editors, **Encyclopedia of Ocean Science**, Vol. 1, 308-317. Academic Press, San Diego.

Widder, E.A and S. Johnsen (2000) 3D spatial point patterns of bioluminescent plankton: A map of the "minefield" **J. Plank. Res.** 22(3): 409-420.

Widder, E.A., S. Johnsen, S. A. Bernstein, J. F. Case, D. J. Neilson. (1999) Thin layers of bioluminescent copepods found at density discontinuities in the water column. **Mar. Biol.** 134: 429-437.

Widder, E. A. Co-holder of **U.S. Patent # 5264906**, awarded in 1993 for the HIDEX-BP (High Intake Defined Excitation Bathyphotometer) which remains the standard in the U.S. Navy for the measurement of bioluminescence in the oceans.

Johnsen, S., E.J. Balser and E.A. Widder. (1999) Modified suckers as light organs in a deep-sea octopod. **Nature** 398:113-114.

d. Synergistic Activities:

2005-present Co-founded and served as President and Sr. Scientist for the Ocean Research & Conservation Association a 501(c)(3) dedicated to protecting marine ecosystems and the species they sustain through the development of innovative technologies and science based conservation action www.teamorca.org .

2008 Profile – NOVA SCIENCE NOW http://www.pbs.org/wgbh/nova/sciencenow/0305/04.html

2003-2008 Curator of 5000 sq ft traveling museum exhibit on bioluminescence. Glow: Living Lights is scheduled for a 5 year North American tour and will be seen by over 5 million viewers. http://www.glowexhibit.com/

2002-present Editorial Board Member – Marine Technology Society Journal

2000 Wrote and produced "Marine bioluminescence: Secret Lights in the Sea". 26 min educational video. Winner of the 2001 Silver Reel Award in Media Excellence and the 2002 Silver CINDY (Cinema In Industry) Award from the International Association of Audio-Visual Communicators >1000 sold.

1998 Wrote and produced "The Bioluminescence Coloring Book". ISBN# 0-9659686-0-X. over 5000 sold. Now in second edition.

1997 Produced educational web site on bioluminescence www.biolum.org. Winner of the National Science Teachers Association sciLINKS award in 2001 and selected for the American Library Association's Great Web Sites for Kids in 2003.

e. Collaborators and Other Affiliations:

(i) Collaborators and Co-Editors preceding 48 months:

T. M. Frank (HBOI); C.L. Frey (HBOI) S.H.D. Haddock (MBARI); S. Johnsen (Duke); J. Katz (JHU); D. Lindsay (JAMSTEC); E. Malkiel (JHU); N.J. Marshall (U. of Queensland); Mikhail V. Matz, (U. of Texas); B.H. Robison (MBARI); A.V. Suntsov (NOAA); T.T. Sutton (VIMS); E.D. Thosteson (ORCA); E. Warrant (Lund Univ.)

(ii) Graduate and postdoctoral advisor:

James F. Case Prof. Emeritus Univ. of Calif. Santa Barbara

(iii) Thesis Advisor and Postgraduate-Scholar Sponsor last 5 years:

Graduate Students Advised: Karen Breitlow, M.S. Florida Atlantic University, Erika Raymond, Ph.D. Johns Hopkins University, Miranda Hoover, Ph.D. candidate FAU
Co-Advised: Jessie W. Davis, M.S. Florida Institute of Technology, Nicole McMullen, M.S. FAU; Beth Whitehill, M.S. FAU; Kyle Bartow, Ph.D. candidate FAU; Megan Geidner, M.S. candidate FAU
Postdocs Advised: Sönke Johnsen, HBOI Post Doc, Now tenured prof Duke Univ.

BIOGRAPHICAL SKETCH - SÖNKE JOHNSEN

Associate Professor, Biology Department, Duke University, Durham, NC, (919) 660-7321

Education:

B.A.	Mathematics, Swarthmore College: with Distinction (highest honor), Phi Beta Kappa and National Merit Scholarship.	1984-1988
Ph.D	Biology, University of North Carolina at Chapel Hill: 1996, Dr. William M. Kier, advisor.	1990-1996

Selected awards:

University Distinguished Teaching Award, Duke University	2006
Julius Thomas Hansen Lecturer, University of California, Berkeley	2005
George A. Bartholomew Award for Research in Comparative Physiology, Society for Integrative and Comparative Biology.	2001
Woods Hole Postdoctoral Scholarship, Woods Hole Oceanographic Institution.	1999-2000
Harbor Branch Postdoctoral Fellowship, Harbor Branch Institution.	1997-1998

Thirty-two relevant recent publications:

Lim, M. L. M., Sodhi, N. S., Johnsen, S. Endler, J. A. (in review). The importance of sensory ecology to conservation biology. *Trends in Ecology and Evolution*.

Matz. M. V., Frank, T. M., Marshall, N. J., Widder, E. A., Johnsen, S. (2008) Giant deep-sea protist produces bilaterian-like traces. *Current Biology*. (in press)

Speiser, D. I., and S. Johnsen (2009). Comparative morphology of the mirror-based eyes of scallops (Pectinoidea). *American Malacological Bulletin*. (in press)

Speiser, D. I., and S. Johnsen (2008). Scallops visually respond to the presence and speed of virtual particles. *Journal of Experimental Biology*. 211: 2066-2070.

Johnsen, S., and K. J. Lohmann (2008). Magnetoreception in animals. *Physics Today*. 61(3): 29-35.

Gagnon, Y. L., Shashar, N., Warrant, E. J., and S. Johnsen (2007). Light scattering from pelagic zooplankton: measurements at different angles and modeling corresponding sighting distances. *Journal of Experimental Biology* 210: 3728-3735.

Leech, D. M. and S. Johnsen (2007). Light, photoreceptors and UV vision. In *Encyclopedia of Inland Waters*, (ed. G. E. Likens).

Sweeney, A., Haddock, S. H. D., and S. Johnsen (2007). Comparative visual acuity of coleoid cephalopods. *Integrative and Comparative Biology* 47: 808-814.

Johnsen, S., Mattern, E., and T. Ritz (2007). Light-dependent magnetoreception: quantum catches and opponency mechanisms of possible photosensitive molecules. *Journal of Experimental Biology* 210: 3171-3178.

Martin, C. H. and S. Johnsen (2007). A Field Test of the Hamilton-Zuk Hypothesis in the Trinidadian guppy (*Poecilia reticulata*). *Behav. Ecol. Sociobiol.* 61: 1897-1909.

Johnsen, S. (2007). Does new technology inspire new directions? Examples drawn from pelagic visual ecology. *Integrative and Comparative Biology* 47: 799-807.

Cummings, M. M. and S. Johnsen (2007). Light in the rocky shores. Pp. 327-331 in *Encyclopedia of Tidepools and Rocky Shores*, (M. Denny and S. Gaines Eds.), University of California Press.

Sweeney, A. M., Des Marais, D. L., Ban, Y. A. and S. Johnsen (2007). Evolution of Graded Refractive Index in Squid Lenses. *Journal of the Royal Society Interface* 4: 685-698.

Leech, D. and S. Johnsen (2006). UV vision and the Feeding Ecology of Juvenile Bluegill Sunfish, *Lepomis machrochirus*. *Canadian Journal of Fisheries and Aquatic Sciences*. 63: 2183-2190.

Tuthill, J. and S. Johnsen (2006) Polarization sensitivity in the red swamp crayfish *Procambarus clarkii* enhances the detection of moving transparent objects. *Journal of Experimental Biology*. 209:1612-1616.

Johnsen, S., Kelber, A., Warrant, E. J., Sweeney, A. M., Lee, R. H. Jr., Hernández-Andrés, J. (2006). Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. *Journal of Experimental Biology* 209: 789-800.

Johnsen, S. and K. J. Lohmann (2005). The physics and neurobiology of magnetoreception. *Nature Reviews Neuroscience* 6: 703-712.

Johnsen S. (2005). Visual ecology on the high seas. *Marine Ecology Progress Series* 287: 281-285.

Johnsen, S. (2005). The red and the black: Bioluminescence and the color of animals in the deep sea. Integrative and Comparative Biology 45: 234-246.

Johnsen, S. and H. M. Sosik (2004). Shedding light on light in the ocean. *Oceanus* 43: 24-28.

Johnsen, S., Widder, E. A., and C. D. Mobley (2004). Propagation and perception of bioluminescence: factors affecting the success of counterillumination as a cryptic strategy. *Biological Bulletin* 207: 1-16.

Johnsen, S. (2003). Lifting the cloak of invisibility: the effects of changing optical conditions on pelagic crypsis. *Integrative and Comparative Biology* 43: 580-590.

Johnsen, S. and H. M. Sosik (2003). Cryptic coloration and mirrored sides as camouflage strategies in near-surface pelagic habitats: implications for foraging and predator avoidance. *Limnology and Oceanography* 48: 1277-1288.

Leech, D. and S. Johnsen (2003). Avoidance and UV vision. Pp. 457-481 in *UV Effects in Aquatic Organisms and Ecosystems*, (W. Helbling, H. Zagarese eds.).

Johnsen, S. (2002). Cryptic and conspicuous coloration in the pelagic environment. *Proceedings of the Royal Society of London: Biological Sciences* 269: 243-256.

Johnsen, S. (2001). Hidden in plain sight: the ecology and physiology of organismal transparency. *Biological Bulletin* 201: 301-138.

Johnsen, S., and E. A. Widder (2001). Ultraviolet absorption in transparent zooplankton and its implications for depth distribution and visual predation. Marine Biology 138: 717-730.

Johnsen, S. (2000). Transparent animals. *Scientific American* 282(2): 62-71.

Johnsen, S., and E. A. Widder (1999). The physical basis of transparency in biological tissue: ultrastructure and the minimization of light scattering. *Journal of Theoretical Biology* 199: 181-198.

Johnsen, S., Balser, E. J., and E. A. Widder (1999). Light-emitting suckers in an octopus. *Nature* 398: 113-114.

Johnsen, S., Balser, E. J., Fisher, E. C., and E. A. Widder (1999). Bioluminescence in the deep-sea cirrate octopod *Stauroteuthis syrtensis* Verrill (Mollusca: Cephalopoda). *Biological Bulletin* 197: 26-39.

Johnsen, S., and E. A. Widder (1998). The transparency and visibility of gelatinous zooplankton from the north west Atlantic and Gulf of Mexico. *Biological Bulletin* 195: 337-348.

CURRICULUM VITAE (Abridged)

Charles Garrett Messing **(Oct 2008)**
Professor
Oceanographic Center
Nova Southeastern University.
8000 N Ocean Drive, Dania Beach, Florida 33004

1470 Sheridan St., #16C
Hollywood, FL 33019
DOB: 5 July 1948, Bronx, New York

Education

Ph. D. (1979) Biological Oceanography, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Coral Gables, FL
M. S. (1975) Biological Oceanography, RSMAS, University of Miami, Coral Gables, FL
B. A. (1970) Biological Science, Rutgers: The State University, New Brunswick, NJ

Recent Professional Scientific Experience

2000-present Professor, Nova Southeastern Univ. Oceanographic Center, Dania Beach, FL
1994-2000 Adjunct Assoc. Prof., Dept. Geological Science, Univ. Miami, Coral Gables, FL
1993-2000 Associate Professor, Nova Southeastern Univ. Oceanographic Center, Dania, FL
1990-1993 Assistant Professor, Nova University Oceanographic Center, Dania, FL

Recent Awards

2000, 2005 Visiting Scientist, Museum National d'Histoire Naturelle, Paris
2001 Visiting Scholar, University of Sydney (November)
1998 Visiting Scientist, Museum National d'Histoire Naturelle, Paris

Memberships

Paleontological Society
Biological Society of Washington
National Center for Science Education
Sigma Xi
American Geophysical Union
The Explorers Club

Selected Recent Peer-reviewed Journal Articles

Messing CG, Reed JK, Walker BK, Brooke SD and Dodge RE (in review) Remotely-Operated Vehicle surveys of benthic macrofauna across the outer southeastern Florida Shelf. *Bull. Mar. Sci.*

Messing CG, Reed JK, Brooke SD & Ross SW. 2008. Deep-water coral reefs of the United States. Pp. 767-791 *In*: Riegl B & Dodge RE (eds), *Coral Reefs of the USA*. Springer-Verlag.

Rankin DL & Messing CG. 2008. A revision of the comatulid genus *Stephanometra* A.H. Clark with a rediagnosis of the genus *Lamprometra* A.H. Clark (Echinodermata: Crinoidea). *Zootaxa* 1888:1-35

Messing CG, David J, Roux M, Améziane N & Baumiller TK. 2007. In situ stalk growth rates in tropical western Atlantic sea lilies (Echinodermata: Crinoidea). *J Exp. Mar. Biol. Ecol.* 353 (2007) 211–220.

Messing CG. 2007. The crinoid fauna (Echinodermata: Crinoidea) of Palau. *Pacific Science* 61(1): 91-111.

Messing CG, Meyer, DL, Siebeck U, Jermiin LS, Vaney, DI & Rouse GW. 2006. A modern, soft-bottom, shallow-water tropical crinoid fauna (Echinodermata) from the Great Barrier Reef. *Coral Reefs* 25(1):164-168.

Messing CG. 2004. Biozonation on deep-water carbonate mounds and associated hardgrounds along the western margin of Little Bahama Bank, with notes on the Caicos Platform island slope. Pp. 107-115 IN Lewis, R.D. & Panuska, B.C. (eds.) *11th Sympos. Geology of the Bahamas and other Carbonate Regions*, Gerace Research Center, San Salvador, Bahamas.

Selected Recent Conference Abstracts

Shirur K, Messing CG, Rodriguez C, Feingold J, Reed JK and Brooke SD. 2008. Quantitative Habitat Characterization and Benthic Assemblage Structure of Deep-water Scleractinian Reefs off Eastern Florida. 11th Intl. Coral Reef Sympos., Ft. Lauderdale, FL.

Messing CG, Brooke SD, Reed JK, Gilmore RG. 2008. A possible role for agglutinating foraminiferans in the growth of deep-water coral bioherms. American Geophysical Union Annual Meeting, Fort Lauderdale, FL 27-30 May.

Reed JK, Pomponi SA, Messing CG & Brooke SD. 2008. Habitat, Fauna, and Conservation of Florida's Deep-Water Coral Reefs. American Geophysical Union Annual Meeting, Fort Lauderdale, FL 27-30 May.

Brooke SD, Messing CG, Reed JK & Gilmore RG. 2006. Exploration of deep-sea coral ecosystems along the east coast of Florida. 11th International Deep-Sea Biology Symposium, Southampton, UK, 10-14 July, Abstracts pp 35-36.

Messing CG. 2005. Growth and ecology of stalked crinoids: summary of a decade of in situ experiments. 2nd Echinoderm Symposium, Muséum national d'Histoire naturelle, Paris, 5-7 Sep., "Ecology and Evolution in Echinoderms."

Selected Recent Grants and Contracts (Principal Investigator except where noted)

NOAA Office of Ocean Exploration: Florida's Deep-water Oases: Exploration and Characterization of Deep-Reef Ecosystems, Jun 2007-May 2009

Suez Energy North America: Calypso U.S. Pipeline, LLC, Mile Post (MP) 31 - MP 0: Deep-water Marine Benthic Video Survey, 2006-2007

Suez Energy North America: Marine Benthic Video Survey, Calypso Florida DWP Project, 2006-2007

NOAA Office of Ocean Exploration: Mapping and Characterization of Deep Sea Coral Ecosystems off the Coast of Florida. Nov 2005-Oct 2006 (Co-Principal Investigator; Sandra D. Brooke, P.I.)

Ecology & Environment, Inc.: Tractebel/Blue Marlin natural gas pipeline biological habitat survey, 0-200 m depth, off Freeport, Grand Bahama Island. Jun 2003-Jun 2007 (Co-investigator; R.E. Dodge, P.I.).

Ecological Services Program, URS Corporation, Miami Springs, FL 33166: URS Tractebel/Calypso natural gas pipeline biological habitat survey, 65-200 m depth, off Fort Lauderdale, FL. Jun 2003-Jun 2004 (Co-investigator; R.E. Dodge, P.I.).

Selected Recent Educational Products

NOAA Office of Ocean Exploration: *Hidden Oases: Florida's Deep-Sea Reefs*. Wrote and produced 48-min educational video and teacher's guide.

BIOGRAPHICAL SKETCH – STEVEN HADDOCK

EDUCATION

Harvey Mudd College, Claremont, California. "Systems and Policy" BS 1988. Honors in Biology. National Merit Scholar. Dean's List.

UC Santa Barbara. Evolution, Ecology, and Marine Biology Ph.D. 1997. Thesis title: Bioluminescence in the open ocean and deep sea: gelatinous zooplankton and marine snow. Advisor: James F. Case.

Jan 1998-Dec. 1999: Post doc: UCSB. Detecting biouminescence with an autonomous underwater vehicle (REMUS). Advisor: James Case

Jan 1999 - Jun. 2001: Postdoctoral Research Associate. Monterey Bay Aquarium Research Inst. Two-year fellowship to study ecology of midwater zooplankton and molecular biology of photoproteins.

RECENT PROFESSIONAL

Jul 2001 - present: Research Scientist. MBARI. Gelatinous plankton: phylogenetics and luminescence.

2005-present: Adjunct Assistant Prof: UCSC Dept. Earth & Marine Sci.

Fall 1996: Visiting Faculty. Evergreen State College, WA. Co-developed and taught *Invertebrate Zoology, Entomology, and Systematics.* Lectures and labs on invertebrates and phylogenetics.

SELECTED RECENT PUBLICATIONS

Dunn, C.W., [...] S.H.D. Haddock, [...] M.Q. Martindale and G. Giribet. (2008) Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452: 745-749

Schnitzler, C.E., R.J. Keenan, R. McCord, A. Matysik, L.M. Christianson, and S.H.D. Haddock. (2008) Spectral diversity of fluorescent proteins from the anthozoan Corynactis californica. *Marine Biotech.* 10: 328-342

Haddock, S.H.D. (2007) Comparative feeding behavior of planktonic ctenophores. *Integr. Comp. Biol*. 47: 847-853

Mills, C.E. and S.H.D. Haddock. (2007) Key to the Ctenophora. p. 189-199 in *Light and Smith's Manual: Intertidal invertebrates of the central California coast*. J.T. Carlton, ed. U.C. Press.

Haddock, S.H.D. (2006) "Luminous marine organisms" in *Photoproteins in Bioanalysis*, S.Daunert & S.Deo, eds. Wiley-VCH. New York, p. 25-47.

Haddock, S.H.D., C.W. Dunn, P.R. Pugh. and C.E. Schnitzler. (2005) Bioluminescent and red-fluorescent lures in a deep-sea siphonophore. *Science*. 309:263.

Haddock, S.H.D., C.W. Dunn, and P.R. Pugh. (2005) A reexamination of siphonophore terminology and morphology, applied to the description of two new prayine species with remarkable bio-optical properties. *J. Mar. Biol. Assoc. U.K.* 85:695-707.

Haddock, S.H.D. and J.N. Heine. (2005) *Scientific blue-water diving*. Calif. Sea Grant, La Jolla, California.

Haddock, S.H.D., T.J. Rivers, and B.H. Robison. (2001) Can coelenterates make coelenterazine? Dietary requirement for luciferin in cnidarian bioluminescence. *Proc. Nat. Acad. Sci*. 98: 11148-11151.

Haddock, S.H.D. and J.F. Case (1999) Bioluminescence spectra of shallow

and deep-sea gelatinous zooplankton: medusae, ctenophores, and siphonophores. *Mar. Biol*.133: 571-582.

OUTREACH AND ACTIVITIES

Public Outreach via Popular Media (examples below):

Bioluminescence Web Page - http://lifesci.ucsb.edu/~biolum/
Web site noted by *Science*, *Natural History*, *Scientific American*, *National Geographic*, etc. for delivering scientific information about luminescence. Top rank in every major search engine.; front page: >100,000 hits annually.

Sep. 2008. History Channel interview on communication.
Jul. 2007. PBS TV interview and photos for *Jim Lehrer News Hour*
Apr. 2008. Photo and short research description in *National Geographic*
Nov. 2007 *Nature* article by Mark Schrope highlighting research
Nov. 2007. Interview with TFN French television news.
Oct. 2007. Hour-long live radio interview for NOAA *Ocean currents*
Sep. 2007. Three days of interviews with French TV show *Thalassa*.
Nov. 2006. McGraw Hill 2007 *Yearbook of Science*. Deep siphonophores.
Oct. 2006. *Abysses*. edited by Claire Nouvian. Major contributor to large-format book about the deep-sea
Jul. 2005. NPR radio interview about siphonophore research.
April 2005. "The complex world of siphonophores" by Haddock & Dunn. *JMBA Global News*.

Board Member/Councillor/Advisor

- Editorial Board of the journal *Luminescence*, 2003-2006
- Int'l Steering Ctty, CoML *Census of Marine Zooplankton*, 2004-now
- Councilor: Int'l. Society for Biolum. and Chemiluminescence 2002-
- Program Committee: 11th Int'l Symp. on Biolum. and Chemilumin.

Mentor, MBARI Internship program*:* 2000-07. Mentored 10 students (2 male, 8 female) with diverse backgrounds, from across the country

Major Research Cruises (one week or longer)

Research activities include blue-water diving, manned-submersibles and ROVs, AUVs, and were conducted in the Pacific, Atlantic, and Mediterranean from 1989 to 2008.

- Non-MBARI cruises: 31, with 7 as chief scientist
- MBARI cruises: 24, with 11 as chief scientist

Programming: data collection and analysis tools

Numerous free programs for data-collection, instrumentation interface, and conversion written in C, PERL, G (LabView), for MacOS, Unix, PalmPilot (*e.g*, palmPCR, molecular sequence and tree file converters).

CURRENT POSTDOCS (2) AND STUDENTS (3)

Postdocs

Rebecca Hoover (MBARI) - Associations between jellies and crustaceans
Nathan Shaner (MBARI) – Fluorescent proteins, luciferases, and luciferins

Graduate Students

Meghan Powers (UCSC) - Cloning of fluorescent- and photoproteins
Warren Francis (CMU) – Luciferin biochemistry
Freya Goetz (Evergreen) – Chaetognath luciferase

BUDGET AND BUDGET JUSTIFICATION

*** this budget is larger than the preproposal budget, due solely to the dramatic increases in travel, shipping and insurance costs to Samoa since the preproposal was submitted**

Salaries – Tamara Frank, chief scientist, 0.75 month		**$ 5577**
o fringe benefit rate for faculty = 30%		
Subcontracts		**$ 31286**
o Ocean Research and Conservation Org. – Edith Widder, 0.5 month	$ 7851	
o Duke University - Sönke Johnsen, 0.5 month	$ 8100	
o Nova Southeastern Univ. – Charles Messing, 0.5 month	$ 7646	
o Monterey Bay Aquarium Res. Inst. – Steven Haddock, 0.5 month	$ 7689	
Travel – travel for subcontractors will be paid directly		**$ 16880**
o RT travel from FL, USA to Pago Pago, Samoa		
- Tamara Frank, Edith Widder, Erika Heine, Charles Messing		
- Orlando to Apia@ $2490 x 4	$ 9960	
- Apia to Pago Pago @$130 x 4	$ 520	
o RT travel from NC, USA to Pago Pago, Samoa		
- Sonke Johnsen, Duke University		
- Raleigh to Apia -	$ 2790	
- Apia to Pago Pago -	$ 130	
o RT travel from CA, USA to Pago Pago, Samoa		
- Steve Haddock, MBARI		
- Monterey to Apia	$ 2090	
- Apia to Pago Pago	$ 130	
o 3 nights lodging/food in Apia (flights to Apia and between Apia and Pago Pago are biweekly and variable) @$70/day x 3 x 7 =	$ 1260	
Supplies		**$ 25420**
o preservatives, sample jars	$ 500	
o computer/camera storage media	$ 1500	
o 6 week rental of Nikon D3 camera	$ 1600	
o Nikon 50mm f/1.4D AF Nikkor Lens	$ 350	
o Electrophysiology electrodes @$210/box of 12	$ 1050	
o Lab and expedition supplies for 5 labs	$ 2500	
o Postage for shipment of samples to taxonomic experts	$ 400	
o Shipping of equipment to and from Samoa	$17120	
o Ocean freight RT between Florida and Pago Pago + storage in Pago Pago + insurance for 6 palettes, 2400 lbs ~ $15000		
- 3 shipping trunks as excess overweight baggage between North Carolina and Pago Pago ~ $1120		

- 2 shipping trunks as excess overweight baggage between California and Pago Pago ~ $ 1000

Other	**$312000**
o Ship and submersible time – 10 days @$31200 – rate includes IDC	
Total Direct Cost	**$391163**
Indirect Cost	**$ 33644**
o Indirect cost rate = 42.5% of modified total direct cost excluding ship and subtime	
Total Proposal Cost	**$424807**