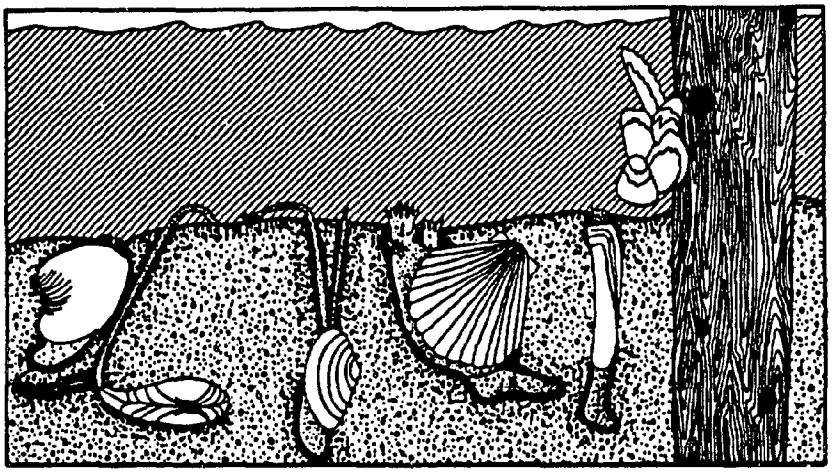
QH
541.5
.C65
T44
year 6
(Dec.
1992)

NOAA STATUS AND TRENDS
Mussel Watch Project
Year 6 Technical Report

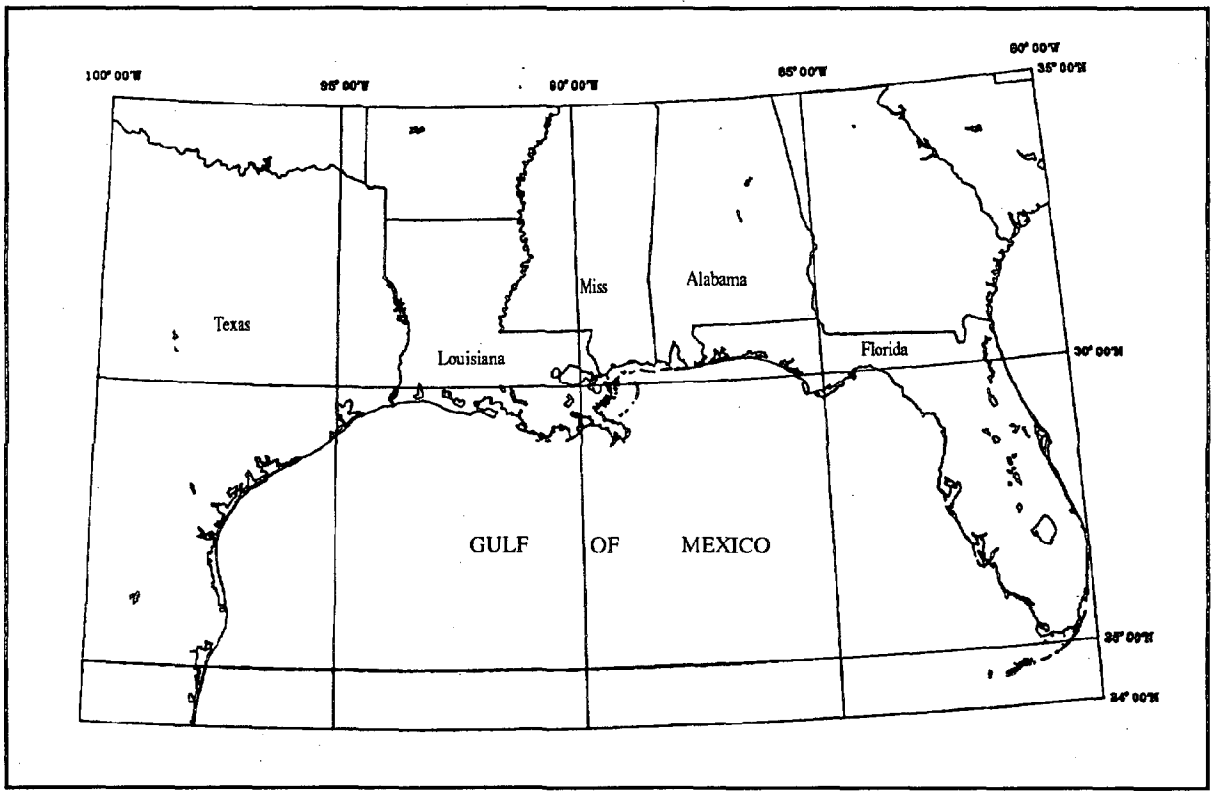
The Geochemical and Environmental Research Group

Texas A&M Research Foundation



Submitted to:

U.S. Department of Commerce
National Oceanic & Atmospheric Adm.
Ocean Assessment Division
6001 Executive Blvd., Rm. 323
Rockville, Maryland 20852



December 1992

NOAA
STATUS AND TRENDS

Mussel Watch Project

Year 6 Technical Report

Property of CSC Library

Prepared by

The Geochemical and Environmental Research Group
Texas A&M University
833 Graham Road
College Station, Texas 77845

Submitted to

U.S. Department of Commerce
National Oceanic & Atmospheric Administration
Ocean Assessment Division
6001 Executive Blvd., Rm 323
Rockville, Maryland 20852

December 1992

QH541.5.C65 T44 year 6 (Dec. 1992)
OCT 2 1992
U.S. DEPARTMENT OF COMMERCE NOAA
COASTAL SERVICES CENTER
2234 SOUTH HOBSON AVENUE
CHARLESTON, SC 29405-2413

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY 1-1

REPRINT 1: *Processes Controlling Temporal Trends in Gulf of Mexico Oyster Health and Contaminant Concentrations* 1-9

REPRINT 2: *International Mussel Watch: the Initial Implementation Phase* 1-17

2.0 INTRODUCTION 2-1

2.1 Project Relevance and Direction 2-1
2.2 Study Objectives 2-4

3.0 POLYNUCLEAR AROMATIC HYDROCARBON RESULTS 3-1

REPRINT 3: *Trace Organic Contamination in Galveston Bay: Results from the NOAA National Status and Trends Mussel Watch Program* 3-33

REPRINT 4: *Toxic Contamination of Aquatic Organisms in Galveston Bay* 3-37

REPRINT 5: *Transplanted Oysters as Sentinel Organisms in Monitoring Studies* 3-41

REPRINT 6: *The Effects of the Apex Barge Oil Spill on the Fish of Galveston Bay* 3-43

PREPRINT 1: *Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990)* 3-47

PREPRINT 2: *Sources of Local Variation in Polynuclear Aromatic Hydrocarbon and Pesticide Body Burden* 3-85

4.0 CHLORINATED HYDROCARBONS 4-1

REPRINT 5: *Chlorinated Hydrocarbons in Gulf of Mexico Oysters: Overview of the First Four Years of the NOAA's National Status and Trends Mussel Watch Program (1986-1989)* 4-21

PREPRINT 3: *National Status and Trends Mussel Watch Program: Chlordane-Related Compounds in Gulf of Mexico Oysters, 1986-1989* 4-39

PREPRINT 4: *Concurrent Chemical and Historical Analyses: Are They Compatible?* 4-61

PREPRINT 5: *Environmental Significance of the Uptake and Depuration of Planar PCB Congeners by the American Oyster (Crassostrea virginica)* 4-61

5.0 TRACE METALS RESULTS **5-1**

5.1 Laboratory Intercalibration Results 5-1
5.2 Trace Metals in Year 6 Oysters 5-1
5.3 Summary and Conclusions from Six Years of Trace Metals in Oysters Data 5-6

REPRINT 8: *Trace Metals in Galveston Bay Oysters* 5-21

6.0 BUTYLTIN RESULTS **6-1**

PREPRINT 6: *Butyltin Concentrations on Oysters from the Gulf of Mexico during 1989-1991* 6-5

7.0 BIOLOGICAL RESULTS **7-1**

7.1 Condition Index/Shell Length 7-1
7.2 Gonadal/Somatic Index 7-1
7.3 Long-Term Changes 7-3

PREPRINT 7: *Spatial and Temporal Distributions of Contaminant Body Burden and Disease in Gulf of Mexico Oyster Populations: The Role of Local and Large-Scale Climatic Controls* 7-21

1.0 Executive Summary

The purpose of the Mussel Watch Project is to determine the long-term temporal and spatial trends of selected environmental contaminant concentrations in bays and estuaries. The key questions in this regard are: (1) What is the current condition of the nation's coastal zone?; and (2) Are these conditions getting better or worse? This report contains the first six years of data from a multi-year project. The first question has been addressed in detail as evidenced by the scientific papers and reports (Table 1.1) that have resulted from the Geochemical and Environmental Research Group's (GERG) interpretations of the Gulf Coast data. Publications not included in GERG's Year 4 or 5 Technical Report are appended to the appropriate sections.

This report is an estimate of the current condition of the Gulf of Mexico coastal zone, based on results from Years 1 thru 6 of the NOAA Mussel Watch Project. Following is a brief sampling survey of these years:

Year 1 - 51 sites (153 stations) - sediments and oysters
Year 2 - 49 sites (147 stations) - sediments and oysters
Year 3 - 65 sites - oysters. Sediments at new sites only.
Year 4 - 67 sites - oysters. Sediments at new sites only. (the 67 sites were sampled in 26 days.)
Year 5 - 71 sites - oysters. Sediments at new sites only.
Year 6 - 64 sites - oysters. Sediments at new sites only.

Year 6 sites included the original list of sites sampled in Years 1 and 2, some sites first sampled in Year 3, seven new sites sampled for the first time in Year 4, four new sites sampled in Year 5 and two new sites in year 6. Sediments and oysters were collected from all but one of the new sites for Years 4, 5 and 6 sites. Eleven sites were deleted from those sampled in previous years. Three of the Texas sites, which had no oysters in Year 3 due to the freshwater-induced die-off, again had no oysters for collection in Year 4. These sites were in San Antonio and Espiritu Santo Bays (SAPP, SAMP, ESSP). Extensive effort was made to sample the sites but only a few spat, too small and too few for collection, were found.

Although a new site for Year 4 was designated in the lower Laguna Madre at Arroyo Colorado, no oysters were found. Further surveys around Port Mansfield also yielded no oysters. Thus, no samples were collected at the new site designated in the lower Laguna Madre.

Oysters from three stations were collected at all sites where there were oysters except at the Pass A Loutre site on the Mississippi River (MRPL). Two and a half hours of dredging did not provide sufficient samples for three replicate sites of twenty individuals per site. This low productivity, adverse and worsening weather, and one total engine failure combined to result in a short site (not enough replicates for all analyses). In Year 5, twelve sites were eliminated from and five sites were added to the sampling project. In year 6, two new sites were sampled. Details of the sample collection and location of field sampling sites are contained in a separate report titled "Field Sampling and Logistics".

The oyster and sediment samples were analyzed for contaminant concentrations [trace metals, polynuclear aromatic hydrocarbons (PAH), pesticides and PCBs], disease incidence and other parameters that aid in the interpretation of contaminant distributions (grain size, oyster size, lipid content, etc.). The analytical procedures used and the QA/QC Project Plan are detailed in a separate report titled "Analytical Methods". The data that was produced from the sample analyses for Year 6 is found in a separate report titled "Analytical Data".

A complete and comprehensive interpretation of the data from the Status and Trends Project for oyster data coupled with the sediment data is an ongoing process. We have begun and are continuing that process as evidenced by this report and the scientific manuscripts that we have published or submitted for publication (Table 1.1). As part of the data interpretation and dissemination, over forty presentations of the NOAA NS&T Gulf Coast Mussel Watch Project were given at national as well as international meetings. With six years of data, the question of temporal trends of contaminant concentrations can be addressed. Detailed examinations of this question are presented in individual sections of this report. A general conclusion that is found for most contaminants measured is that the concentrations have remained relatively constant over the six-year sampling period (Reprint 1). This general trend, however, is not observed at all sites. Some sites show significant changes (both increases and decreases) between years (Reprint 1). Continued sampling will be required to determine the frequency and rates of these changes.

Exceptions to this general trend are found for DDTs and TBT. When historical data for DDT in bivalves is compared to current NS&T data, a decrease in concentration is apparent. Also based on TBT data collected as part of the NOAA NS&T Mussel Watch Project, a decline in TBT concentration in oysters is apparent. Both declines may be in response to regulatory actions.

During Year 3 of this project, sixteen additional sites were sampled. These sites were chosen to be closer to urban areas, and therefore to the sources of contaminant inputs. These sixteen new sites were not, however, located near any known point sources of contaminant input. These sites were added to better represent the current status of contaminant concentrations in the Gulf of Mexico. Generally the mean contaminant concentration at these new sites was higher than the other 48 or 49 Gulf of Mexico sites.

While sampling sites for this project were specifically chosen to avoid known point sources of contaminant input, the detection of coprostanol in sediment from all sites indicates that the products of man's activities have reached all of the sites sampled. However, when compared to known point sources of contamination, all of the contaminant concentrations reported are, in most cases, many orders of magnitude lower than obviously contaminated areas. The lower concentrations in Gulf of Mexico samples most likely reflect the fact that the sites are far removed from point sources of inputs, a condition which is harder to achieve in East and West Coast estuaries. In fact, new sites added in Years 3, 4, 5 and 6 to be closer to urban areas generally had higher contaminant concentrations. An important conclusion derived from the extensive NS&T data set is that contamination levels in Gulf Coast Near Shore areas remain the same or are getting better, and most areas removed from point sources are not severely contaminated.

In addition to analyzing and synthesizing data from the Status and Trends portion of the "International Mussel Watch," GERG was also involved in its initiation. The objectives of that program, funded by NOAA through UNEP/IOC, have been summarized in Reprint 2.

This document represents one of four report products as part of Year 6 of the NS&T Gulf of Mexico projects. The other three reports are entitled:

- Analytical Methods
- Analytical Data
- Field Sampling and Logistics

Table 1.1 **GERG/NOAA NS&T PUBLICATIONS**

	Included in Year Report
Wade, T.L., B. Garcia-Romero and J.M. Brooks (1988) **Tributyltin Contamination of Bivalves from U.S. Coastal Estuaries.** *Environmental Science and Technology,* 22:1488-1493.	IV
Wade, T.L., E.L. Atlas, J.M. Brooks, M.C. Kennicutt II, R.G. Fox, J. Sericano, B. Garcia-Romero and D. DeFreitas (1988) **NOAA Gulf of Mexico Status and Trends Program: Trace Organic Contaminant Distribution in Sediments and Oysters.** *Estuaries* 11:171-179.	IV
Wade, T.L., B. Garcia-Romero and J.M. Brooks (1988) **Tributyltin analyses in association with NOAA's National Status and Trends Mussel Watch Program.** *OCEANS '88 Conference Proceedings,* Baltimore, MD, 31 Oct. - 2 Nov. 1988, pp 1198-1201.	IV
Wade, T.L., M.C. Kennicutt, II and J.M. Brooks (1989) **Gulf of Mexico Hydrocarbon Seep Communities: III: Aromatic Hydrocarbon Burdens of Organisms from Oil Seep Ecosystems.** *Marine Environmental Research* , 27:19-30.	IV
Wade, T.L. and J.L. Sericano (1989) **Trends in Organic Contaminant Distributions in Oysters from the Gulf of Mexico.** *Proceedings, Oceans '89 Conference,* Seattle, WA, 585-589.	IV
Wade, T.L. and B. Garcia-Romero (1989) **Status and Trends of Tributyltin Contamination of Oysters and Sediments from the Gulf of Mexico.** *Proceedings, Oceans '89 Conference,* Seattle, WA, 550-553.	IV
Wade, T.L. and C.S. Giam (1989) **Organic contaminants in the Gulf of Mexico.** In: *Proceedings, 22nd Water for Texas Conference,* Oct. 19-21, 1988, South Shore Harbour Resort and Conference Center, League City, TX (R. Jensen and C. Dunagan, Eds.), pp. 25-30.	V
Craig, A., E.N. Powell, R.R. Fay and J.M. Brooks (1989) **Distribution of *Perkinsus marinus* in Gulf coast oyster populations.** *Estuaries,* 12:82-91.	IV
Presley, B.J., R.J. Taylor and P.N. Boothe (1990) **Trace Metals in Gulf of Mexico Oysters.** *The Science of the Total Environment,* 97/98, pp. 551-553.	IV

Sericano, J.L., E.L. Atlas, T.L. Wade and J.M. Brooks.(1990) **NOAA's Status and Trends Mussel Watch Program: Chlorinated Pesticides and PCB's in Oysters (Crassostrea virginica) and Sediments from the Gulf of Mexico, 1986-1987** *Marine Environmental Research* 29:161-203. IV

Wade, T.L., B. Garcia-Romero, and J.M. Brooks (1990) **Butyltins in Sediments and Bivalves from U.S. Coastal Areas.** *Chemosphere* 20:647-662. IV

Brooks, J.M., M.C. Kennicutt II, T.L. Wade, A.D. Hart, G.J. Denoux and T.J. McDonald (1990) **Hydrocarbon Distributions Around a Shallow Water Multiwell Platform.** *Environmental Science and Technology*, 24:1079-1085. IV

Sericano, J.L., T.L. Wade, E.L. Atlas and J.M. Brooks (1990). **Historical Perspective on the Environmental Bioavailability of DDT and Its Derivatives to Gulf of Mexico Oysters.** *Environmental Science and Technology*, 24:1541-1548. IV

Wade, T.L., J.L. Sericano, B. Garcia-Romero, J.M. Brooks, and B.J. Presley (1990) **Gulf Coast NOAA National Status & Trends Mussel Watch: The First Four Years,** *MTS'90 Conference Proceedings*, Washington, D.C., 26-28 September 1990, pp. 274-280. IV, V

Brooks, J.M., T.L. Wade, B.J. Presley, J.L. Sericano, T.J. McDonald, T.J. Jackson, D.L. Wilkinson and T.F. Davis (1991) **Toxic Contamination of Aquatic Organisms in Galveston Bay,** *Proceedings Galveston Bay Characterization Workshop*, February 21-23, 1991, pp. 65-67. VI

Wade, T.L. J.M. Brooks, J.L. Sericano, T.J. McDonald, B. Garcia-Romero, R.R. Fay, and D.L. Wilkinson (1991) **Trace Organic Contamination in Galveston Bay: Results from the NOAA National Status and Trends Mussel Watch Program,** *Proceedings Galveston Bay Characterization Workshop*, February 21-23, 1991, pp. 68-70. VI

Presley, B.J., R.J. Taylor and P.N. Boothe (1991) **Trace Metals in Galveston Bay Oysters,** *Proceedings Galveston Bay Characterization Workshop*, February 21-23, 1991, pp. 71-73. VI

Sericano, J.L., T.L. Wade and J.M. Brooks (1991) **Transplanted Oysters as Sentinel Organisms in Monitoring Studies**, *Proceedings Galveston Bay Characterization Workshop*, February 21-23, 1991, pp. 74-75. VI

McDonald, S.J., J.M. Brooks, D. Wilkinson, T.L. Wade, and T.J. McDonald (1991) **The Effects of the Apex Barge Oil Spill on the Fish of Galveston Bay**, *Proceedings Galveston Bay Characterization Workshop*, February 21-23, 1991, pp. 85-86. VI

Wade, T.L., J.M. Brooks, M.C. Kennicutt II, T.J. McDonald, G.J. Denoux and T.J. Jackson (1991) **Oysters as Biomonitors of Oil in the Ocean.** *Proceedings 23rd Annual Offshore Technology Conference* (6529) (Houston, TX, May 6-9, 1991), p. 275-280. V

Brooks, J.M., M.A. Champ, T.L. Wade, and S.J. McDonald (1991) **GEARS: Response Strategy for Oil and Hazardous Spills.** *Sea Technology*, April 1991, pp. 25-32. V

Sericano, J.L., T. L. Wade and J.M. Brooks (1991) **Chlorinated hydrocarbons in Gulf of Mexico oysters: Overview of the first four years of the NOAA's National Status and Trends Mussel Watch Program (1986-1989)** In: *Water Pollution: Modelling, Measuring and Prediction.* Wrobel, L.C. and Brebbia, C.A. (Eds.) Computational Mechanics Publications, Southampton, and Elsevier Applied Science, London. pp. 665-681. V,VI

Wade, T.L., B. Garcia-Romero, and J.M. Brooks (1991) **Bioavailability of butyltins.** In: *Organic Geochemistry - Advances and applications in the natural environment.* Manning, D.A.C. (Ed.). Manchester University Press, Manchester. pp. 571-573. V

Wilson, E.A., E.N. Powell, M.A. Craig, T.L. Wade and J.M. Brooks (1991) **The distribution of *Perkinsus marinus* in Gulf coast oysters: Its relationship with temperature, reproduction and pollutant body burden.** *Int. Reuve der Gesantan Hydrobioligie,* 75:533-550. IV

Sericano, J.L., A.M. El-Husseini, and T.L. Wade (1991) **Isolation of planar polychlorinated biphenyls by carbon column chromatography.** *Chemosphere*, 23(7):915-924. V, VI

Wade, T.L., B. Garcia-Romero, and J.M. Brooks (1991) **Oysters as Biomonitors of Butyltins in the Gulf of Mexico.** *Marine Environmental Research*, 32:233-241. IV

Powell, W.N., J.D. Gauthier, E.A. Wilson, A. Nelson, R.R. Fay, and J.M. Brooks (1992) **Oyster disease and climate change. Are yearly changes in *Perkinsus marinus* parasitism in oysters (*Crassostrea virginica*) controlled by climatic cycles in the Gulf of Mexico?** PSZNI: *Mar. Ecol.* (In Press). IV

Sericano, J.L., T.L. Wade, and J.M. Brooks (1993) **The Usefulness of Transplanted Oysters in Biomonitoring Studies.** *Proceedings of The Coastal Society Twelfth International Conference*, Oct. 21-24, 1990, San Antonio, TX (In Press). V

Wade, T.L., J.L. Sericano, J.M. Brooks, and B.J. Presley (1993) **Overview of the First Four Years of the NOAA National Status and Trends Mussel Watch Program.** *Proceedings of The Coastal Society Twelfth International Conference*, Oct. 21-24, 1990 (San Antonio, TX) (In Press). V

Sericano, J.L., T.L. Wade, B. Garcia-Romero, and J.M. Brooks, **Environmental Accumulation and Depuration of Tributyltin by the American Oyster, *Crassostrea virginica*.** *Marine Biology* (Submitted). IV

Sericano, J.L., T.L. Wade, E.N. Powell and J.M. Brooks, **Concurrent Chemical and Histological Analyses: Are They Compatible?** *Chemistry and Ecology* (in press). V,VI

Wilson, E.A., E.N. Powell, T.L. Wade, R.J. Taylor, B.J. Presley, and J.M. Brooks, **Spatial and Temporal Distributions of Contaminant Body Burden and Disease in Gulf of Mexico Oyster Populations: The Role of Local and Large-Scale Climatic Controls.** *Helgol. Meeresunters* (in press). V

Sericano, J.L., T.L. Wade, J.M. Brooks, E.L. Atlas, R.R. Fay and D.L. Wilkinson. **National Status and Trends Mussel Watch Program: Chlordane-related compounds in Gulf of Mexico oysters: 1986-1990.** Environmental Pollution (in press). V,VI

Hofmann, E.E., J.M. Klinck, E.N. Powell, S. Boyles, M. Ellis. **Modeling oyster populations II. Adult size and reproductive effort.** (in preparation). V

Hofmann, E.E., E.N. Powell, J.M. Klinck, E.A. Wilson. **Modeling oyster populations III. Critical feeding periods, growth and reproduction.** (in preparation). V

Jackson, T.J., T.L. Wade, T.J. McDonald, D.L. Wilkinson and J.M. Brooks. **Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990)** Environmental Pollution (in press). VI

Sericano, J.L., T.L. Wade, A.M. El-Husseini and J.M. Brooks. **Environmental significance of the uptake and depuration of planar PCB congeners by the American oyster (*Crassostrea virginica*).** *Marine Pollution Bulletin* (in press). VI

Wade, T.L., E.N. Powell, T.J. Jackson and J.M. Brooks (1992) **Processes Controlling Temporal Trends in Gulf of Mexico Oyster Health and Contaminant Concentrations.** *Proceedings MTS '92*, Marine Technology Society, Oct. 19-21, 1992, Washington, D.C. 223-229. VI

Ellis, M.S., K.-S. Choi, T.L. Wade, E.N. Powell, T.J. Jackson and D.H. Lewis. **Sources of local variation in polynuclear aromatic hydrocarbon and pesticide body burden in oysters *(Crassostrea virginica)* from Galveston Bay, Texas.** *Estuaries* (Submitted). VI

Tripp, B.W., J.W. Farrington, E.D. Goldberg, and J.L. Sericano (1992) **International Mussel Watch: the initial implementation phase.** *Marine Pollution Bulletin*, 24:371-373. VI

Garcia-Romero, B., T.L. Wade, G.G. Salata, and J.M. Brooks. **Butyltin concentrations in oysters from the Gulf of Mexico during 1989-1991.** *Environmental Pollution* (in press). VI

Reprint 1

Processes Controlling Temporal Trends in Gulf of Mexico Oyster Health and Contaminant Concentrations

Terry L. Wade, Eric N. Powell, Thomas J. Jackson, and James M. Brooks

PROCESSES CONTROLLING TEMPORAL TRENDS IN GULF OF MEXICO OYSTER HEALTH AND CONTAMINANT CONCENTRATIONS

Terry L. Wade, Eric N. Powell, Thomas J. Jackson and James M. Brooks

Geochemical and Environmental Research Group
Texas A&M University
833 Graham Road
College Station, TX 77845 USA

ABSTRACT

The concentrations of PAH, DDT, PCB, dieldrin and chlordane in Gulf of Mexico oysters as cumulative % for five consecutive years are discussed. Gulf-wide changes in contaminant concentrations are observed. PAH and DDT co-vary while PCB has a different distribution. The body burden of PAH and pesticides in oysters is correlated with latitude. Available evidence suggest a linkage between oyster health (infection intensity), reproductive effort and contaminant body burden. Analyses of oyster gonadal material confirms the reproductive process can purge contaminants from oysters, which confounds interpretation of contaminant body burden data.

INTRODUCTION

The National Status and Trends (NS&T) Mussel Watch Program was instituted in 1986/87 by the National Oceanic and Atmospheric Administration (NOAA). The purpose of this program is to determine the current status and long-term trends of selected contaminants in U.S. coastal waters using bivalves as sentinel organisms. This approach has been used successfully in the past as recently summarized in a worldwide mussel watch literature survey (1). The NS&T program has already provided a good description of the current status of selected contaminants in bivalves and sediments (2,3,4,5,6) from U. S. coastal areas.

The continued collection of data will allow for the resolution of possible temporal trends in the contaminant concentrations. The objective of this paper is to examine polynuclear aromatic hydrocarbon (PAH), selected pesticides, and polychlorinated biphenyl (PCB) data for the first five years of the NS&T Gulf of Mexico program for temporal trends and to summarize correlations between oyster health and contaminant concentrations. These trends will continue to be reassessed as additional years of data become available.

METHODS

The collection and analytical techniques used have been described elsewhere (3,6) and will only be briefly described here. Homogenized oyster tissue is extracted with CH_2Cl_2 in the presence of NA_2SO_4 (to remove water). The pesticide/PCB/PAH are isolated from other organic materials using silica gel/alumina column chromatography and high performance

liquid chromatography with phenogel columns. The purified samples are then analyzed by gas chromatography with a mass selective detection for PAH and an electron capture detector for pesticides/ PCBs. The accuracy and precision of these methods have been established by several intercalibration exercises overseen by the U.S. National Institute of Standards and Technology. These intercalibrations document the comparability of the data between sampling years and between participating analytical laboratories.

RESULTS AND DISCUSSION

The geographical distributions of PAH/PCB/pesticides for the most southern Texas site and continuing to the most southern Florida site have been reported (3,4,5,6). In general, no consistent temporal trends in concentration have been observed for most contaminants measured as part of the NS&T program (4), with the exception of tributyltin which has decreased from 1989 to the present (7).

Bar graphs (5) or crossplots (4) of data comparing one year's data versus another have been used to display the general trend for PAH/PCB and pesticide data (4,5,8). The variations in concentration for a particular site are easily visualized using these data presentations. However, a cumulative frequency function can be used to examine the heterogeneous distribution of contaminants in Gulf of Mexico oysters (9). This approach has the advantage of examining the Gulf of Mexico as a single environmental system, determining the percentage of sites exposed to a particular threshold concentration, and providing information for environmental evaluation.

The distributions of the PAH/PCB and pesticide concentrations are described by a lognormal distribution, i.e. the distribution of data is skewed to low concentrations and has a fraction which extends to high concentrations. The lognormal distribution, typical of environmental data, has been used (2) to define "high" concentrations as those with logarithmic values more than the mean plus one standard deviation of the logarithms for all concentrations.

Distribution functions are useful measures of environmental quality data in that changes with time can be determined without being influenced by "outliers". For the cumulative distribution plot, the data are sorted from lowest value to highest value, similar to rank transformation (10). Each observation is 1/n fraction of the data set, where n is the number of samples in the data set. The sum of the fraction of samples less than the concentration is plotted against the concentration. From this plot the median can be determined, since it is defined as the 50th percentile. The interquatrile range (IQR) is used as a measure of variability. The IQR is the 75th percentile minus the 25th percentile and equals 1.35 times the standard deviation for a normal distribution (11).

The cumulative % distribution for DDTs is shown in Figure 1. Similar distribution plots were made for all the contaminants measured, but their distributions will only be summarized here. All of the DDT plots are smooth "S" shaped curves, indicating the log data is a normal distribution. There is a slight decrease in DDT in Year 2 at lower concentrations compared to Year 1, but almost identical distributions at higher concentrations. The

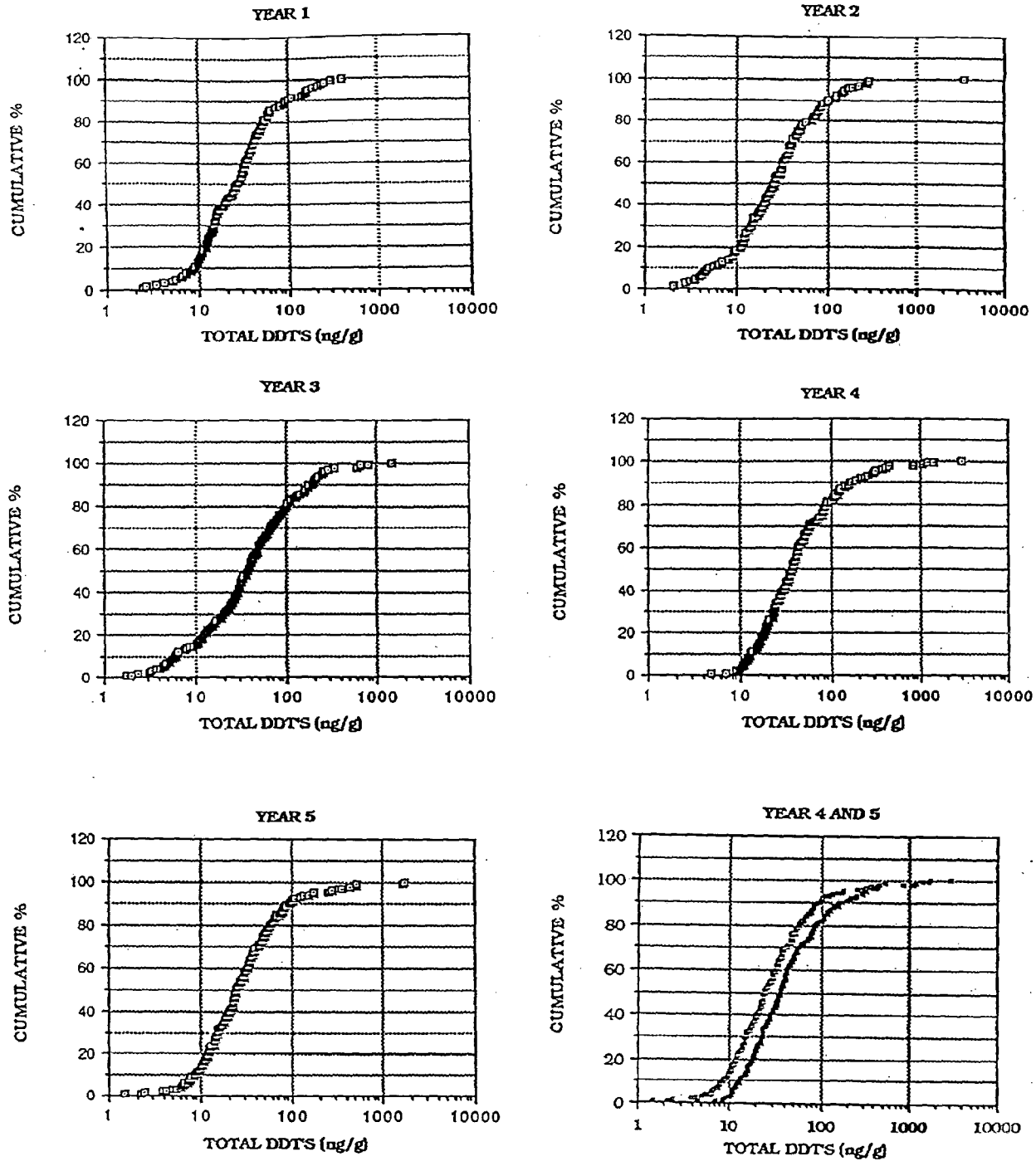


Figure 1. Cumulative percent of total DDT (ng/g).

distribution for Years 3 and 4 indicates increased concentrations throughout the entire Gulf of Mexico. In Year 5 a return to a similar distribution to that seen in Years 1 and 2 was observed. It is easier to see changes between years when the plots can be superimposed. The last graph in Figure 1 shows DDTs for Years 4 and 5 on the same plot. It is easy to see that both are normal distributions, but Year 4 (farthest from the right) had higher concentrations throughout the entire Gulf.

Because of space limitations, not all of the distributions are presented here. However, the geometric median of the distributions for PAH, PCB, DDT, chlordane and dieldrin are plotted vs. sampling year in Figure 2. This figure summarizes the distributions as seen from comparison of the DDT distribution in Figure 1 to the DDT plot in Figure 2. In Figure 2 the geometric mean concentration for DDT decreases slightly between Years 1 and 2, increases in Years 3 and 4 and is back to concentrations similar to Years 1 and 2 in Year 5. This was expected based on Figure 1. The distribution for the total of the 18 PAHs measured as part of the NS&T program shows the same distribution as the DDT (Figure 2). The PAH distribution has been described in detail (12). The other contaminant classes displayed different distributions. Total PCBs distribution for the Gulf of Mexico had a slight decrease between Year 1 and Year 2, then a steady increase from Year 2 to Year 5. It therefore appears that the PCBs have different source functions than the DDTs and PAHs. The dieldrin and chlordane concentrations change little between the years, except for the possibility of a slight decrease over the five years of sampling.

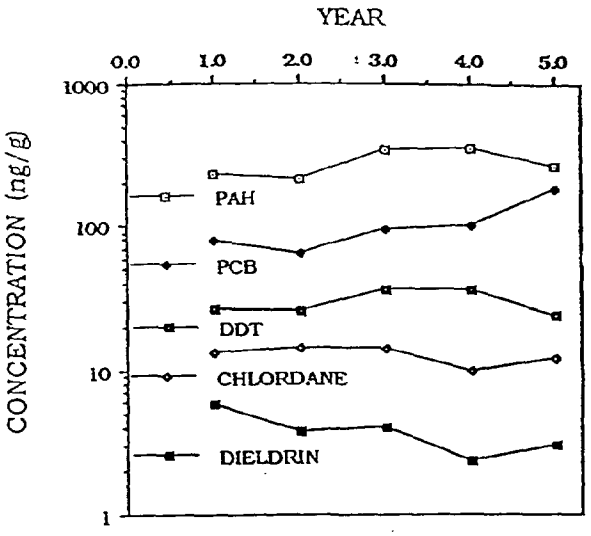


Figure 2. Median vs. year.

The NS&T program was designed to address the question of temporal trends in the contaminant loadings of U. S. coastal waters. Examination of the first five years of data indicates that for the entire Gulf of Mexico contaminant concentrations appear to be remaining relatively constant. However, there are yearly fluctuations above and below this "normal concentration". These concordant Gulf-wide fluctuations suggest that climatic factors exert a strong influence on contaminant body burdens and on biological attributes of oysters (13).

The NS&T program was not designed to answer the question of what effect the contaminant loading has on the health of oysters. The program does, however, measure several indicators of oyster health, including condition index, disease incidence and reproductive stage. Since the NS&T

study was not designed to answer these questions, they can not be answered rigorously with the data available, but can be partially answered.

Biological and environmental factors may effect the rate and extent of bioaccumulation. These biological factors include differential growth rate (14,15), reproductive stage (14,16,17), stress and disease (18,19,20). These biological factors make spatial and temporal comparisons designed to evaluate the status and trends of contaminant loading more difficult.

Analysis of the first four years of NS&T data has shown that the body burden of polynuclear aromatic hydrocarbons (PAHs) and pesticides in oysters is correlated with latitude in the Gulf of Mexico. Wilson et al. (21) suggested that the latitudinal temperature gradient in the Gulf produced variation in reproductive effort and that this variation in reproductive effort effected PAH body burden sufficiently to override the effect of local variation in contaminant loading in many cases. Wilson et al. (13), in a more thorough analysis, showed that PAH body burden responds to climate change and that biological factors, climate's effect on temperature, and freshwater inflow may effect the final body burden of PAHs.

Likely biological factors are reproduction and disease (*Perkinsus marinus*) infection intensity. Reproduction has frequently been suggested as an important route of depuration (22) because lipid loss peaks at this time. Parasites and pathogens are less frequently implicated (23), but parasites and pathogens should have an effect; if for no other reason, they frequently reduce reproductive effort (24). In oysters, both reproductive rate and disease are significantly effected by temperature and salinity (25) and thus could serve as important intermediaries between climate change and contaminant body burden.

Recently, newly developed techniques have enabled us to determine the concentration of organic contaminants in oyster gonadal tissues (26). These analyses revealed that eggs and sperm are enriched in PAH and PCB compared to somatic tissue. Eggs, but not sperm, were enriched in DDTs and chlordane. Dieldrin was not detected in these oyster samples. This evidence indicates that the frequency of spawning and timing of oyster collection during their spawning cycle may effect body burden of contaminants. These processes may explain the latitudinal gradient in PAH and pesticide body burdens observed for the Gulf of Mexico (21) and the relationship of PAH body burden and climate change.

These complexities as oysters makes interpretation of NS&T data a challenge. The more we can learn about the NS&T sentinel organisms, *C.virginica*, the more likely we will be able to meet that challenge.

ACKNOWLEDGMENTS

This research was supported by the National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce, Grants 50-DGNC-5-00262 and 50-DGNC-0-00047 from the Office of Ocean Resources Conservation and Assessment.

REFERENCES

1. Cantillo, A. Y. Mussel Watch worldwide literature survey. NOAA Technical Memorandum NOS ORCA 63, 1991, 136 p.

2. O'Connor, T. P. Coastal Environmental Quality in the United States, 1990. Chemical Contamination in Sediment and Tissues. A Special NOAA 20th Anniversary Report, 1990, 34 p.

3. Wade, T. L., Atlas, E. L., Brooks, J. M., Kennicutt II, M. C., Fox, R. G., Sericano, J., Garcia-Romero, B. and Defreitas, D. A. NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediments and oysters. *Estuaries, 11*, 1988, pp. 171-179.

4. Wade, T.L. and Sericano, J. Trends in Organic Contaminant Distributions in Oysters from the Gulf of Mexico. *Oceans '89 Proceedings*, 1989, pp. 585-589.

5. Wade, T. L., Sericano, J. L., Garcia-Romero, B., Brooks, J. M. and Presley, B. J. Gulf Coast NOAA National Status & Trends Mussel Watch: The First Four Years. *Proc. Mar. Tech. Soc.*, 1990, pp. 274-280.

6. Sericano, J. L., Wade, T. L., Atlas, E. L. and Brooks, J. M. Historical Perspective on the Environmental Bioavailability of DDT and Its Derivatives to Gulf of Mexico Oysters. *Environ. Sci. Technol.*, 77, 1990, pp. 1541-1548.

7. Wade, T. L., Garcia-Romero, B., and Brooks, J. M. Oysters as Biomonitors of Butyltins in the Gulf of Mexico. *Marine Environ. Res.*, 32, 1991, pp. 233-241.

8. Wade, T. L., Brooks, J. M., Kennicutt II, M. C., Denoux, G.J. and Jackson, T. J. Oysters as Biomonitors of Oil in the Ocean. *Proceedings of the 23rd Annual Offshore Technology Conference*, OTC 6529, 1991, pp. 275-280.

9. Mackay, D. and Paterson, S. Spatial concentration distributions. *Environ. Sci. Technol.*, *18*, 1984, pp. 207A-214A.

10. Conover, W. J. and Iman, R. L. Rank Transformations as a Bridge between Parametric and Nonparametric Statistics. *Amer. Statistician*, 35, 1981,pp.124-129.

11. Hensel, D. R. Less than obvious. Statistical treatment of data below the detection limit. *Environ. Sci. Technol.*,24, 1990, pp. 1766-1774.

12. Jackson, T.J., Wade. T. L., McDonald, T.J., Wilkinson, D. L. and Brooks, J. M. Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990). *Environmental Pollution* (in press).

13. Wilson, E.A., Powell, E.N., Wade, T. L., Taylor, R. J., Presley, B.J. and Brooks, J.M. Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: The role of local and large-scale climatic controls. *Helgol. Meeresunters.* (submitted).

14. Cunningham, P.A. and Tripp, M.R. Factors affecting the accumulation and removal of mercury from tissues of the

American oyster *Crassostrea virginica*. *Mar. Biol. (Berl.), 31*, 1975, pp. 311-319.

15. Boyden, C.R. Effect of size upon metal content of shellfish. *J. Mar. Biol. Assoc. U.K., 57*, 1977, pp. 675-714.

16. Frazier, J.M. The dynamics of metals in the American oyster, *Crassostrea virginica*. I. Seasonal effects. *Chesapeake Sci., 16*, 1975, pp. 162-171.

17. Martinicic, D., Nürnberg, H.W., Stoeppler, M., and Branica, M. Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Sea). *Mar. Biol. (Berl.), 81*, 1984, pp. 177-188.

18. Shuster, Jr., C.N. and Pringle, B.H. Trace metal accumulation by the American eastern oyster, *Crassostrea virginica*. *Proc. Natl. Shellfish. Assoc., 59*, 1969, pp. 91-103.

19. Sindermann, C.J. An examination of some relationships between pollution and disease. *Rapp. P-V. Réun. Cons. Int. Explor. Mer., 182*, 1983, pp. 37-43.

20. Moore, M.N., Livingstone, D.R. and Widdows, J. Hydrocarbons in marine mollusks: Biological effects and ecological consequences. In: U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, FL, 1989, pp. 291-329.

21. Wilson, E.A., Powell, E.N., Craig, M.A., Wade, T.L. and Brooks, J.M. The distribution of *Perkinsus marinus* in Gulf Coast oysters: Its relationship with temperature, reproduction, and pollutant body burden. *Int. Rev. Gesamten Hydrobiol., 75*, 1990, pp. 533-550.

22. Cossa, D. A review of the use of *Mytilus* spp. as quantitative indicators of cadmium and mercury contamination in coastal waters. *Oceanol. Acta, 12*, 1989, pp. 417-432.

23. Khan, R.A. Effects of chronic exposure to petroleum hydrocarbons on two species of marine fish infected with a hemoprotozoan, *Trypanosoma murmanensis*. *Can. J. Zool., 65*, 1987, pp. 2703-2709.

24. Barber, B.J., Ford, S.E. and Haskin, H.H. Effects of the parasite MSX (*Haplosporidium nelsoni*) on oyster (*Crassostrea virginica*) energy metabolism. I. Condition index and relative fecundity. *J. Shellfish Res., 7*, 1988, pp. 25-31.

25. Soniat, T.M. and Gauthier, J.D. The prevalence and intensity of *Perkinsus marinus* from the mid northern Gulf of Mexico, with comments on the relationship of the oyster parasite to temperature and salinity. *Tulane Stud. Zool. Bot., 27*, 1989, pp. 21-27.

26. Ellis, M.S., Choi, K.S., Wade, T.L., Powell, E. N., Jackson, T.J. and Lewis, D.H. Sources of local variation in polynuclear aromatic hydrocarbon and pesticide body burden in oysters (*Crassostrea virginica*) from Galveston Bay, Texas. *Estuaries* (submitted).

Reprint 2

International Mussel Watch: the Initial Implementation Phase

Bruce W. Tripp, John W. Farrington, Edward D. Goldberg, and José Sericano

International Mussel Watch: the initial implementation phase

As a consequence of increasing population and intensifying industrial development on a global scale, the world's coastal waters will continue to receive societal waste. The goals of the International Mussel Watch Project are to assess the extent and severity of contamination of the coastal waters of the world with respect to selected chemicals, and to develop an international infrastructure of cooperating scientists and laboratories for research and monitoring of contaminants in coastal waters worldwide in the future.

The International Oceanographic Commission of UNESCO (IOC), in collaboration with the United Nations Environment Program (UNEP) and the US National Oceanographic and Atmospheric Administration (NOAA) are jointly funding the International Mussel Watch Program and have initiated a monitoring programme in Central and South America in 1991–92. The programme is being directed by the International Mussel Watch Committee (Table 1) and administered by the Project Secretariat office based at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, 02543, USA.

TABLE I

Members of the International Mussel Watch Committee.

Members	Ex Officio
Edward D. Goldberg, Chairman Scripps Institution of Oceanography, USA	Bruce W. Tripp, Executive Officer CRC/Woods Hole Oceanographic Institution, USA
John W. Farrington, Vice Chairman Woods Hole Oceanographic Institution, USA	José Sericano, Field Scientific Officer GERG/Texas A&M University, USA
Roger Dawson Chesapeake Biological Laboratory, USA	Anthony H. Knap, UNESCO-GEMSI Liaison Bermuda Biological Station for Research, Bermuda
Arne B. Jernelov Water & Air Pollution Research Laboratory, Sweden	
Laurence D. Mee International Atomic Energy Agency, Monaco	
Eric Schneider National Oceanic and Atmospheric Administration, USA	

The need for an International Mussel Watch project was recognized in 1975, when Professor Edward Goldberg in his Marine Pollution Bulletin editorial, called for a global marine monitoring programme to serve as a

"springboard for action'. He outlined a fiscally reasonable, global scale monitoring programme based on the sentinel organisms concept. This monitoring programme must be capable of detecting spatial and temporal trends in concentrations of several important chemical contaminants. Since the late 1960s, scientists have been using bivalve filter-feeding molluscs to monitor for selected chemical contaminants in coastal marine waters and an extensive 'mussel watch' literature has developed from that work. Such contamination of coastal waters might result in changes that are deleterious, over the long term, to both the integrity of the coastal environment and to human health. Because of their sedentary habits and their ability to bioconcentrate the pollutants of interest, mussels and other bivalve species appear to be appropriate sentinel organisms even considering complexities such as age, season, organism health and interspecies differences. The mussel watch approach has been adopted as one of several coastal environmental quality monitoring strategies by several national programmes and by UN programmes. The International Mussel Watch Project will build on this cumulative experience. A world-wide literature search has recently been completed by the US NOAA Status and Trends Program and is available as a special report.

Particularly important among the monitoring programmes that were established during the 1970s were those of the International Council for the Exploration of the Sea (ICES). The United Nations Environment Program has also created its Regional Seas Program which has placed a major emphasis on the development of host country capabilities for measuring the levels of contaminants in coastal and marine environments. The IOC sponsored the formation of a Task Team on Marine Pollution Research and Monitoring in the West Pacific region. National governments in many countries have initiated their own coastal monitoring programmes to provide technical information that can be used to protect coastal resources from the deleterious effects of chemical contamination. In the United States, the 'Mussel Watch' Program was begun by the US EPA in the mid 1970s and involved academic scientists from several academic research institutions. This programme used mussels and oysters as indicators of the local levels of four classes of pollutants in US coastal waters, including synthetic organics, fossil fuel compounds and their derivatives, several metals, and the transuranic radioactive elements produced in the nuclear fuel cycle and by fallout from nuclear weapons tests. Mussel Watch became an operational contaminant monitoring programme in the United States in 1986 and is presently directed by US NOAA as a component of the Status and Trends Program.

In a 1978 workshop in Barcelona, the members of the US Mussel Watch Program joined with scientists of other countries to assess the methodologies employed for the detection and measurement of pollutants in coastal zones through the sentinel organism approach. The participants at the Barcelona workshop decided that continuing international collaboration and communication would be worthwhile, and elected a committee charged with the task of planning for a future meeting. Communication at the international level was continued at a second meeting held in Hawaii in November of 1983. Participants at the Hawaii meeting examined the conceptual approaches used by the Mussel Watch programmes and assessed the potential for expansion of this approach to a global scale and especially to the southern hemisphere. The need for the International Mussel Watch Project was reaffirmed at the Hawaii meeting. Planning momentum was maintained by the International Mussel Watch Committee during the next few years.

The International Mussel Watch Project is being implemented initially in the Central-South America and Caribbean region and will focus on organochlorine biocide contaminants and PCBs (Table 2). Plans are being made to expand the programme to other contaminants and to other regions so that all countries that wish to participate, may do so. We invite anyone interested in participating in subsequent phases of International Mussel Watch to contact the Project Secretariat in Woods Hole. Currently available funding does not permit a global-scale programme or a Western Hemisphere program that includes all types of chemical contaminants. This initial implementation phase will focus on organochlorine biocides because of their continued used in agricultural and public health applications in several tropical and sub-tropical areas and because we know very little about production and use or about the resulting coastal contamination. The experienced gained from this initial phase will be useful in implementing an expansion of the programme.

In May, 1991 members of the International Mussel Watch Committee and representatives of three regional monitoring programmes met at the University of Costa Rica to finalize the initial implementation phase of International Mussel Watch. At that meeting, sampling sites and participating national scientists were selected. The Project Secretariat coordinates the work of two central analytical facilities. International Laboratory for Marine Radioactivity (ILMR) in Monaco and Geochemical and Environmental Research Group (GERG) at Texas

TABLE 2

Chlorinated hydrocarbons to be analysed in collected tissue samples.

We envisage that about 70–80 sites will be sampled for indigenous bivalves and tissue samples will be analysed for a variety of chlorinated pesticides and selected chlorinated biphenyls:

Aldrin	Heptachlor
Endrin	Heptachlor epoxide
Dieldrin	Hexachlorobenzene (HCB)
Chlordanes	α-Hexachlorocyclohexane (α-HCH)
	β-Hexachlorocyclohexane (β-HCH)
o,p'-DDD	Lindane (γ-HCH)
p,p'-DDD	Trans-nonachlor
o,p'-DDE	Methoxychlor
p,p'-DDE	
o,p'-DDT	
p,p'-DDT	

NOTES: Mirex and Kelthane may be added to the suite of contaminants analysed if funding for analysis becomes available.

A common set of individual chlorobiphenyls (PCBs) will be chosen for analysis following the assessment of the results of the first round of intercalibration exercises of IOC/ICES/JMG. Total PCBs will be estimated from these data.

A&M University, will analyse the collected samples for organochlorine contaminants. Tissue samples and extracts will be archived for later analysis of other contaminants if funding is available. IOMR will also supervise the Field Scientist responsible for sample collection. The International Mussel Watch Project will complement regional monitoring programmes where they are established, thus linking the existing programmes and increasing their effectiveness. These existing regional programmes provide a base on which to build an international programme and their support and collaboration is critical to the success of the international programme. In the initial implementation phase, samples will be collected throughout the region with the assistance of host-country scientists. These scientists will form the nucleus of an international marine monitoring network through which the results of the project will be disseminated.

Host-country scientists and IMW sampling sites will be coordinated by the Woods Hole-based Project Secretariat, working with the Field Scientific Officer. All sampling and sample logistics will be supervised by the Field Scientific Officer and the host-country scientists will work directly with him. The field sampling is currently underway, and collection have already been completed in much of Central America and South America. The Project Secretariat and the Field Scientific Officer will provide technical support to host-country scientists as resources permit. The International Mussel Watch Committee, in concert with the Project Secretariat, the Field Scientific Officer, and the contract laboratories will provide data interpretation, taking into account comments from host-country scientists. An international meeting, involving participating host-country scientists is being organized for early 1993. For those scientists with analytical expertise, tissue samples will be available for in-country analysis and inter-laboratory comparison. Host-country scientists will be asked to assemble production and use data as well, from available sources in their respective countries.

This initial implementation phase will: *1.* generate high quality data on chlorinated pesticides and estimate PCB concentrations in the Central-South America-Caribbean coastal region, *2.* serve as a 'field-test' of a large-scale international marine monitoring programme for chemical contaminants, *3.* create an international network of coastal environmental scientists, *4.* provide a forum for training and for discussion of analytical results, and *5.* create the institutional structure for a global scale coastal monitoring programme.

Continuation (and expansion) of this project will be considered when the programme is assessed at the conclusion of the initial implementation phase. Host-countries and the entire UN family will benefit from the scientific results generated during this initial phase and will have an opportunity to expand local monitoring activities with technical support from the Project as well as to integrate these activities into regional and global-scale programmes.

BRUCE W. TRIPP
Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.
JOHN W. FARRINGTON
Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.
EDWARD D. GOLDBERG
Scripps Institution of Oceanography, La Jolla, CA 92093, USA.
JOSÉ SERICANO
International Laboratory for Marine Radioactivity, Monaco.

2.0 Introduction

This document is one volume of the Sixth Annual Report prepared by the Geochemical and Environmental Research Group (GERG), in the College of Geosciences and Maritime Studies at Texas A&M University, for the U.S. Department of Commerce National Oceanic and Atmospheric Administration's Mussel Watch Project for the Gulf of Mexico. This section discusses the background and relevance of the proposed project and reviews the study objectives.

The overall goal of the national Mussel Watch Project is to assess and document the status and long-term changes in the environmental quality of coastal and estuarine environments along the East and West coasts of the United States and the Gulf of Mexico coast. In order to meet this goal, a series of systematic observations of selected chemical contaminants (e.g., trace metals, PAHs, PCBs, and pesticides) in representative samples of bivalves and sediments has been undertaken. GERG's portion of the project deals with U.S. Gulf of Mexico coastal sites. This document presents the results obtained during the first six years of the project. Three other documents as part of the sixth year study include:

- Analytical Methods
- Analytical Data
- Field Sampling and Logistics

2.1 Project Relevance and Direction

Over the last several decades, problems associated with chemical contamination of the marine environment have received increasing attention. Numerous studies have been undertaken to identify the inputs, transport, and effects of a variety of elements and compounds. Among the major contaminants studied are petroleum hydrocarbons, halogenated organic compounds, and a suite of trace metals including cadmium (Cd), lead (Pb), zinc (Zn), mercury (Hg), and others. Particular attention has focused on the coastal zone and estuaries near large population centers. These areas potentially experience the largest impact from chemical contamination and may be most sensitive to the accumulation of toxic compounds.

One approach for monitoring the status of coastal and estuarine pollution on a national scale has been the concept of "sentinel organisms" or "bioindicators". The National Mussel Watch Project initially sponsored by the Environmental Protection Agency was an application of this concept. The project used bivalves to monitor the "health" of marine ecosystems and identify "hot spots" of chemical contamination along the nation's coastline. Some of the results of this

project have been summarized (Farrington *et al.*, 1983; NAS, 1980) and are discussed later in this report.

Farrington *et al.* (1983) summarized the rationale for using common mussels (*Mytilus* sp.), various oyster species (*Crassostrea* and *Ostrea*) and other bivalves as "sentinel" organisms:

1. Bivalves are cosmopolitan (widely distributed geographically). This characteristic minimizes the problems inherent in comparing data for markedly different species with different life histories and relationships within their habitat.

2. They are sedentary and are thus better than mobile species as integrators of chemical pollution at a given area.

3. They concentrate many chemicals by factors of 10^2 to 10^5 compared to seawater concentrations in their habitat. Trace constituent measurements are easier to accomplish in tissues than in seawater.

4. Inasmuch as the chemicals are measured in the bivalves, an assessment of biological availability of chemicals is obtained.

5. In comparison to fish and crustacea, bivalves exhibit low or undetectable activity of those enzyme systems that metabolize many xenobiotics such as aromatic hydrocarbons and polychlorinated biphenyls (PCBs). Thus, a more accurate assessment of the magnitude of xenobiotic contamination in the habitat of the bivalves can be made.

6. They have many relatively stable local populations extensive enough to be sampled repeatedly, providing data on short- and long-term temporal changes in concentrations of pollutant chemicals.

7. They survive under conditions of pollution that often severely reduce or eliminate other species.

8. They can be successfully transplanted and maintained on subtidal moorings or on intertidal shore areas where normal populations do not grow due to a lack of suitable substrate.

9. They are a commercially valuable seafood species on a worldwide basis. Therefore, measurement of chemical contamination is of interest for public health considerations.

An international workshop, "Mussel Watch II", convened to reassess the "Mussel Watch" concept and to evaluate the accomplishments and deficiencies of the EPA Mussel Watch Project that was implemented. Some of the conclusions are summarized below:

Accomplishments:

- An extensive data base of radionuclides in mussels and oysters was obtained. These data allowed the detection of several minor leakages from nuclear reactors.

- The Mussel Watch data base of trace metals has permitted an assessment of the perturbations in the biogeochemical cycles of metals in coastal waters induced by their mobilization by man and by waste discharges.

- Measurements of PCB and DDT compounds established a data base against which future changes can be measured.

- Data from the Mussel Watch Project provided conclusive evidence that polynuclear aromatic hydrocarbons produced from combustion products are not generally accumulated in food webs.

Deficiencies:

- Analytical limitations in trace organic analysis prevented a wider spectrum of organic compounds from being measured. Subsequent analyses have revealed other compounds such as hexachlorobenzene, mirex, and others.

- Data management was inadequate, and consequently data was not promptly available.

- Some samples from the Gulf Coast were never analyzed.

- Statistical design was not established prior to the sampling and analytical project.

- Mussels (or oysters) are unsatisfactory for the identification of new pollutant compounds, and they do not readily accumulate potentially important compounds or compound groups.

The consensus opinion was that a modified, more specifically defined approach to using marine organisms as environmental indicators would be a valuable tool in assessing estuarine and coastal contamination.

2.2 Study Objectives

Reliable and continuous information regarding the status and trends of environmental quality in the nation's coastal and estuarine regions is necessary to make informed decisions involving the use and allocation of resources. The National Status and Trends Project for Marine Environmental Quality was initiated in 1984 by the Ocean Assessment Division of NOAA to provide this environmental quality information. Based on the experience gained during the EPA Mussel Watch Project and on recommendations from a workshop report, the chemical measurements segment of the National Status and Trends Project was developed. During the workshop on chemical measurements, the working hypothesis of the project was worded as follows:

> "Chemical measurements of toxic contaminant levels in environmental samples serve as leading indicators of trends in environmental quality and can reflect trends in inputs of these chemicals into marine systems. Significant correlations have been demonstrated between contaminant levels in marine samples and the health of marine biological components."

Implicit in such a statement is that the chemical measurements are of the highest quality obtainable, are directly comparable between all sites and samples, and have a known statistical variability. Simply stated, the objective of this project is to provide such measurements in sediments and bivalves and to provide sufficient ancillary data to allow meaningful interpretation of the measurements.

There are four stated objectives for the National Status and Trends Project:

1. The primary objective is to establish a national data base using state-of-the-art sampling, preservation, and analysis methodologies which are consistently applied and subject to rigorous quality control and assurance.

2. Use the information in the data base to estimate environmental quality, to establish a statistical basis for detecting spatial and temporal change, and to identify areas of the nation that might benefit from more intensive study.

3. Seek and validate additional measurement techniques, especially those that describe a biological response to the presence of contaminants.

4. Create a cryogenic, archival specimen bank containing environmental samples collected and preserved through techniques that will permit reliable analysis over a period of decades.

In a general sense, scientific objectives relating to the goals of our Mussel Watch portion of the National Status and Trends Project include:

- What is the geographic distribution of contaminant concentration in oysters and sediments at selected sites along the Gulf of Mexico coast?
- Are there "problem" areas?
- Are particular compounds or classes of compounds significant contaminants in broad regions of the Gulf?
- What is the relationship between contaminant concentration in sediments and in oysters?
- What is the relationship between the concentration of specific metals and organic compounds?
- What is the variability in contaminant concentrations within sites and between sites?
- What portion of that variability can be removed by normalizing chemical measurements to other parameters (e.g. to TOC, lipid weight, etc.)?
- Are there unidentified contaminants present in significant quantities in the samples?
- What is the relationship between the "health" of oysters and the concentration of contaminants?
- Are contaminant concentrations increasing or decreasing with time?

The long-term project is designed to examine the above problems in a rigorous manner. Some of these objectives are being pursued as is evidenced by publications that have resulted from this program (Table 1.1). It can be expected, too, that other problems and questions will arise during the course of the project.

Researchers at GERG are pursuing the answers to some of the above questions through our association with this NOAA NS&T Project as well as other programs (i.e. EPA Galveston Bay National Estuary

Program, EPA - EMAP-NC) and through unfunded student thesis and dissertation research. Some of the ongoing research at GERG involves oyster transplant studies in an attempt to better calibrate oysters as detectors of environmental contaminants. GERG has developed techniques to analyze alkylated PAH, PAH metabolites and planer PCBs, and is currently developing methods for dioxins and dibenzofurans. All of these research projects may be of value to the NS&T Project in the future.

3.0 Polynuclear Aromatic Hydrocarbon Results

Polynuclear Aromatic Hydrocarbon (PAH) concentrations are of concern because many of these compounds are known or suspected carcinogens and/or mutagens. The sources of PAH in the environment are petroleum, petroleum products, and combustion of fossil fuels and organic materials (i.e. forest fires). In estuarine systems PAH inputs may come from natural seepage, oil production, oil transportation, atmospheric deposition, combustion products (i.e. creosote), municipal waste, industrial waste and runoff. Although large spills get most of the publicity in the popular press, they account for less than 15% of the total PAH entering the marine environment.

The concentration of 24 Polynuclear Aromatic Hydrocarbons (PAH) are measured as part of the NS&T project in oyster and sediment samples from the Gulf of Mexico. The NS&T data can be used to provide some indication of the relative importance of petroleum verses combustion sources, but analyses of additional alkylated PAH is even more definitive (Preprint 1).

One purpose of the NS&T project is to determine the environmental quality of the nation's coastal zone. This has been fairly well addressed, as described in the recent publications and reports that have resulted from this project (Table1.1 and reprints 3,4,5, and 6). Another purpose of the NS&T project is to determine if the environmental conditions of the U.S. coastal zone are getting better or worse. The continued collection of data is necessary in order to address this last question.

The NS&T sites are chosen to avoid "hot spots" or known point sources of contaminant inputs. The sites are sampled once a year in the winter in an attempt to eliminate seasonal variability. Samples are collected from three stations at each site and analyzed individually. The geographical distribution of Gulf Coast oyster PAHs are shown in Figures 3.1 to 3.29. The total of the PAH measured in all years (Figure 3.1) indicates concentration ranges from below the detection limit (~20 ng/g) to concentration of over 12 mg/g. Based on the total of measured PAH, little change is obvious for the first six years in geographic PAH distribution, when within-site variability is considered. Most sites have lower total measured PAH concentration in Year 6 when compared to the mean of Years 1 to 5. Only three sites had higher concentrations in Year 6. The 2 and 3 ring lower molecular weight PAHs (Figure 3.25) show a similar distribution with only three sites higher in year 6. The high molecular weight 4 and 5 ring PAHs (Figure 3.26) had higher concentrations at only eight sites in Year 6 compared with the mean of the first five years. The 4 and 5 ring PAHs represent the major percentage of PAH present in these samples (Figures 3.27 and 3.28). The total of all 24 PAHs measured

since year 2 (Figure 3.29) shows the same trend as the total of the 18 PAHs.

The concentrations of PAH at most sites did not change when the concentrations for Year 6 are compared to the mean concentrations for Years 1 to 5. The predominant PAHs detected were pyrene, fluoranthene, chrysene and napthalenes. In general, the 4 and 5 member rings predominated; however, there were considerable amounts of 2 and 3 ring aromatics at some sites. The decrease at some sites in total aromatics for Years 1-4 vs. Year 5 was due to a decrease in the 2 and 3 ring aromatics (i.e., BBSD, CBJB and SAWB, Figure 3.25). The presence of the 4 and 5 ring aromatics is indicative of PAH from combustion sources. However, as discussed above, alkylated PAH provide additional resolution of sources (Preprint 1).

There are generally higher PAH concentrations in bay systems that are adjacent to large urban areas with the associated high levels of industrial activities. An example of this is Galveston Bay, Texas. The closer the site is to the urban area, the higher the PAH concentration (Reprint 4).

The general overall conclusion from the PAH data is there is no significant change in PAH concentrations at most of the sites sampled over the six year period. The PAHs found in higher concentrations (pyrene, fluoranthene) are mainly derived from combustion sources. This input should be relatively constant with time and reflect the consistency in PAH concentrations between sampling years. The sites that show large increases in a given sampling year usually show decreases in subsequent years. This indicates that episodic inputs of PAH, possibly from oil spills, account for these increases. Then when the input stops, the ecosystem starts to recover.

We are continuing to look for temporal trends in the PAH data. The data set for the six years of NS&T program is large. Therefore, trends analyses require the use of various techniques including statistical ones. Other complications with data interpretation are caused by the nature of oysters. They can accumulate and depurate contaminants. There is currently only limited data on these processes. GERG is developing more data through several independant resarch projects (Preprint 2 and Reprint 5). We are currently looking at the data in an attempt to detect any gulf-wide temporal trends.

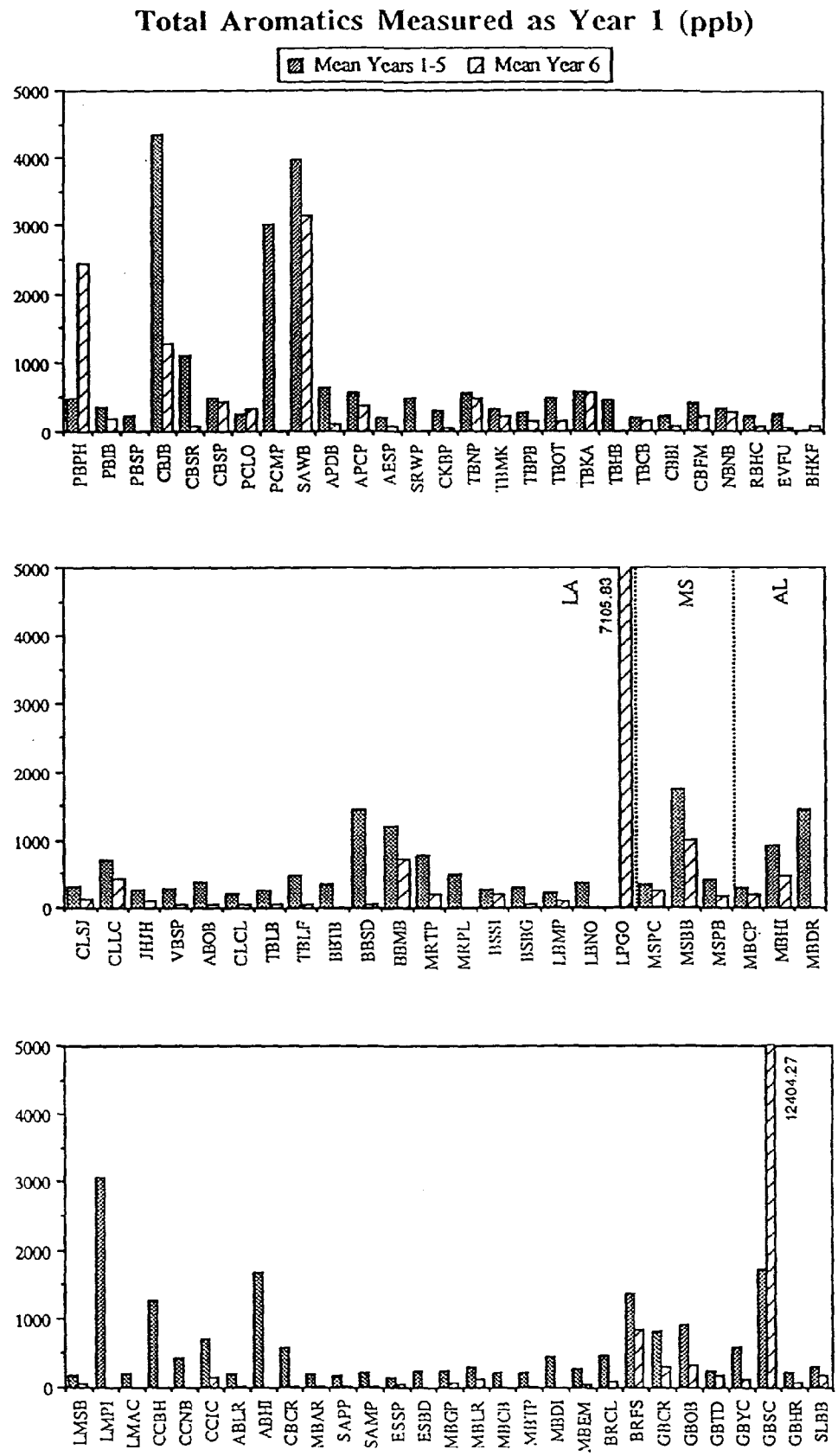


Figure 3.1 Average total aromatics as Year 1 concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

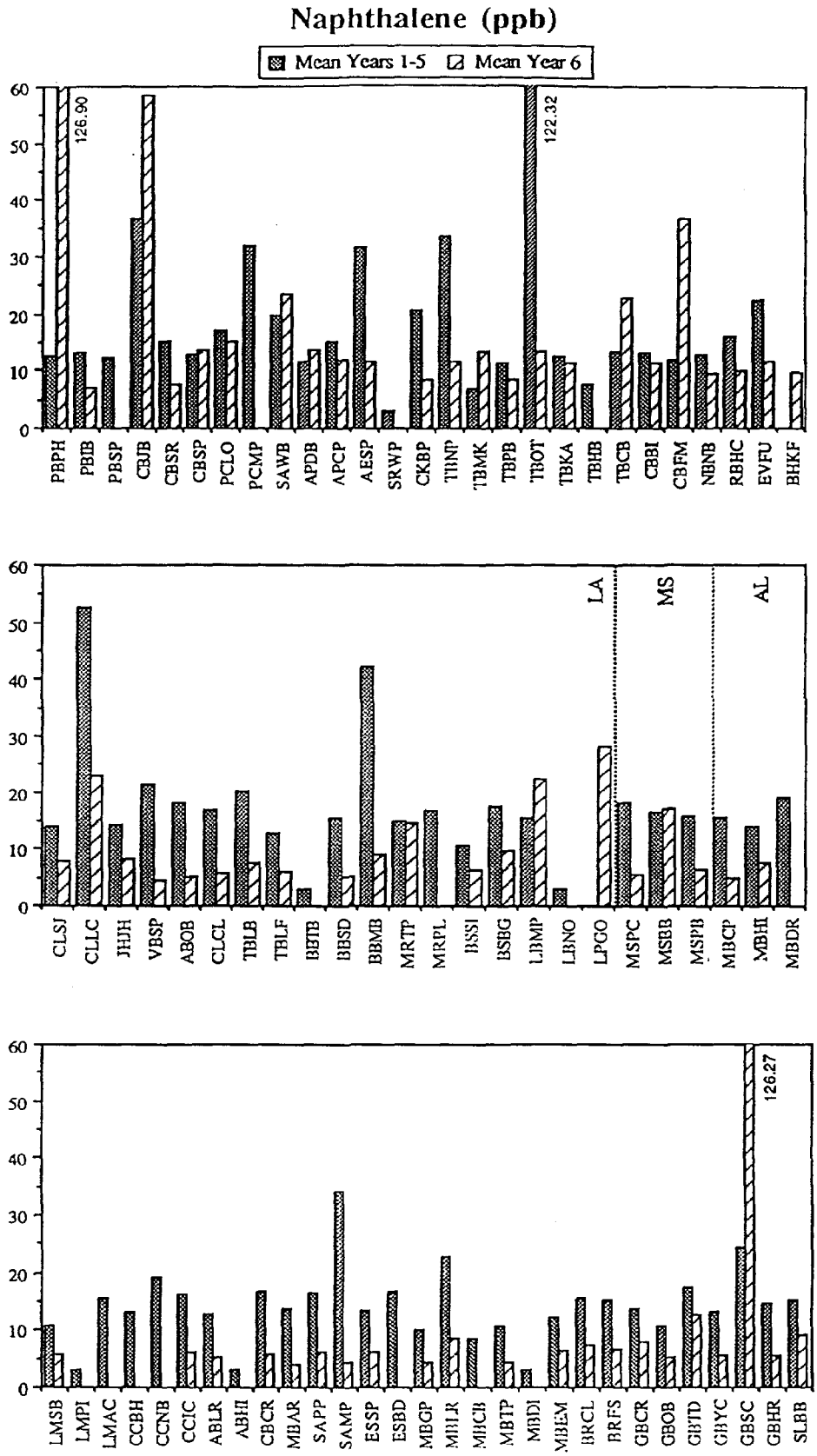


Figure 3.2 Average naphthalene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

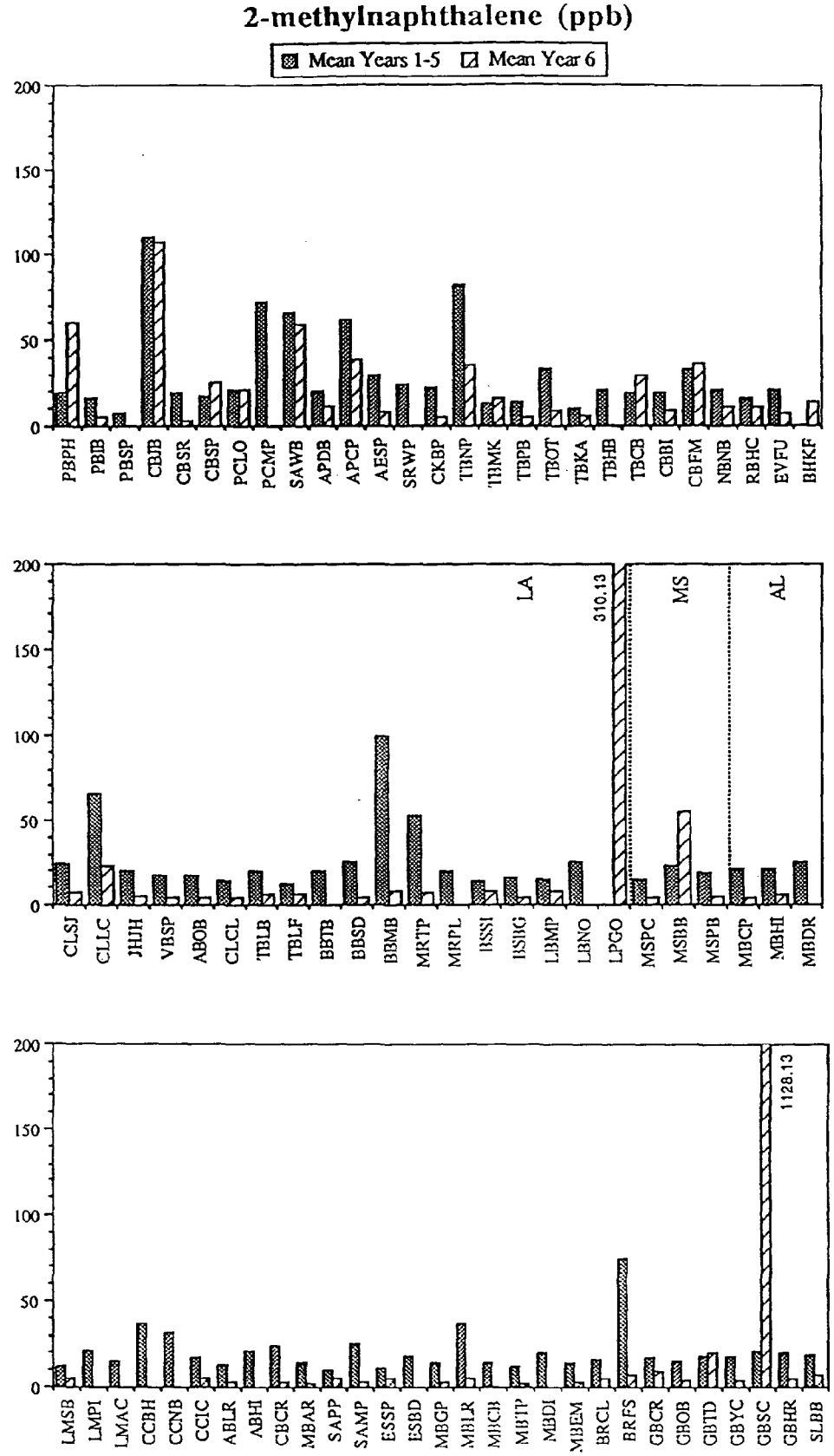


Figure 3.3 Average 2-methylnapthalene concentrations in oysters from each NS&T Mussel Watch gulf of Mexico sampling site for Years 1-5 and Year 6.

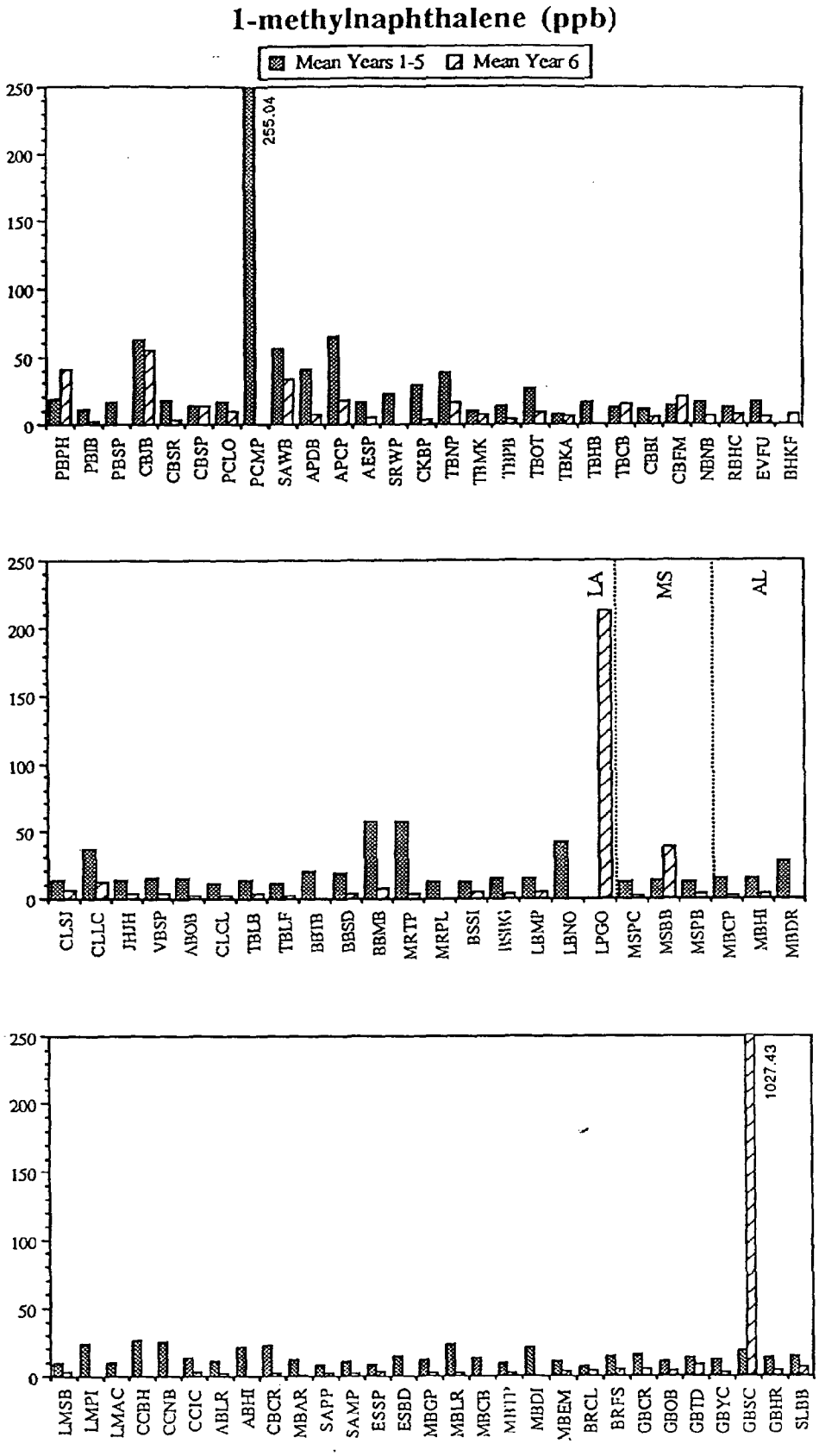


Figure 3.4 Average 1-methylnapthalene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

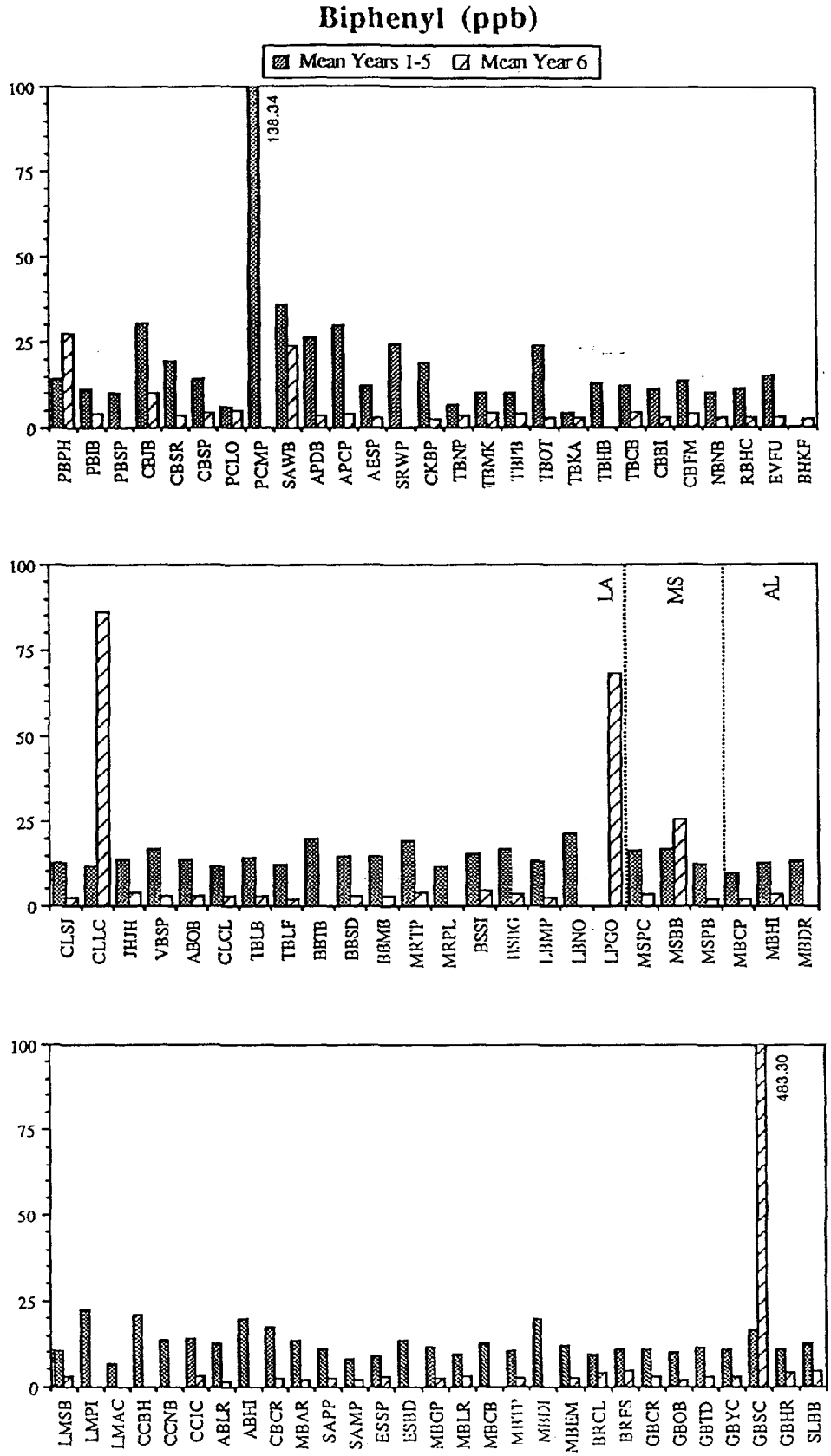


Figure 3.5 Average biphenyl epoxide concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

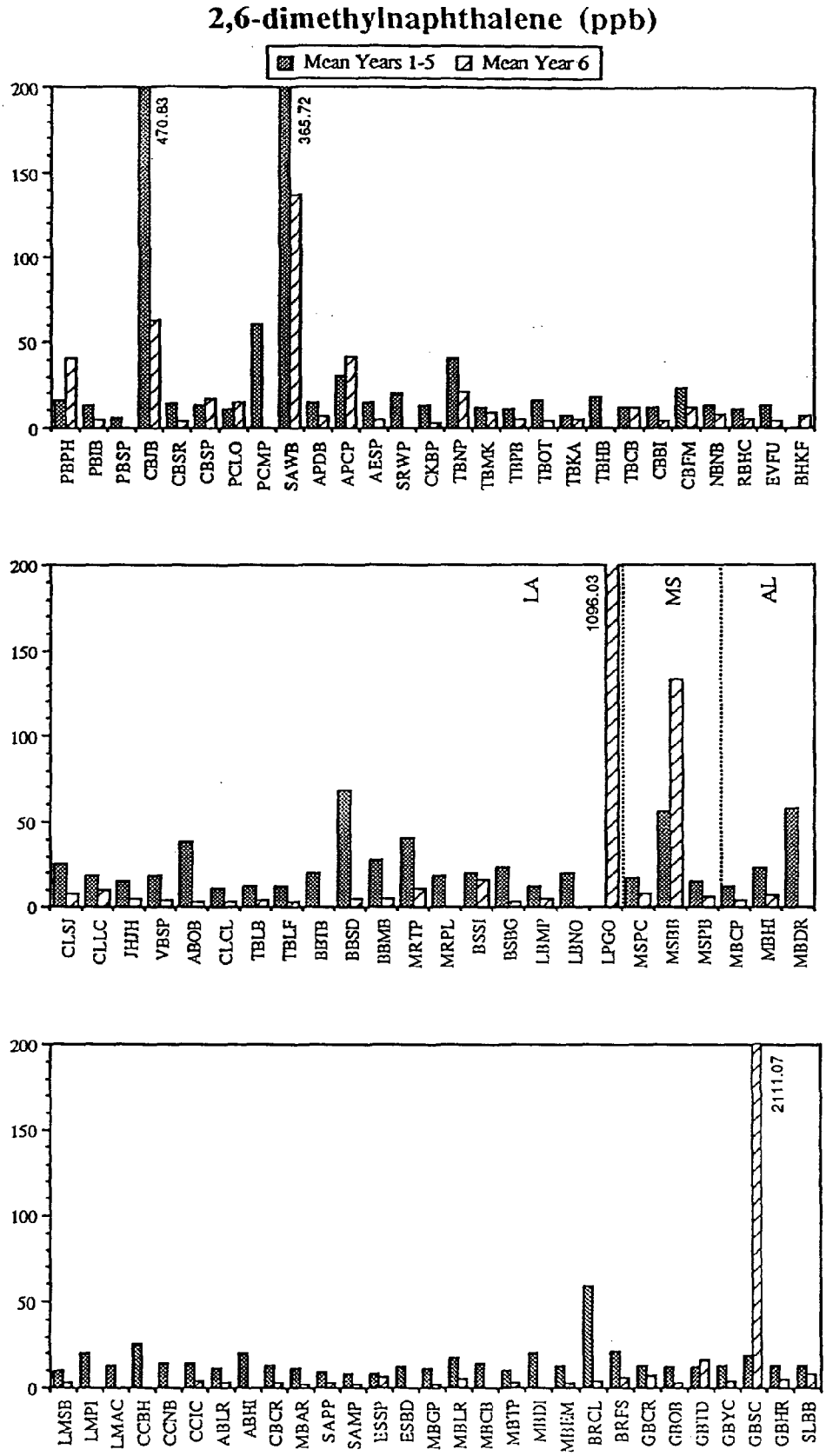


Figure 3.6 Average 2,6-dimethylnapthalene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

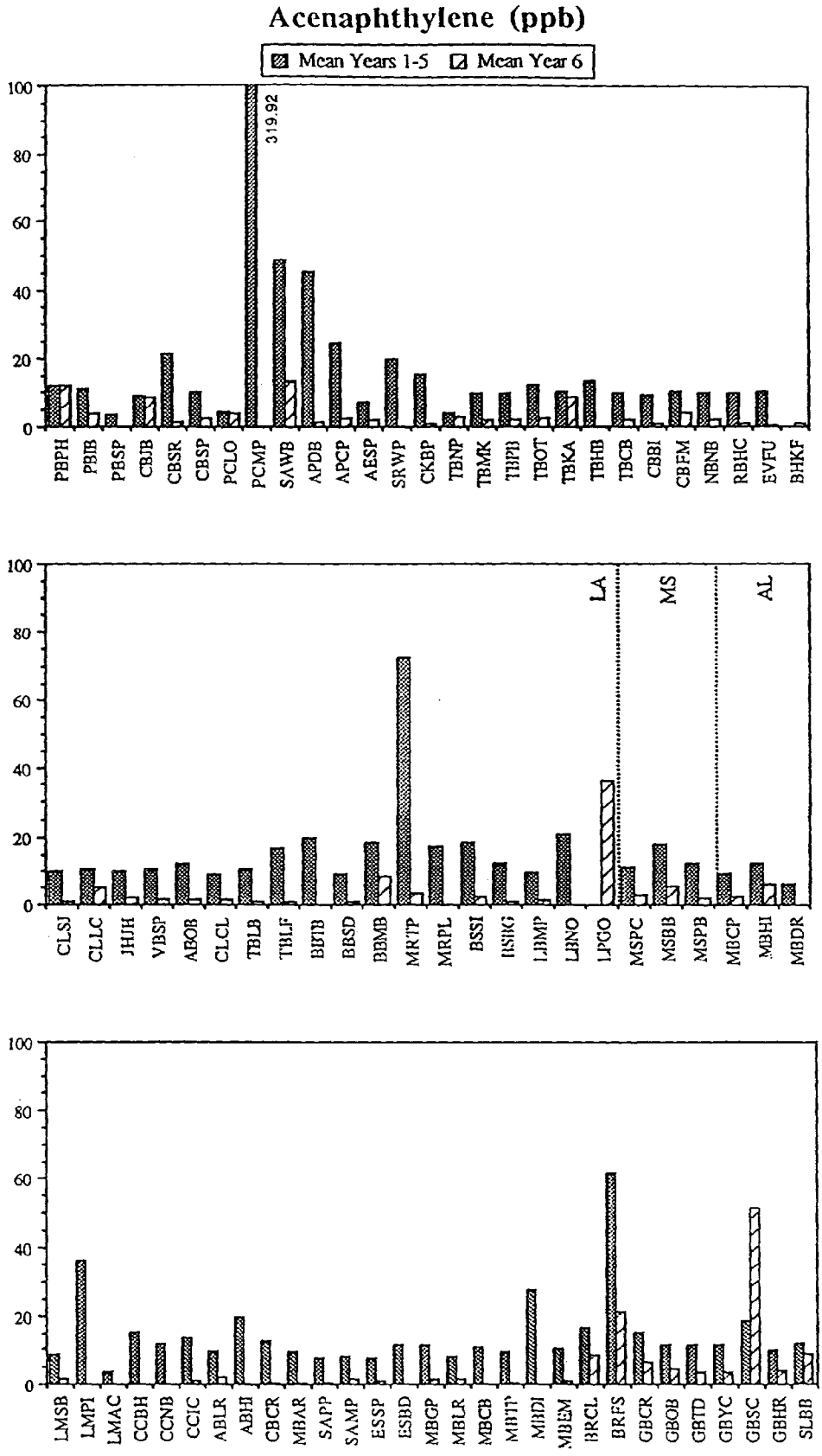


Figure 3.7 Average acenaphthylene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

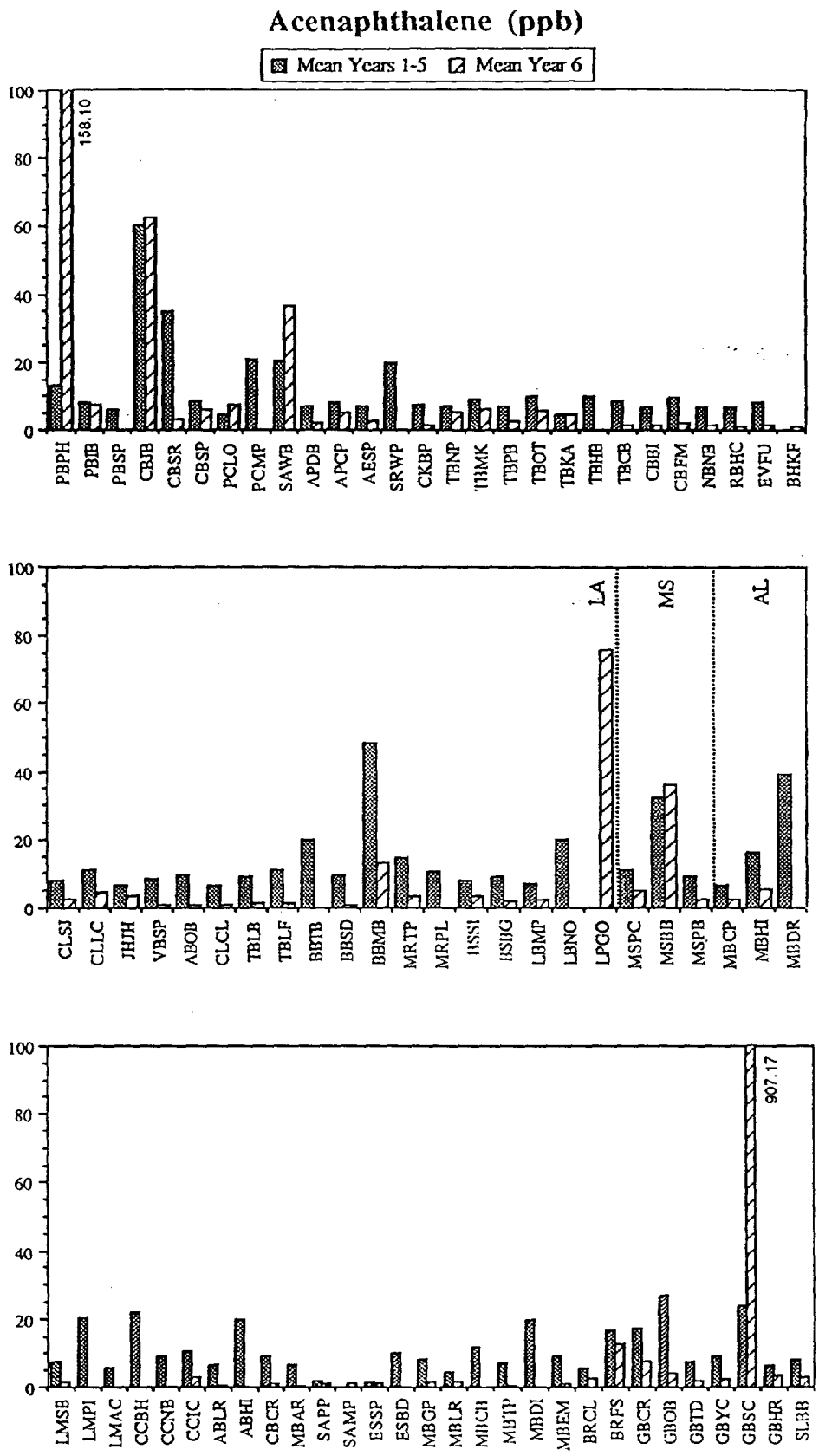


Figure 3.8 Average acenaphthene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

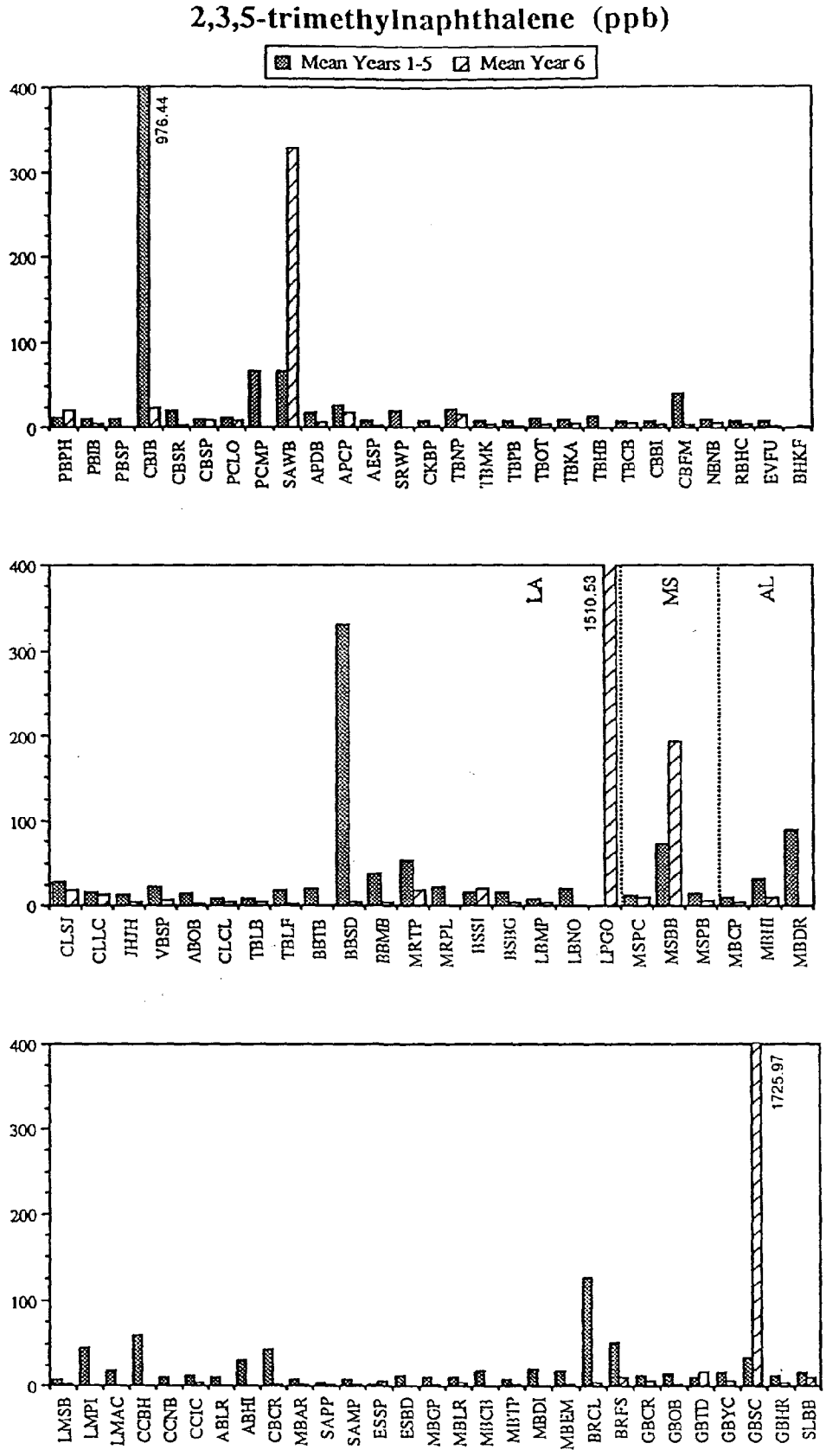


Figure 3.9 Average 2,3,5-trimethylnapthalene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

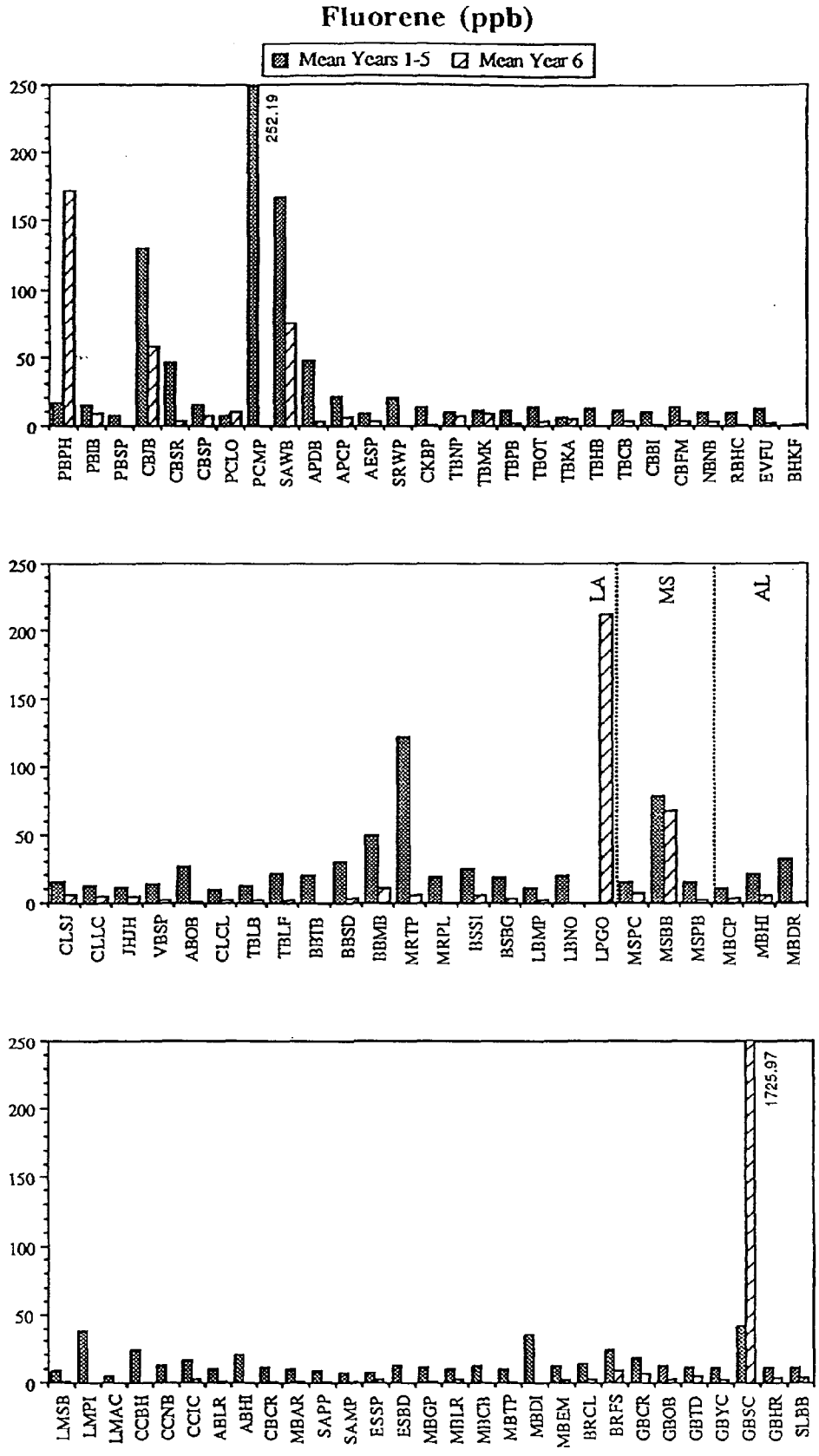


Figure 3.10 Average fluorene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

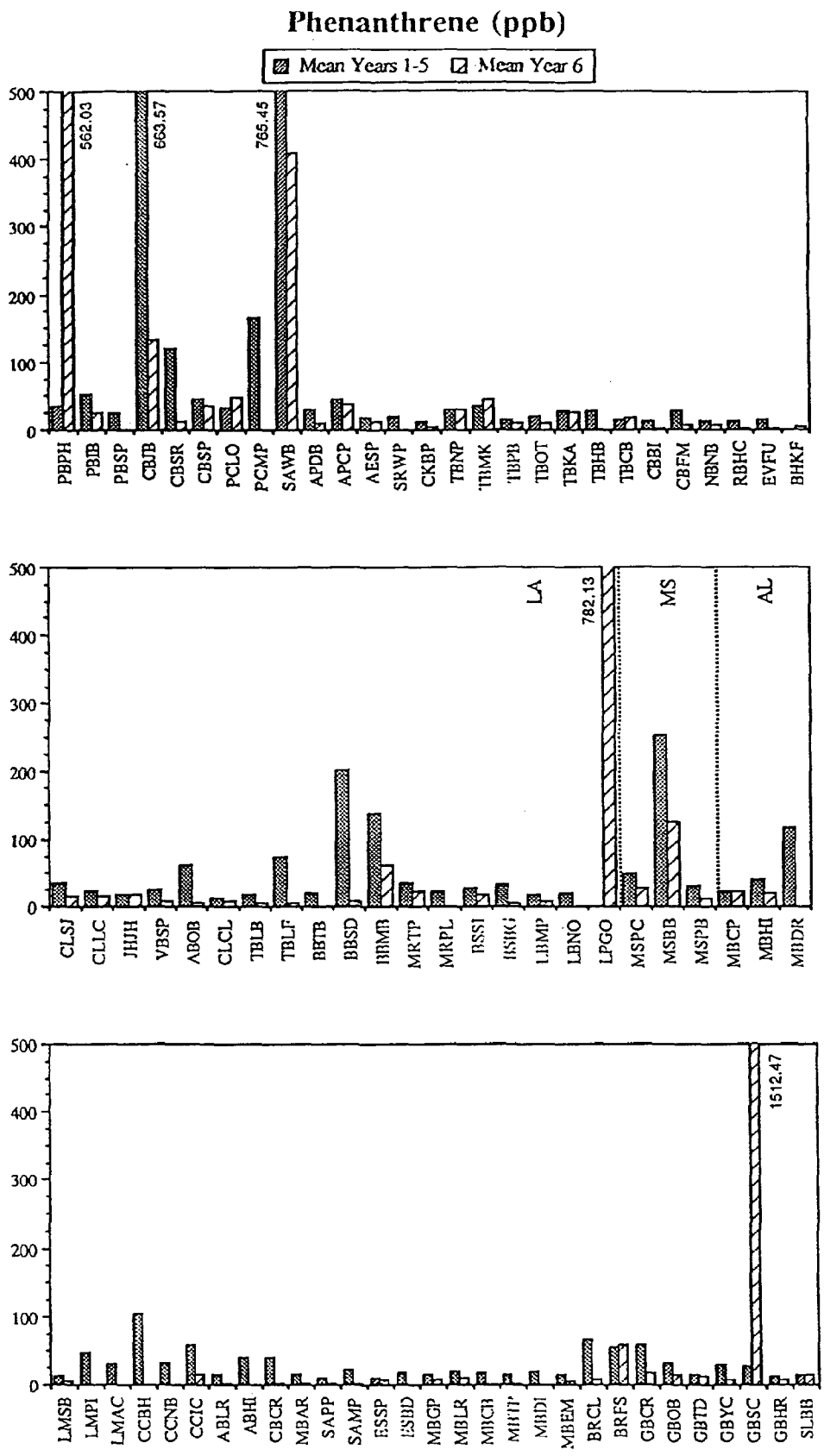


Figure 3.11 Average phenanthrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

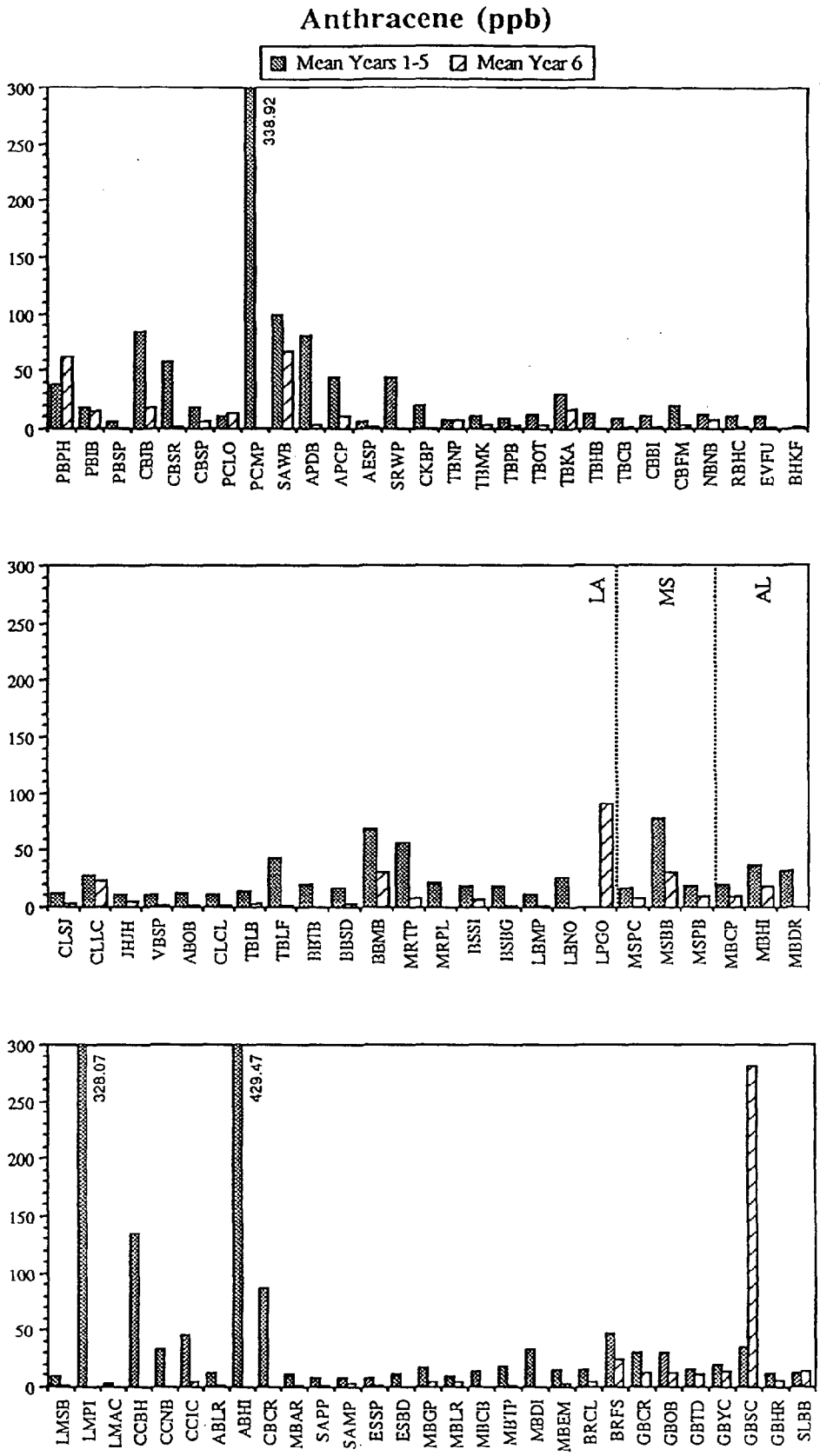


Figure 3.12 Average anthracene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

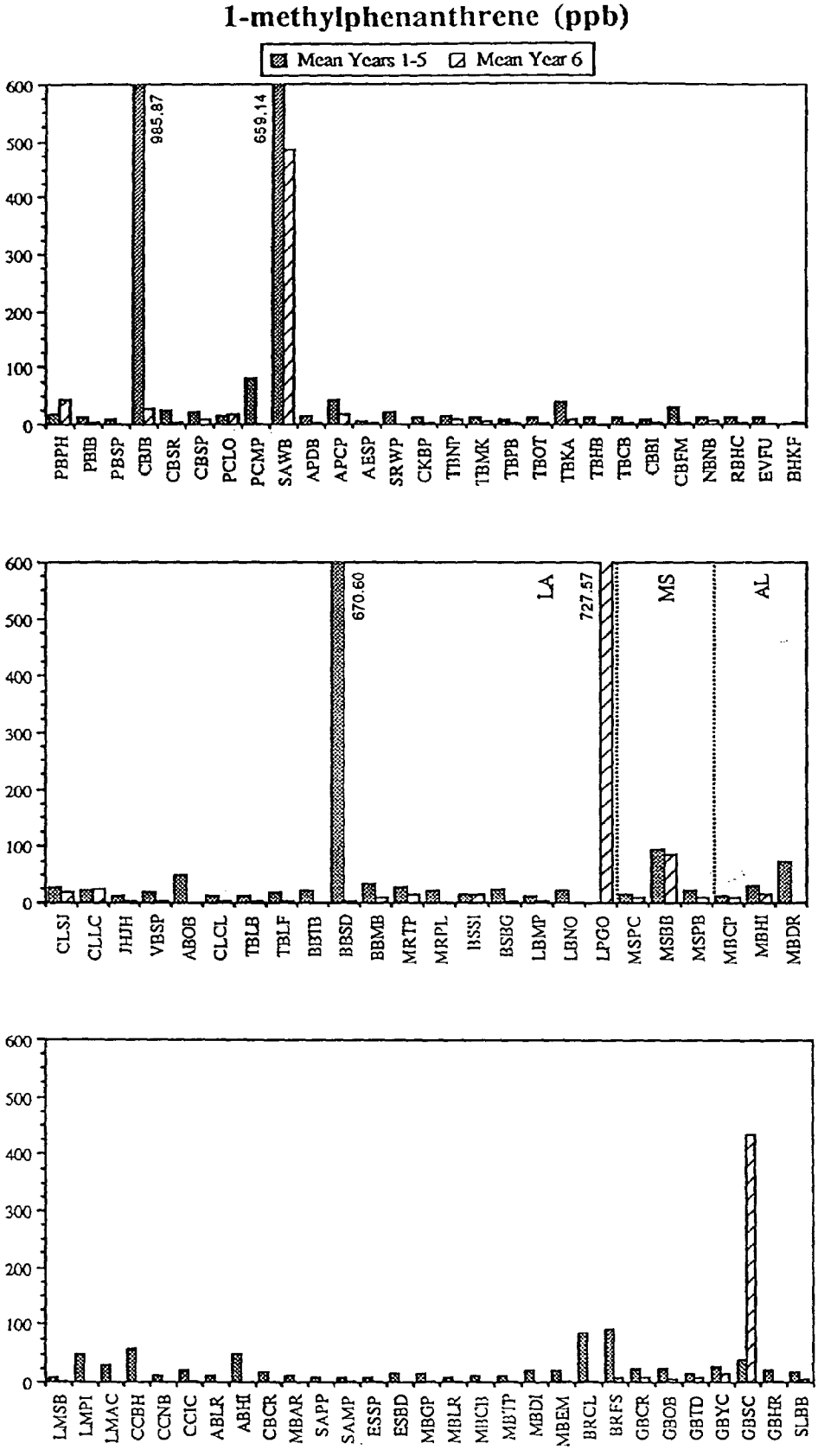


Figure 3.13 Average 1-methylphenanthrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

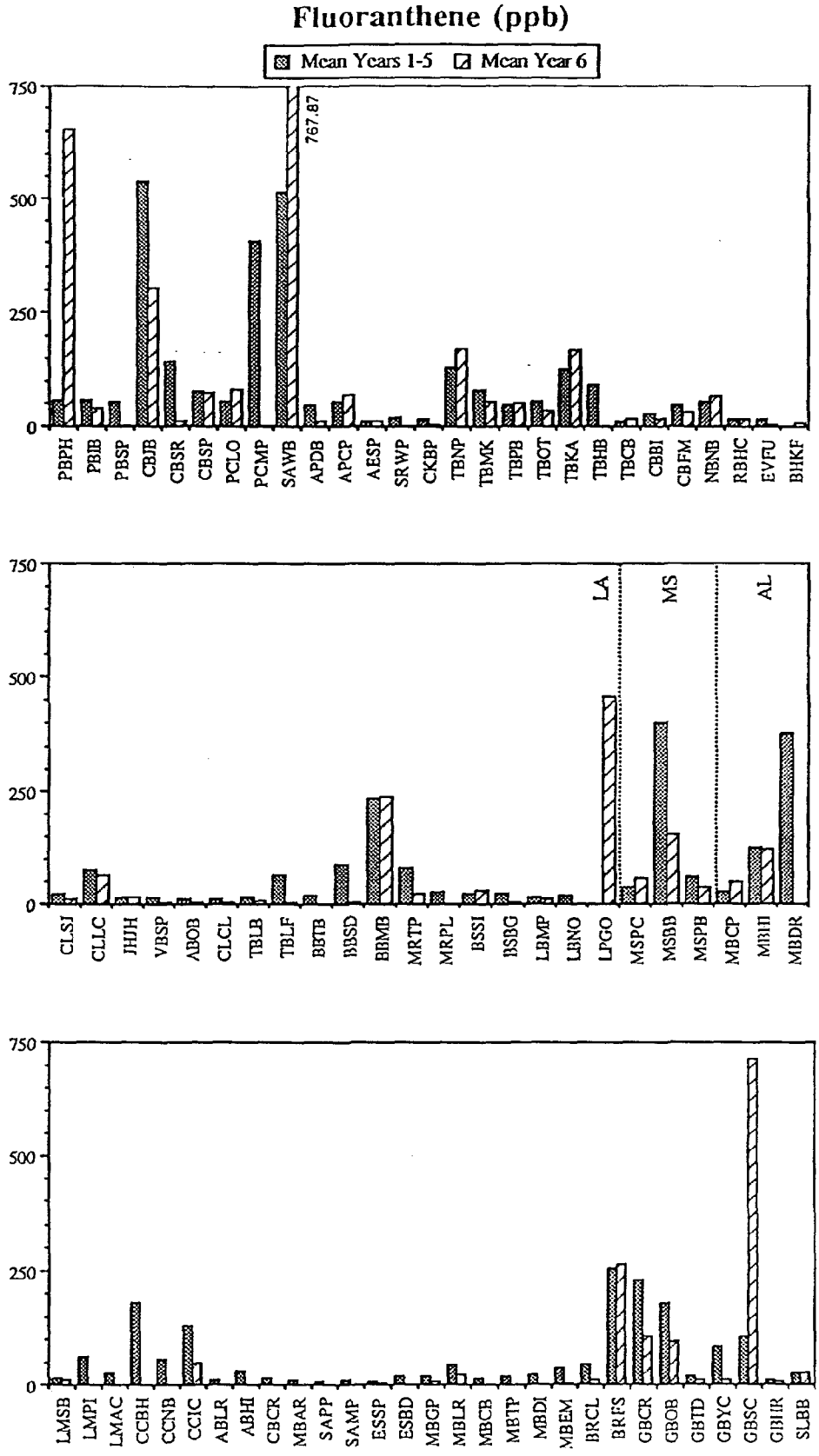


Figure 3.14 Average fluoranthene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

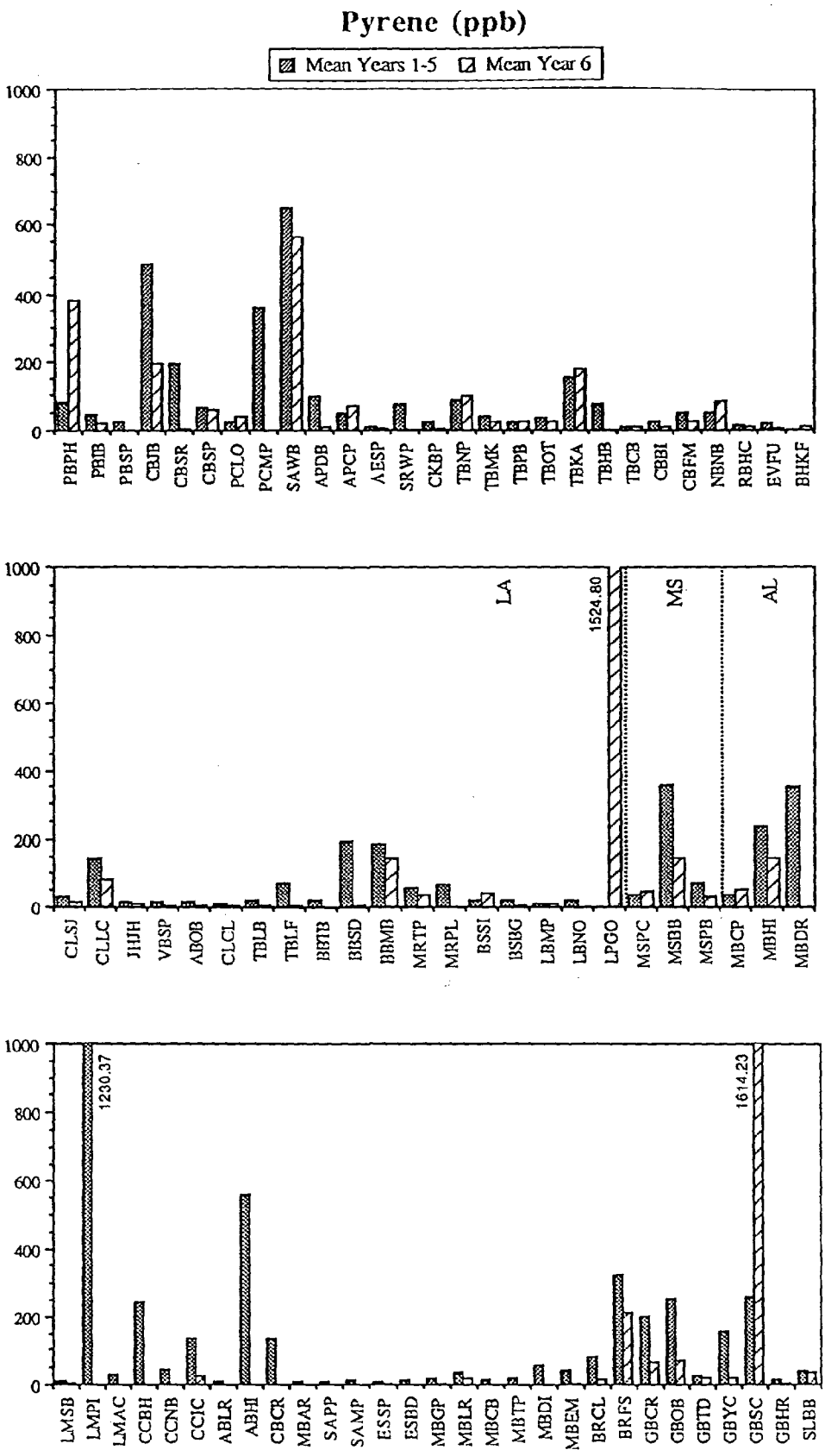


Figure 3.15 Average pyrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

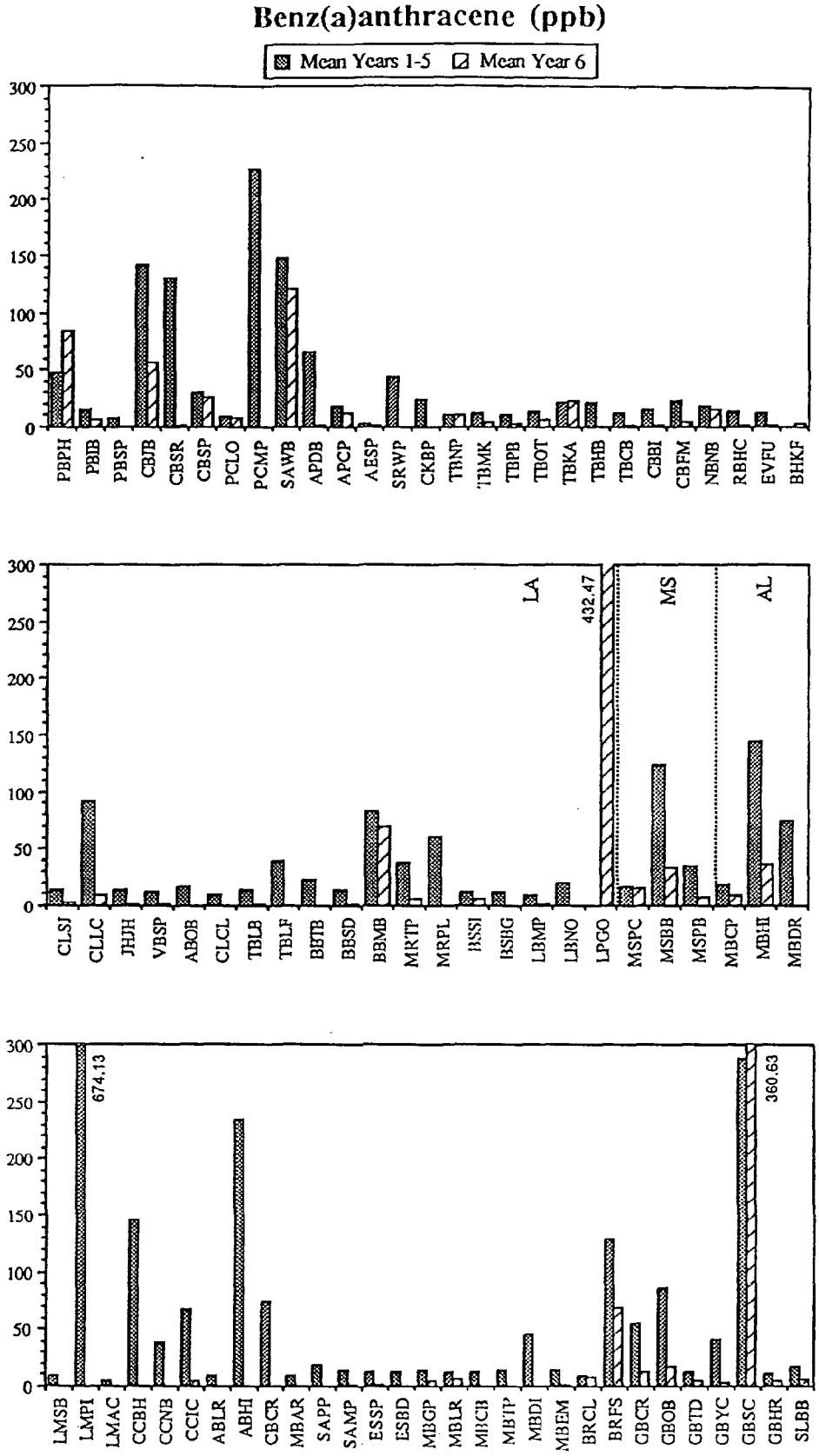


Figure 3.16 Average benz(a)anthracene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

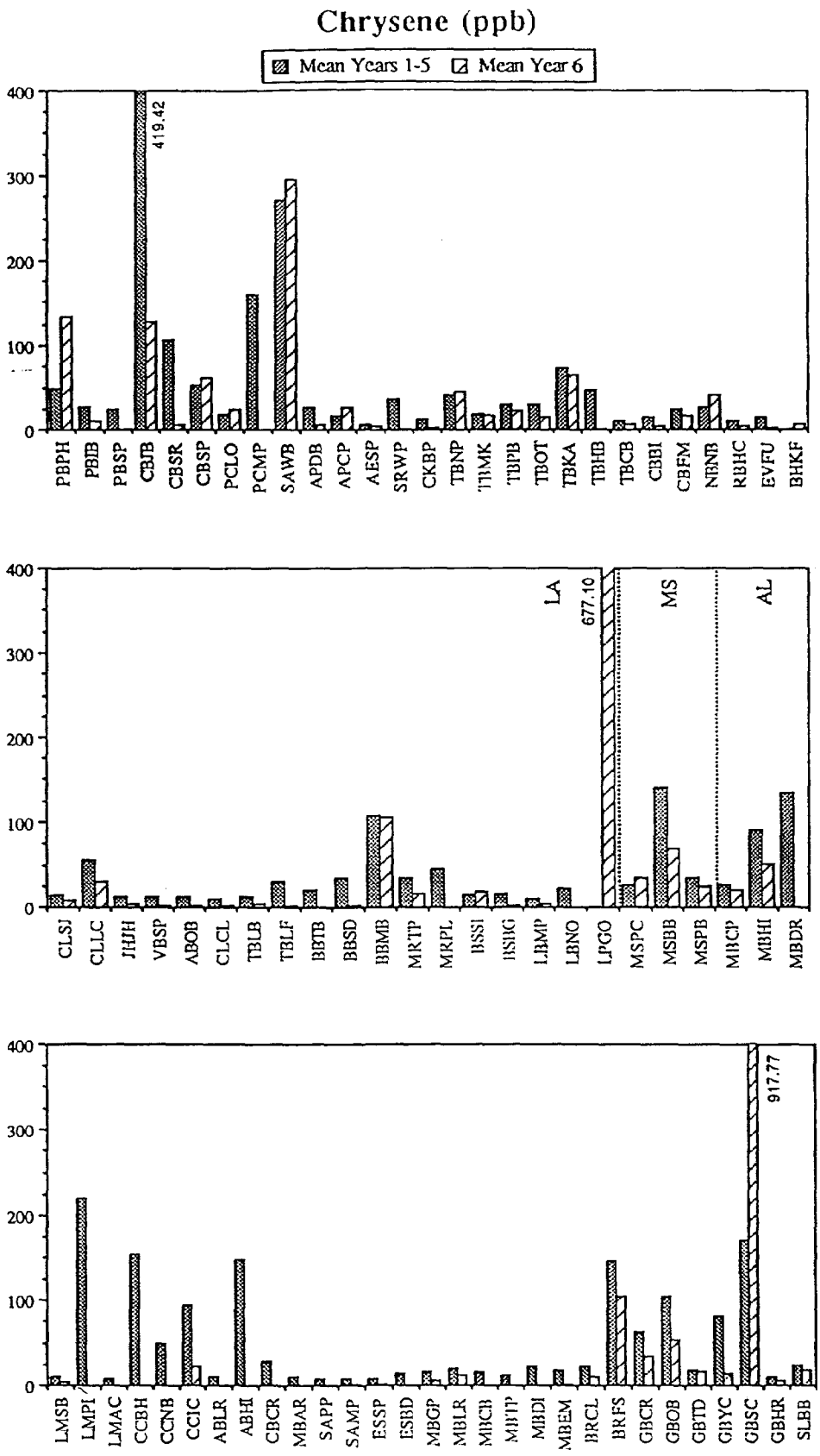


Figure 3.17 Average chrysene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

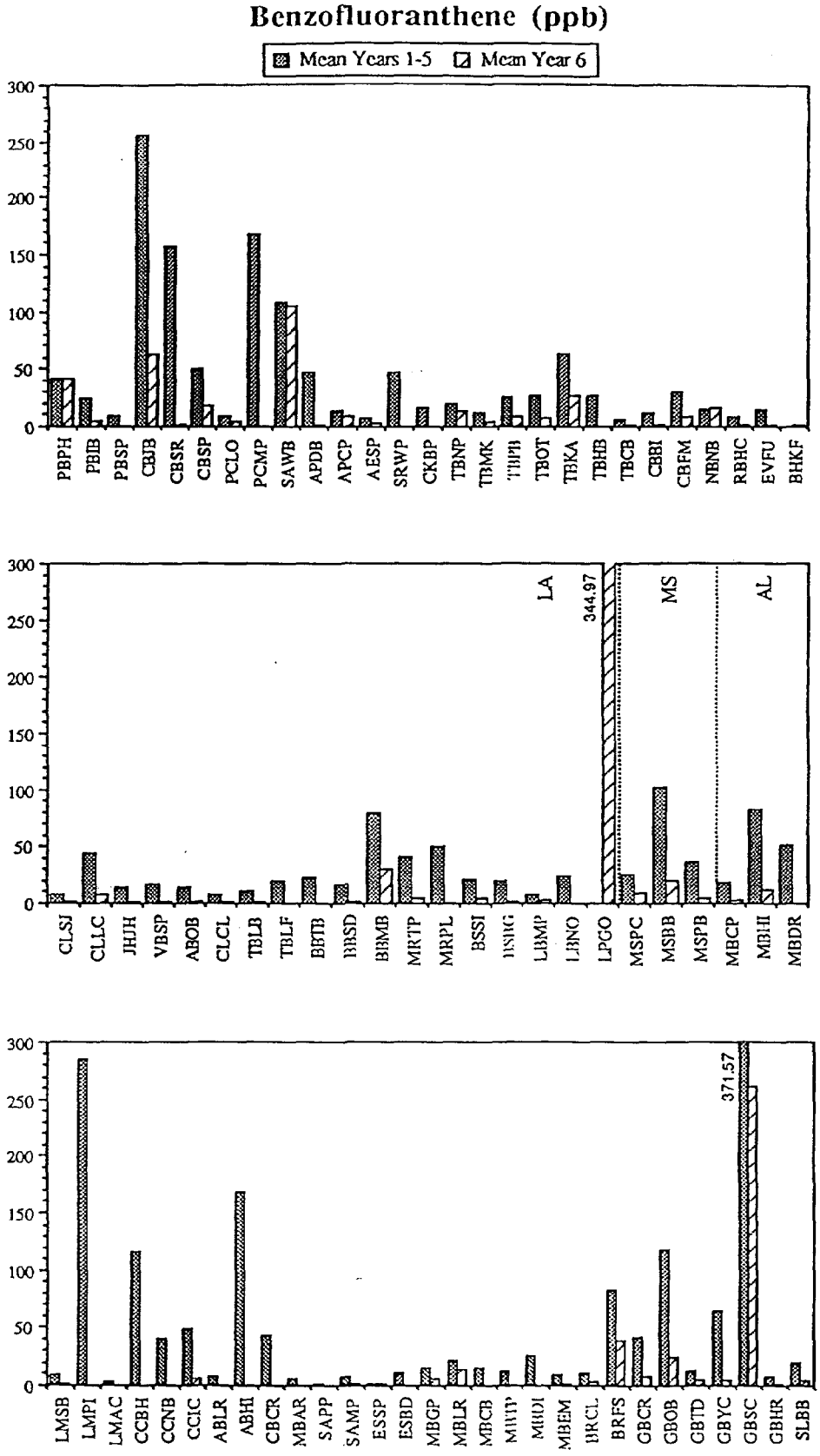


Figure 3.18 Average benzofluoranthene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

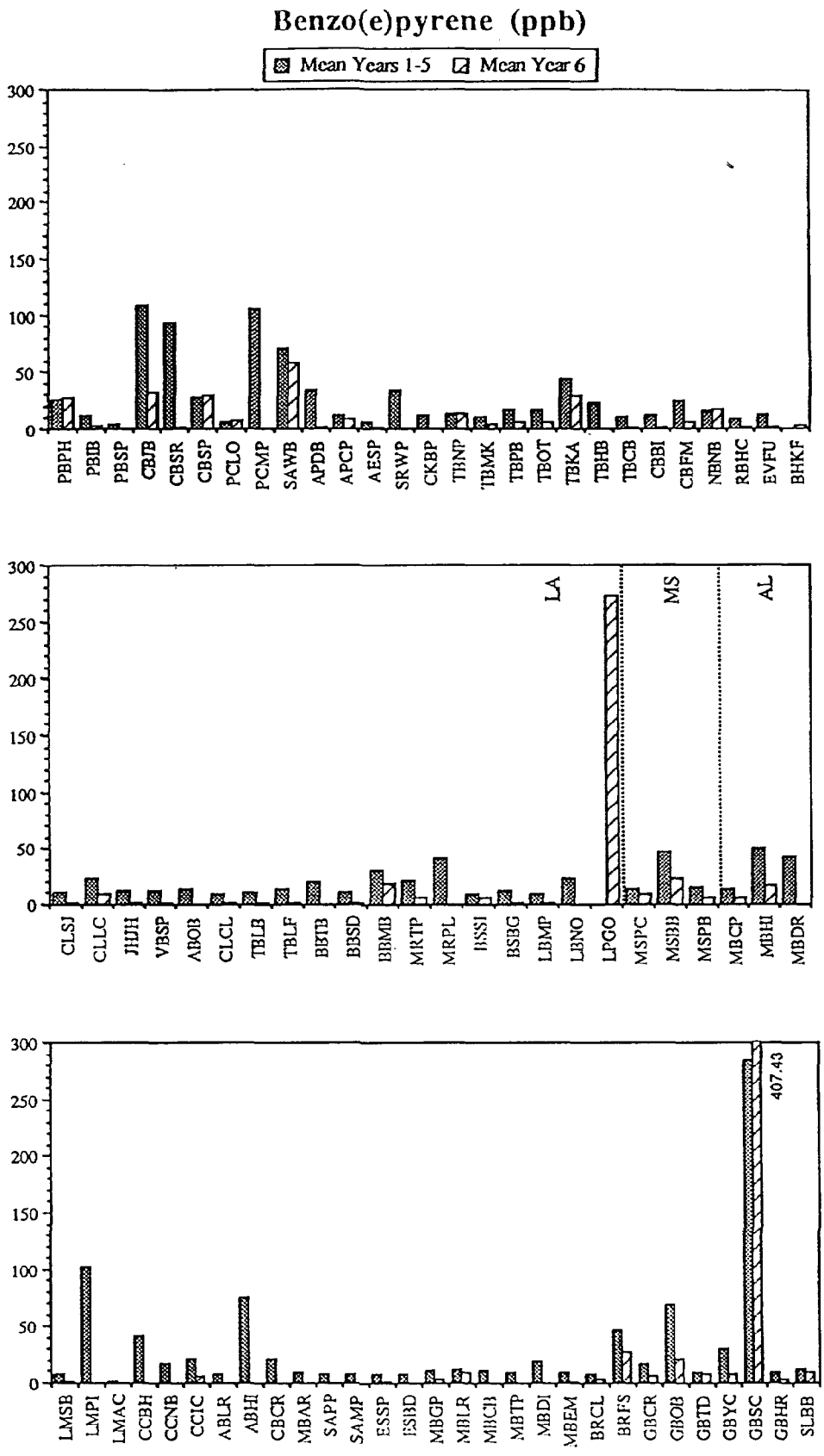


Figure 3.19 Average benzo(e)pyrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

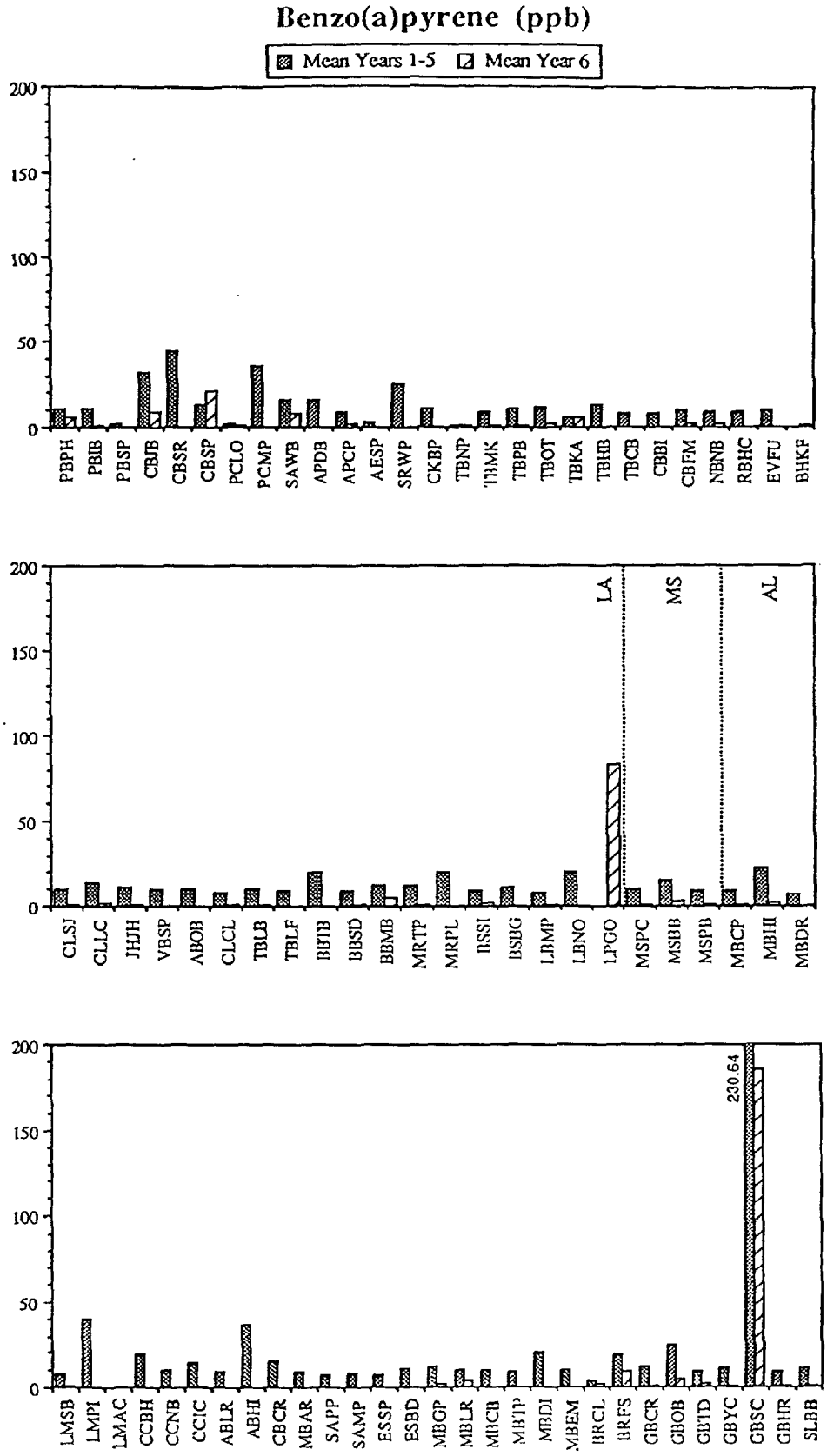


Figure 3.20 Average benzo(a)pyrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

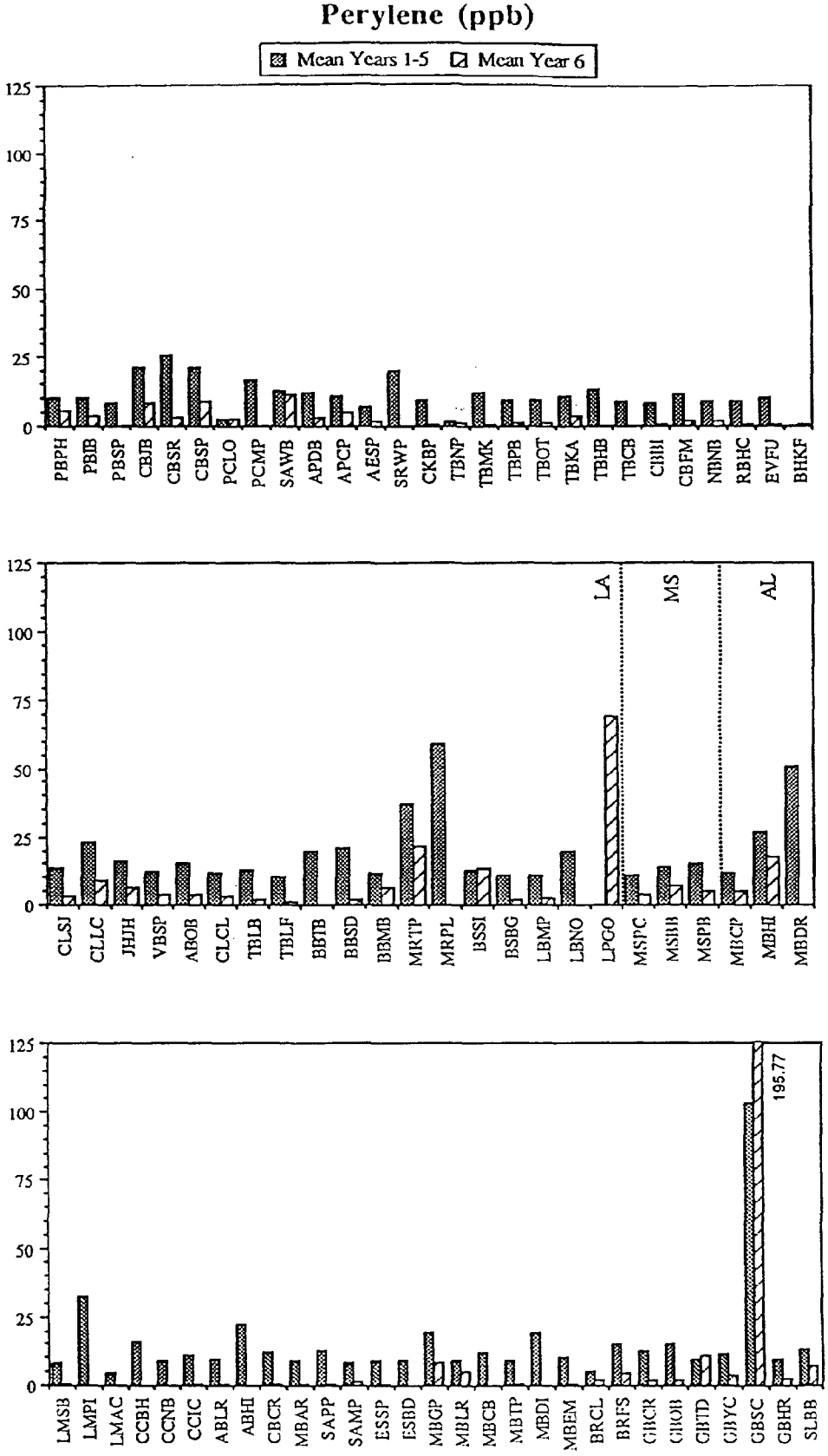


Figure 3.21 Average perylene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

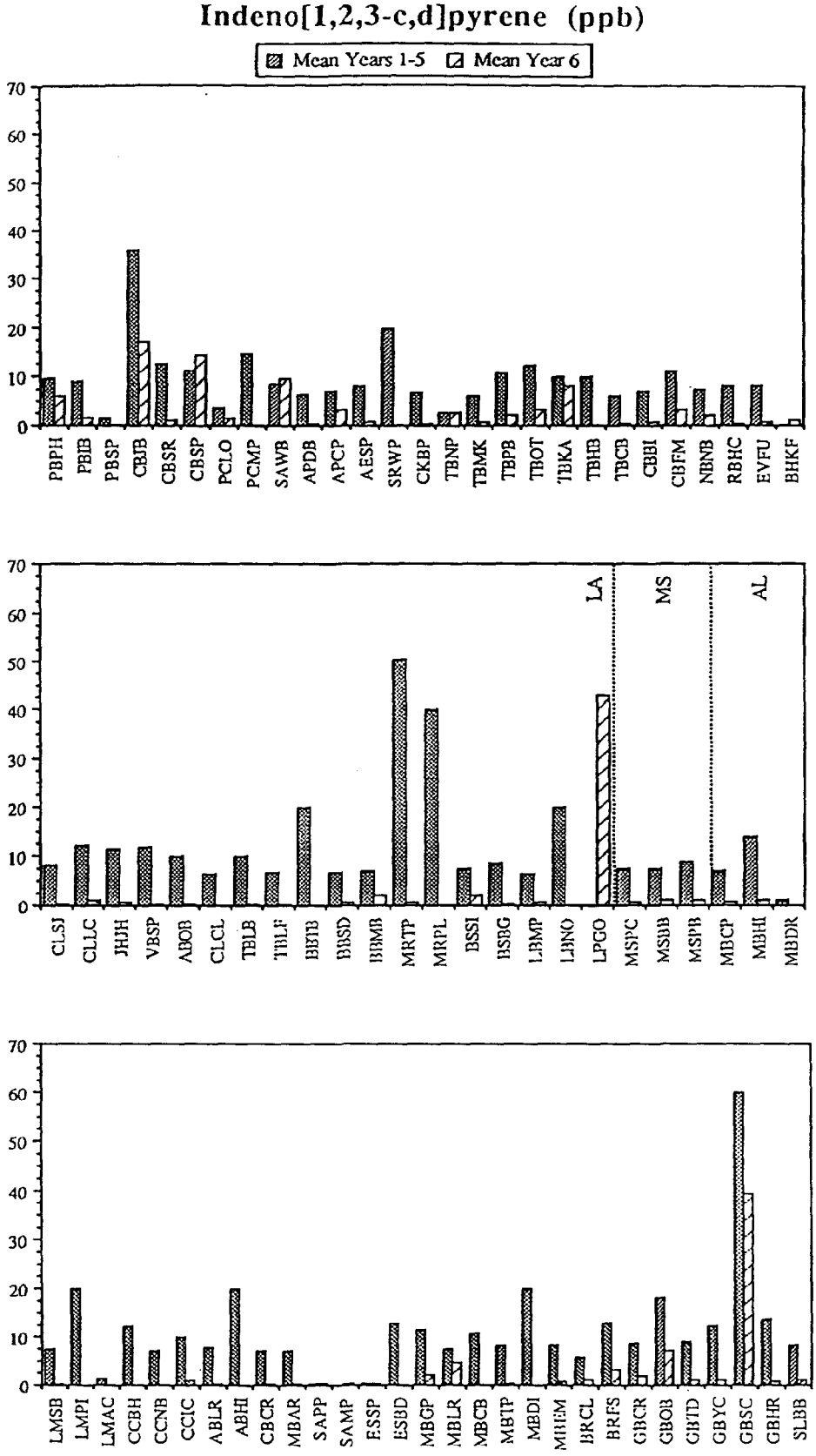


Figure 3.22 Average indeno[1,2,3-c,d]pyrene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

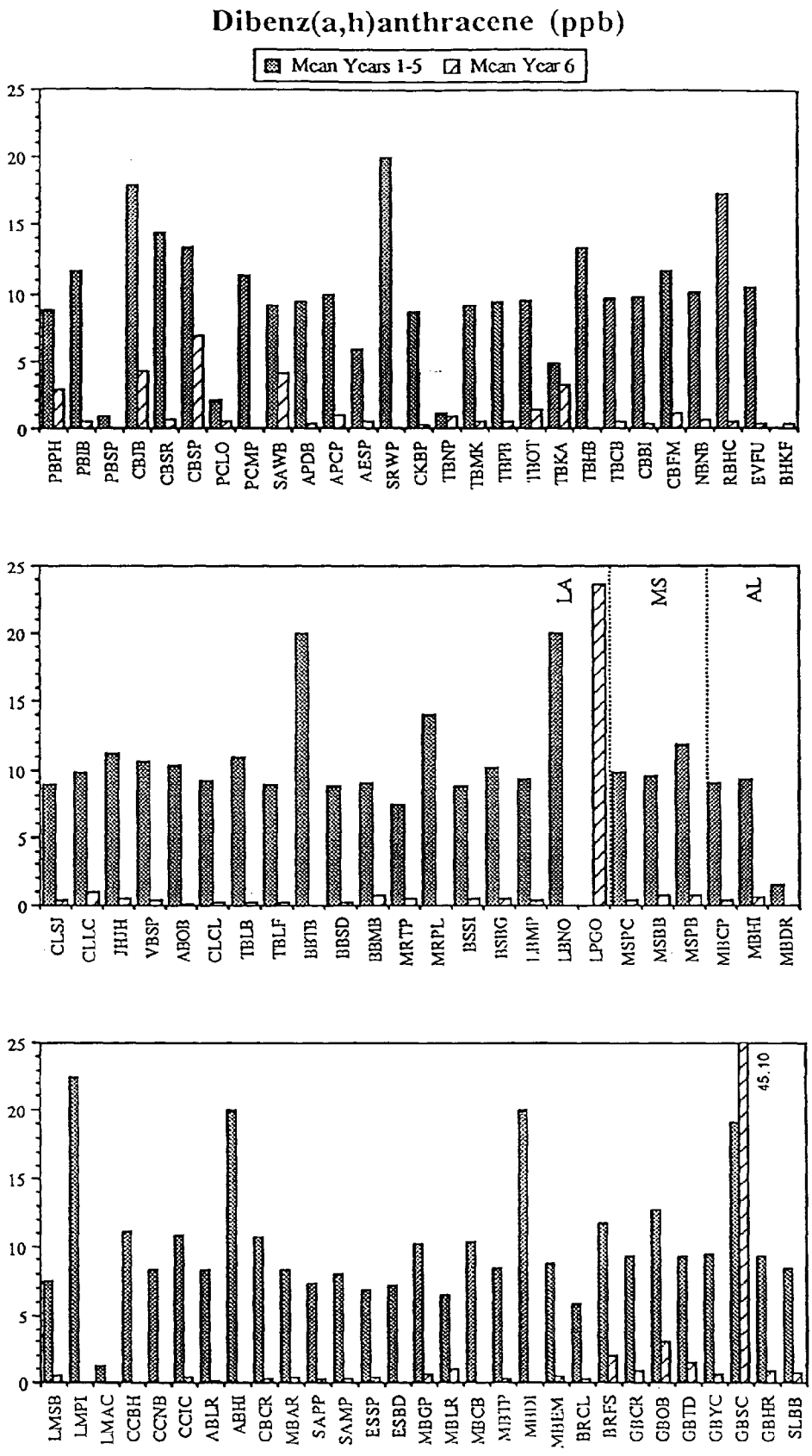


Figure 3.23 Average dibenz(a,h)anthracene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

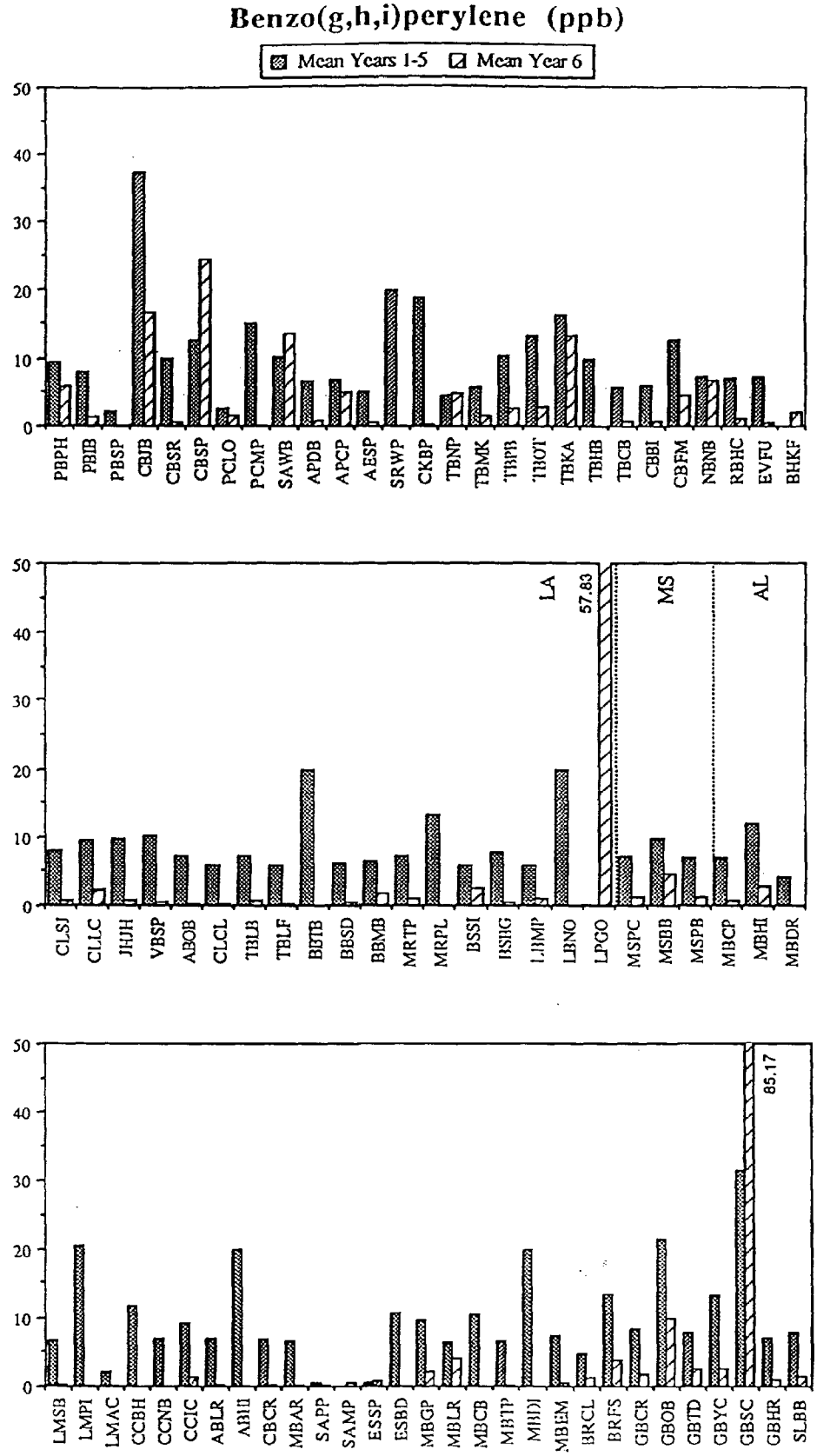


Figure 3.24 Average benzo(ghi)perylene concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

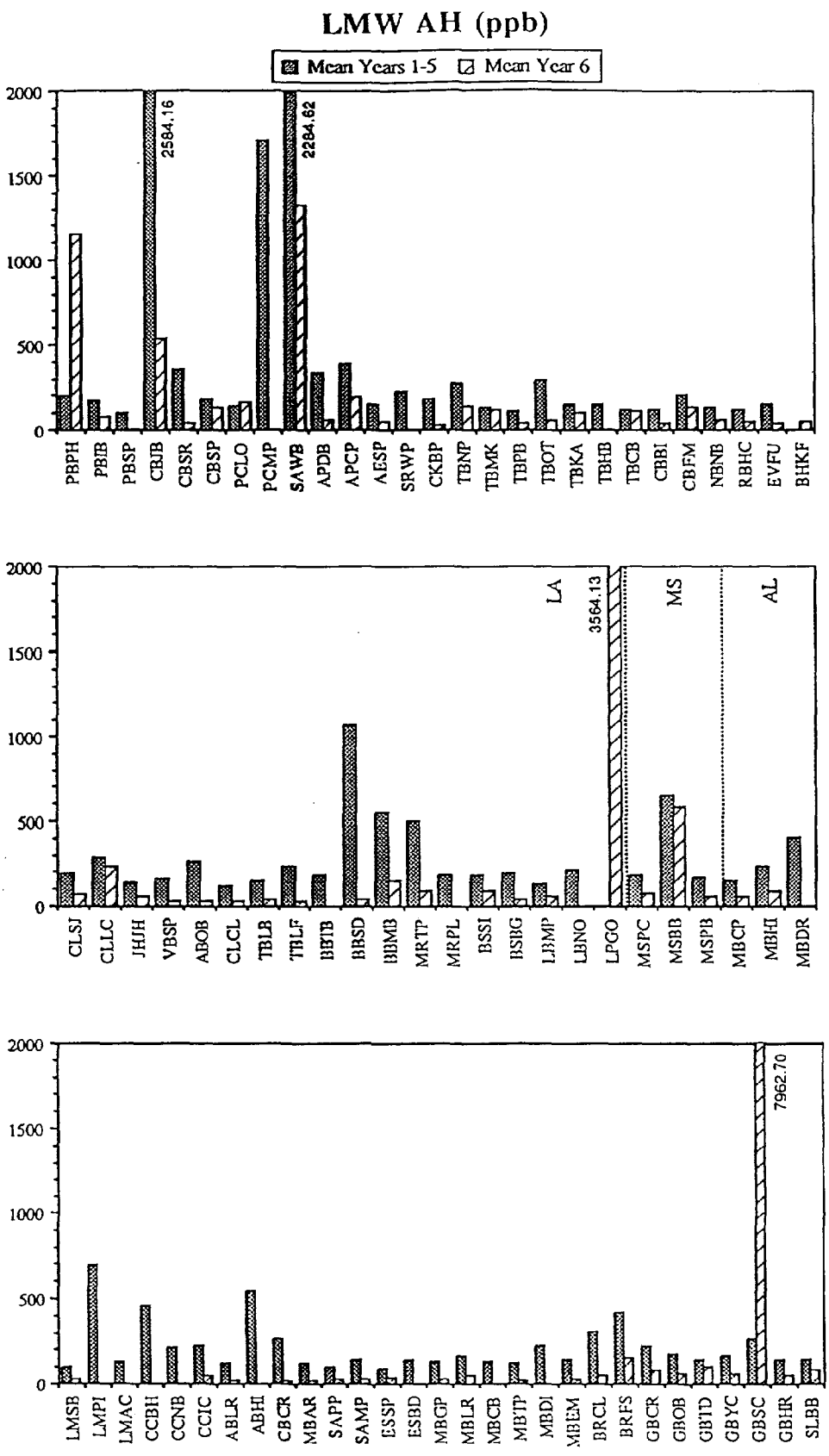


Figure 3.25 Average LMW (2+3 ring) aromatics concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

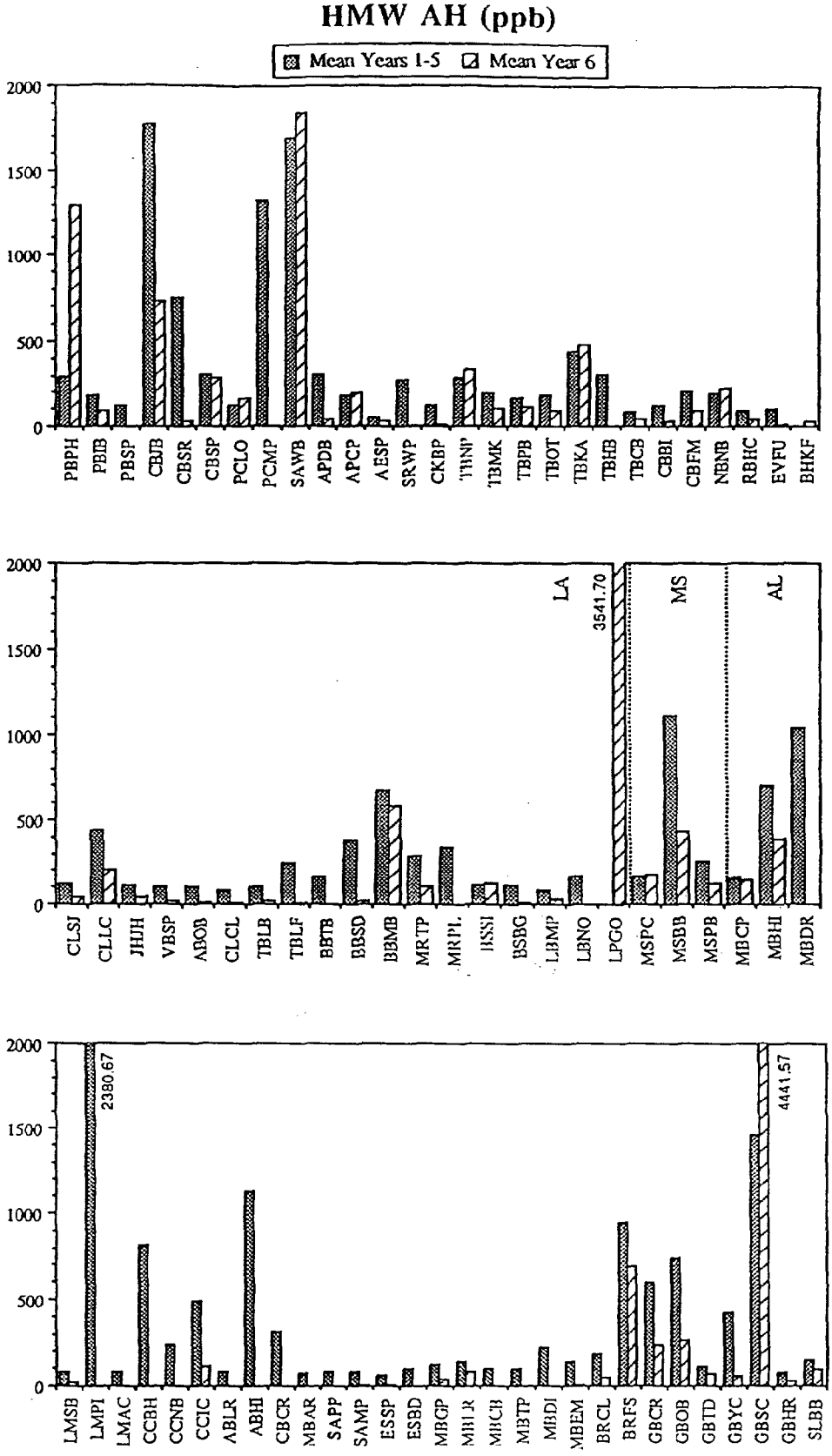


Figure 3.26 Average HMW (4+5 ring) aromatics concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

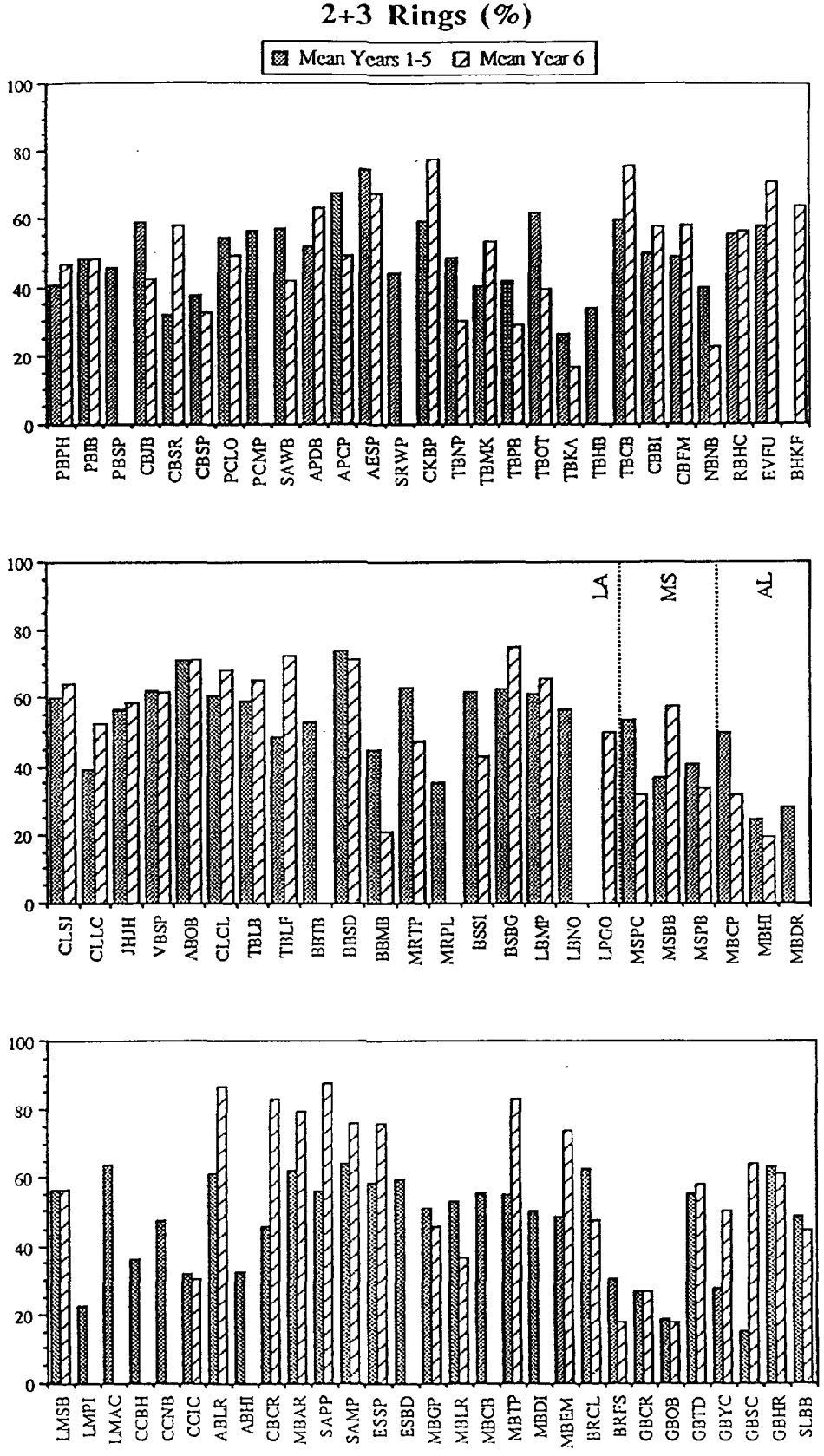


Figure 3.27 Average 2+3 ring aromatics percentages in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

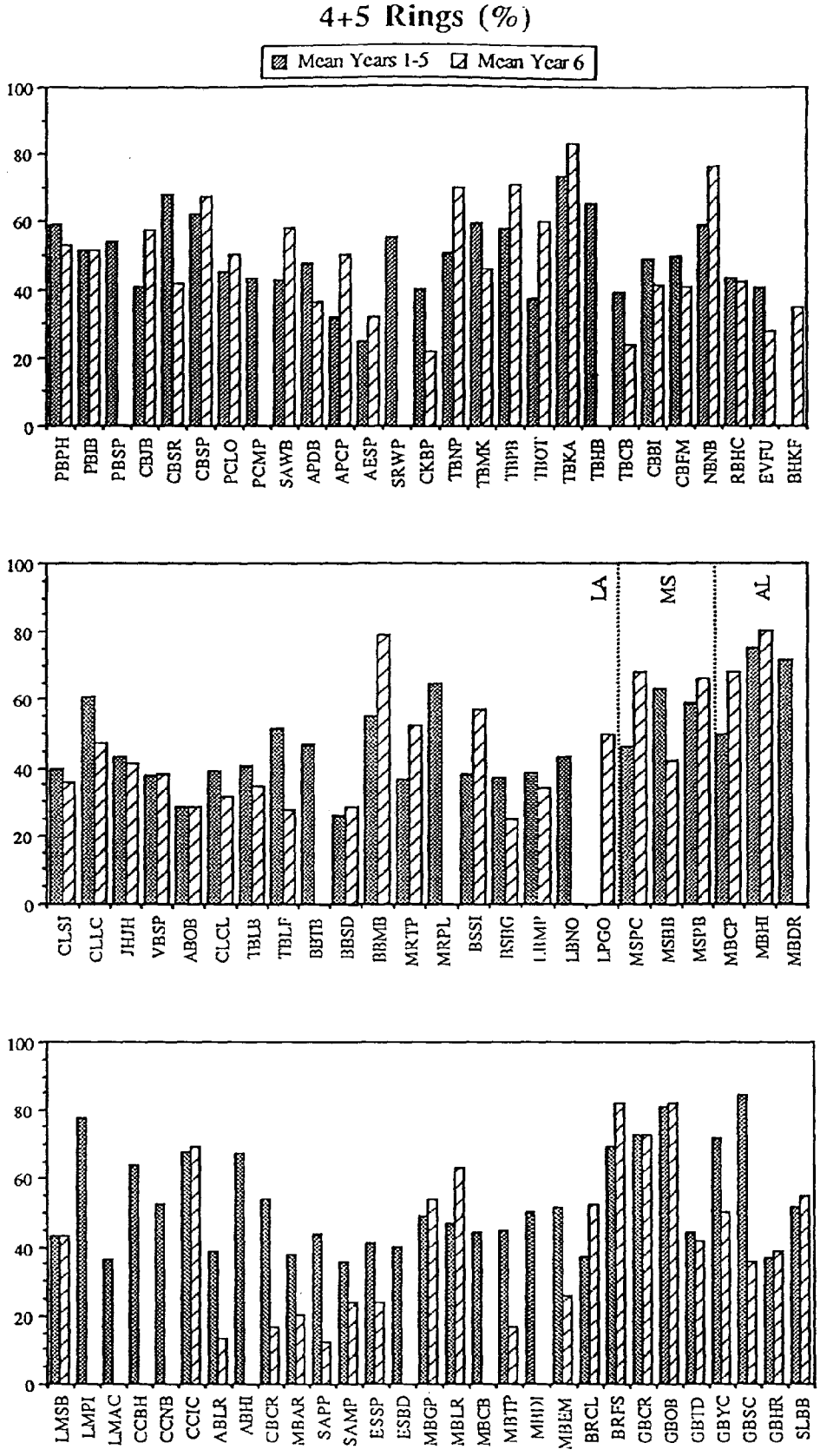


Figure 3.28 Average 4+5 ring aromatics percentages in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

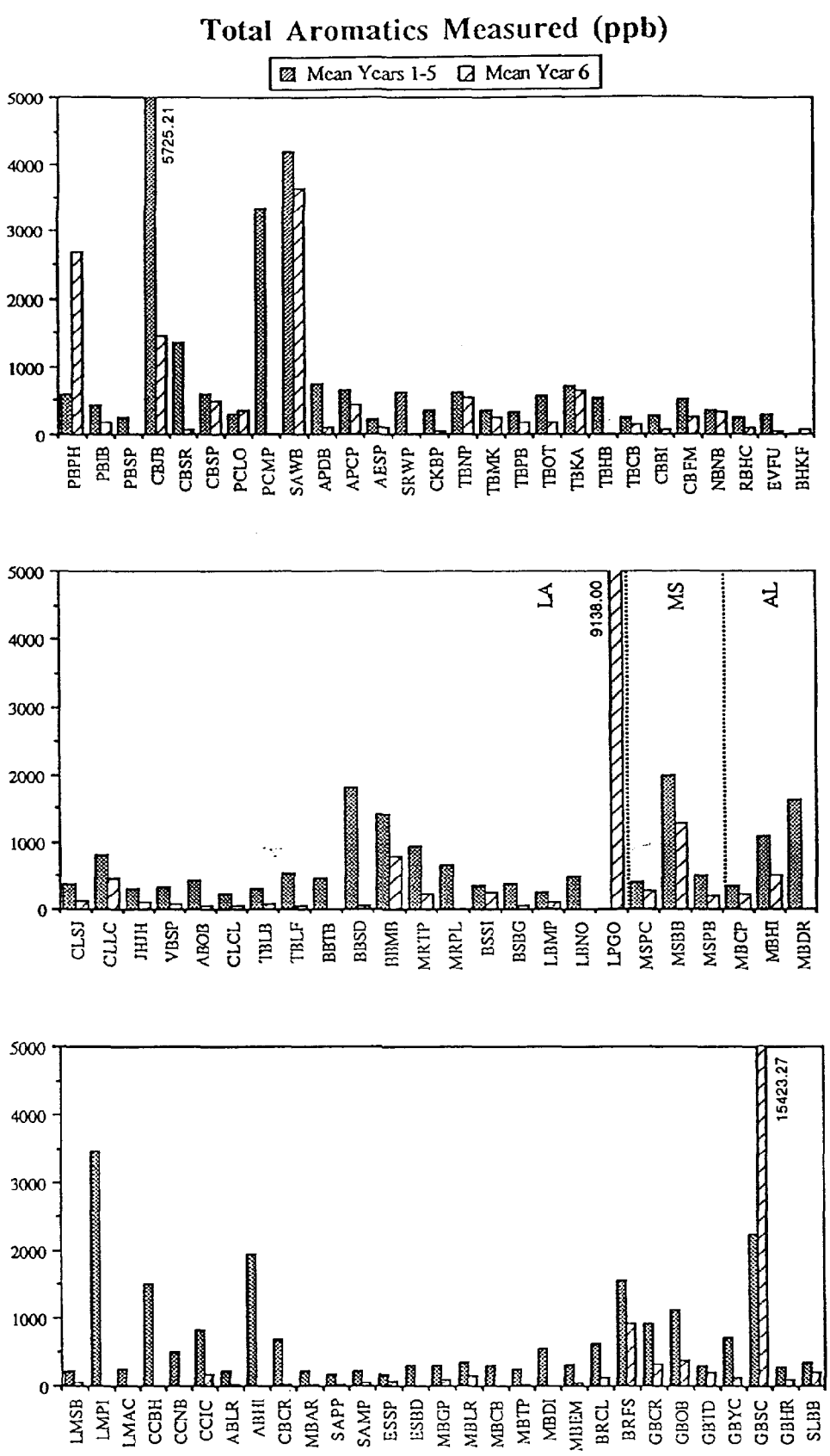


Figure 3.29 Average total aromatics measured in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

Reprint 3

Trace Organic Contamination in Galveston Bay: Results from the NOAA National Status and Trends Mussel Watch Program

Terry L. Wade, James M. Brooks, José L. Sericano, Thomas J. McDonald, Bernardo Garcia-Romero, Roger R. Fay, and Dan L. Wilkinson

Trace Organic Contamination in Galveston Bay: Results from the NOAA National Status and Trends Mussel Watch Program

Terry L. Wade, James M. Brooks, José L. Sericano, Thomas J. McDonald, Bernardo García-Romero, Roger R. Fay, and Dan L. Wilkinson
Geochemical and Environmental Research Group, Texas A&M University

In order to determine the current status and long-term trends for selected environmental contaminants in U.S. coastal areas, the National Oceanic and Atmospheric Administration (NOAA) established the National Status and Trends (NS&T) Mussel Watch Program. As part of the NS&T Program, sediment and oyster samples have been collected and analyzed from over 70 estuarine sites in the Gulf of Mexico representing all major Gulf Coast estuaries. Sampling sites were located in areas not influenced by known point sources of inputs.

Oysters have been employed as sentinel organisms because they are cosmopolitan, sedentary, known to bioaccumulate contaminants of interest, able to provide an assessment of bioavailability, not readily capable of metabolizing contaminants, able to survive pollution loading, readily found as locally stable populations, transplantable, and commercially valuable. Oysters are, therefore, excellent biomonitors for contamination in estuarine areas.

The Galveston Bay system is one of the largest and most economically important estuaries along the Texas Gulf Coast. This area has been the recipient of various contaminant inputs because of an aggressively growing urban and industrial region. Houston, Deer Park, Baytown, Texas City and Galveston, surrounding Galveston Bay to the north and west, are some of the most heavily industrialized areas in Texas. Hundreds of industrial plants bordering the Galveston Bay estuarine system, including petrochemical complexes and refineries, as well as runoff, are likely to introduce significant amounts of organic contaminants into the bay. In general, ecological studies have suggested that the waters of Galveston Bay contained contaminants in sublethal amounts which caused stress to organisms resulting in significant changes in the estuarine community structure.

Samples were collected at six locations in Galveston Bay (Fig. 1). Sampling was conducted each winter and began January of 1986 at four sites (15-18), and in December of 1987 at two other sites (58-59). Additional samples were collected at some of these sites to provide information on seasonal trends in contaminant concentrations. Sediments (top 1 cm) and oysters (20) were collected at three stations at each site and analyzed for polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated pesticides (e.g DDT, chlordane) and tributyltin. All sample analyses were performed using Standard Operating Procedures to provide high quality, precise, accurate and reproducible data. Data quality was further assured by participation in NOAA/NIST intercalibration exercises. This allows for direct comparison of NS&T Gulf Coast data with NS&T data for the East and West Coasts.

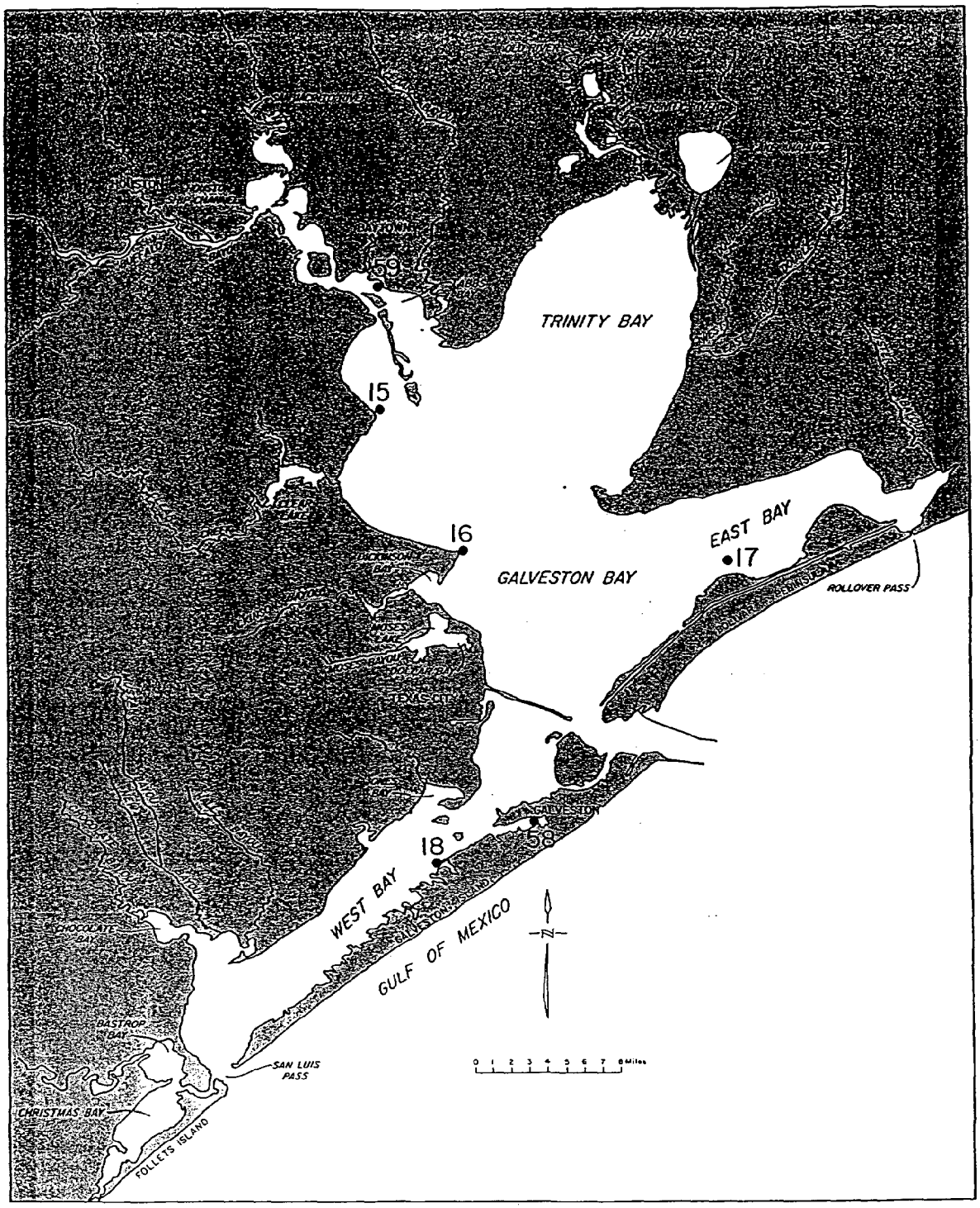


Figure 1. Galveston Bay sampling sites included the Ship Channel (59), Yacht Club (15), Todd's Dump (16), Hanna Reef (17), Offatt's Bayou (58) and Confederate Reef (18).

Total PAH average concentrations ranged from 54 to 2400 ng/g. The higher concentrations were measured in oysters from the upper portion of Galveston Bay (i.e., stations 15 and 59) and near the City of Galveston (i.e., stations 18 and 58). Oyster samples from areas farther away from urban centers (i.e., stations 16 and 17) had average concentrations one to two orders of magnitude lower. In general, these concentrations are in good agreement with those previously encountered during temporal studies in Galveston Bay. Two PAHs, pyrene and fluoranthene, generally accounted for >25% of the total PAHs measured. The predominance of these compounds would suggest that the major source of PAHs in the Galveston Bay area is combustion products.

Average total PCB and DDT concentrations in Galveston Bay oysters were in the 48-1100 and 12-240 ng/g ranges, respectively. Most of the DDT residue is present as metabolites, DDE and DDD. In general, less than 10% of the total contaminant load in oysters is the parent compound, DDT. Samples from stations 15 and 59 were the most contaminated, while oysters from Station 17 had the lowest residue concentrations. These concentrations agree with the ranges reported earlier for Galveston Bay bivalves.

Contaminant concentration patterns were similar for most contaminants. The upper bay sites (15, 59) had higher concentrations than the mid-bay sites (16, 17) for DDT, PAH, PCB and butyltins. Sites from the lower bay (18, 58) had intermediate concentrations. This most likely results from proximity to large urban areas and runoff inputs. The lower contaminant loading in the mid-bay region probably results from dilution effects. The concentrations found in Galveston Bay are similar to the range found throughout the Gulf of Mexico for the NS&T Program. The concentrations in the upper bay are above average for the Gulf of Mexico, mid-bay concentrations are below, and lower bay concentrations are close to the average Gulf of Mexico concentrations. Most of the sites show no consistent temporal trend for the organic contaminants. However, there is a general decrease in concentrations over time at Station 15 for PAH, PCB and DDT. Sample collections at other times of the year indicate some seasonal variability of contamination concentrations.

Reprint 4

Toxic Contamination of Aquatic Organisms in Galveston Bay

James M. Brooks, Terry L. Wade, Bobby J. Presley, José L. Sericano, Thomas J. McDonald, Thomas J. Jackson, Dan L. Wilkinson, and Tamara F. Davis

Toxic Contamination of Aquatic Organisms in Galveston Bay

James M. Brooks, Terry L. Wade, Bobby J. Presley, José L. Sericano, Thomas J. McDonald, Thomas J. Jackson, Dan L. Wilkinson and Tamara F. Davis
Geochemical and Environmental Research Group, Texas A&M University

Little information regarding historical trends and concentrations of heavy metals, hydrocarbons, pesticides and PCBs in aquatic organisms in Galveston Bay has been available to guide decision makers and regulators. Each year millions of pounds of fish and shellfish are caught by commercial and sport fishermen in Galveston Bay and consumed as nutritional seafood. However, little or no testing of edible tissues for toxic contamination by heavy metals, hydrocarbons, pesticides and PCBs has been conducted to assure public health and safety. For this reason, the Galveston Bay National Estuary Program (GBNEP), funded by the U.S. Environmental Protection Agency (EPA) and the State of Texas, undertook this study to characterize contamination in selected aquatic organisms in Galveston Bay.

The sampling design called for the analysis of trace contaminants in five species from four sites in Galveston Bay. Five species of marine organisms were targeted for collection and analyzed as follows: two macroinvertebrates, *Crassostrea virginica*, the oyster, and *Callinectes sapidus*, the blue crab; and three vertebrate marine fishes, *Cynoscion nebulosus*, the spotted sea trout, *Pogonias cromis*, the black drum, and *Paralichthys lethostigma*, the southern flounder. The goal of the program was to collect ten specimens of each target organism of legal market size from each collection site. Standard fisheries data were recorded for all collections. The collection sites for these target species (Fig. 1) were Morgan's Point, at the mouth of the Galveston Ship Channel, Eagle Point off San Leon, Carancahua Reef in West Bay, and Hanna Reef in East Bay.

Four samplings of aquatic organisms have been undertaken for the GBNEP. The first sampling May 23-25 collected oyster and crab samples; however, trawling for fish was not very successful as a result of low salinity water due to Trinity River flooding. A second sampling was undertaken June 6-8 that involved gill netting at the four sites. This sampling had some success in collecting black drum, sea catfish (*Arius felis*), spotted sea trout and southern flounder from some of the sites, although not in sufficient quantities for most analyses. Most fish samples were collected from a sampling from July 30 to August 3 after the bay had returned to a somewhat normal salinity regime. However, late July sampling was complicated by the Apex Barge oil spill that occurred on July 28. Because of this spill, few fish were collected near Eagle Point (close to the oil spill site). A final sampling trip on September 4-6 completed the remaining sampling at Eagle Point.

The analytical program called for the analyses of ten individual specimens of the five target organisms from each site (200 edible muscle tissue samples). Fifty liver samples were composited for analyses from the approximately 120 fishes. Trace contaminants measured included heavy metals, polynuclear aromatic hydrocarbons (PAHs), pesticides and PCBs and a GC-MS scan for other EPA

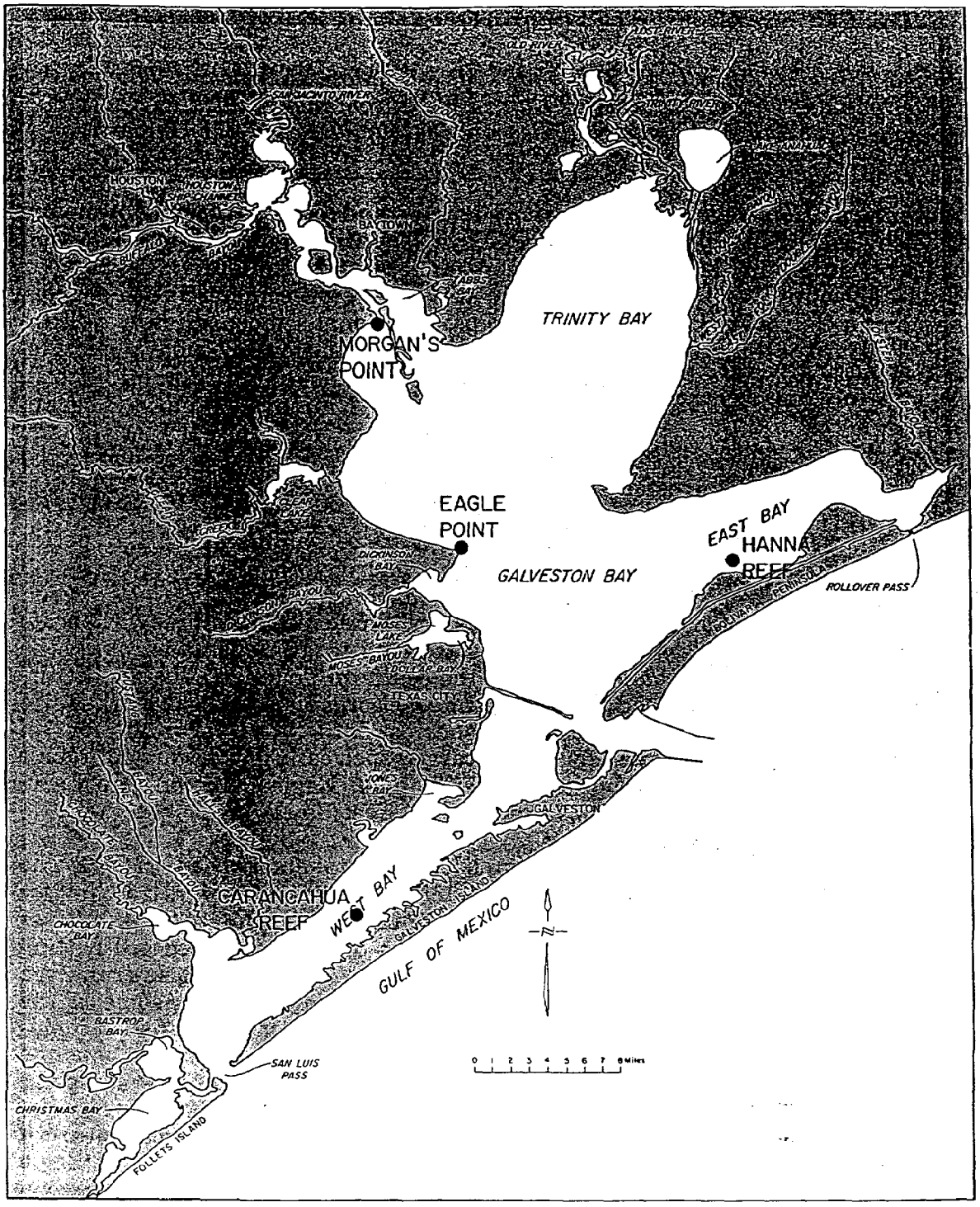


Figure 1. Collection sites for tissue samples.

organic priority pollutants. Trace elements of interest in this study were those on the EPA Priority Pollutant List (PPL) which included: arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), and zinc (Zn). PAHs determined by GC/MS/SIMs included 39 two- to five-ring aromatics and selected alkylated homologs. Pesticides and PCBs were determined by gas chromatography with election capture detection (ECD). Selected chlorinated pesticides (aldrin, chlordane, dieldrin, endrin, heptachlor, BHC, heptachlor epoxide, hexachlorobenzene, lindane, mirex, trans-nonachlor, toxaphene, DDTs, DDDs and DDEs) and individual PCB congeners were quantitated. Analytical methods for trace organic analyses followed those of the NOAA National Status and Trends Mussel Watch Program.

None of the average concentrations of trace metals or trace organic contaminants in fish tissue, oysters, or crabs collected in this study pose a risk to human health associated with consumption of seafood based on the U.S. EPA (1989) guidance manual for assessing human health risks for chemically contaminated fish and shellfish. In general, trace contaminants were higher in oyster and crab tissues than fish tissue. This was especially true for trace organics and certain trace metals such as zinc, lead, nickel, copper, cadmium and silver. Mercury showed the opposite trend with higher concentrations in fish tissue. Most PAHs in Galveston Bay seem to originate from combustion sources (atmospheric deposition or runoff) and not from petroleum inputs based on the distribution of PAHs and their alkylated homologs. The chlorinated hydrocarbons were represented by low levels of DDT and its metabolites (DDD and DDE). As expected, higher contaminant levels were generally found in the upper portion of Galveston Bay (Morgan's Point) near the Houston Ship Channel.

Reprint 5

Transplanted Oysters as Sentinel Organisms in Monitoring Studies

José L. Sericano, Terry L. Wade, and James M. Brooks

Transplanted Oysters as Sentinel Organisms in Monitoring Studies

José L. Sericano, Terry L. Wade and James M. Brooks
Geochemical and Environmental Research Group, Texas A&M University

Coastal marine environment contamination by a number of organic compounds of synthetic or natural origin has received increasing attention over the last several years. Biomonitoring of these compounds in the aquatic environment is well established and bivalves are generally preferred for this purpose. The rationale for the "Mussel Watch" approach using different bivalves, e.g., mussel, oysters and/or clams, has been summarized by different authors and its concept has been applied to many monitoring programs during the last decade.

The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Program, for example, is designed to monitor the current status and long-term effects of selected organic and inorganic contaminants of environmental concern, i.e., polynuclear aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyls (PCBs), and trace metals. Concentrations of these contaminants in bivalves are measured along the coasts of the U.S.A. over several years. During the first five years of this program (1986-1990) the objective was to sample all the locations prescribed by NOAA; however, this goal was compromised by locations depleted of living oysters because of diseases, predators, excessive freshwater runoff, harvesting or dredge material burying entire reefs. Therefore, in some instances, it was not possible to obtain samples. At the end of the first five years of the NS&T program, nearly 20% of the original locations presented some of the above mentioned sampling problems that left the data base with missing values. Transplantation of bivalves to areas where indigenous individuals were not originally present or have been lost because of natural or man-induced actions could be a potentially useful tool in monitoring environmental pollution.

The present study was designed to examine the uptake and depuration of selected organic contaminants, PAHs and PCBs in oysters (*Crassostrea virginica*) through transplantation experiments in two locations in Galveston Bay, Texas.

Approximately 250 oysters of similar dimensions (e.g., 6-8 cm) were collected from a relatively uncontaminated area in Galveston Bay, Hanna Reef, and transplanted in 24x70 cm net bags, containing 25-30 individuals per bag, to a new location near the Houston Ship Channel (HSC) in the upper part of the bay. Composite samples of 20 transplanted and 15 indigenous oysters were collected at zero, three, seven, 17, 30, and 48 days during the first phase of the transplantation experiment. The remaining Hanna Reef oysters were then back-transplanted to their original location in Galveston Bay. At the same time, approximately 150 indigenous oysters from the HSC site were also transplanted to the Hanna Reef area. Composite samples of 20 oysters from each population were collected at three, six, 18, 30, and 50 days after transplantation.

The concentrations of most organic contaminants in oysters transplanted from Hanna Reef to the HSC increased dramatically during the seven-week exposure

period. Comparatively, concentrations of individual PAHs and PCBs in indigenous oysters during the first phase of this experiment were fairly constant. The analyte concentrations in native oysters represent the time-integrated contaminant concentrations available to the oysters in solution, adsorbed onto particles and incorporated into food.

Initial concentrations of total PAHs in transplanted oysters increased from 290 ng/g to a final value of 4360 ng/g. Two- and three-ring PAHs were detected in low concentrations in transplanted and indigenous oysters. Four- and five-ring compounds were accumulated to the highest concentrations in Hanna Reef oysters. By the end of the first 48 days, transplanted oysters accumulated these PAHs to levels that were not statistically differentiable from the concentrations measured in native individuals. The PAHs accumulated to the highest concentrations were: pyrene > fluoranthene > chrysene > benzo(e)pyrene > benzo(b)anthracene.

Hanna Reef and HSC oysters showed statistically significant depuration ($p < 0.05$) of four- and five-ring PAHs after relocation to the Hanna Reef area. Depurations of these aromatic compounds by both groups of oysters were approximately exponential. The half-lives ranged from 10.4 and 12.4 days for pyrene to 25.6 and 38.5 days for fluoranthene in Hanna Reef and HSC oysters, respectively. Most of the values were, however, between ten and 16 days.

PCB concentrations in transplanted oysters increased from 30 ng/g to 850 ng/g after the 48-days exposure period. Pentachlorobiphenyls were the compounds accumulated to the highest concentrations in transplanted and native oysters. In comparison, practically no octa-, nona- or decachlorobiphenyls were detected in either oyster group. Unlike the PAHs, not all the PCB homologs measured in transplanted oysters reached the concentration encountered in indigenous individuals by the end of the first phase of this experiment. While there were no statistically significant differences in the tri- and tetrachlorobiphenyl concentrations measured in transplanted and native oysters, significant differences were observed in the total concentrations of penta- and hexachlorobiphenyls. It is evident that a longer exposure period is needed for the higher molecular weight PCB to reach a steady state concentration.

Hanna Reef and HSC oysters showed statistically significant depuration ($p < 0.05$) of low molecular weight PCBs when relocated to the Hanna Reef area. Originally uncontaminated oysters depurated PCBs at a faster rate than chronically contaminated oysters. The clearance rates of high molecular weight PCBs were significantly slower in both oyster populations Biological half-lives for these PCBs in Hanna Reef and HSC oysters ranged from 21 to 129 days and from 20 days to > year, respectively.

Transplanted oysters can be considered valuable bioindicators of environmental contamination by PAHs and PCBs in areas lacking indigenous oysters. However, in order to avoid misleading interpretations of environmental data collected using transplanted bivalves, it is imperative to understand that some trace organic compounds need extremely long time, i.e., several months, to reach equilibrium concentrations.

Reprint 6

The Effects of the Apex Barge Oil Spill on the Fish of Galveston Bay

Susanne J. McDonald, James M. Brooks, Dan Wilkinson, Terry L. Wade, and Thomas J. McDonald

The Effects of the Apex Barge Oil Spill on the Fish of Galveston Bay

Susanne J. McDonald, James M. Brooks, Dan Wilkinson,
Terry L. Wade, and Thomas J. McDonald
Geochemical and Environmental Research Group, Texas A&M University

On July 28, 1990 the Greek tanker, *Shinoussa*, collided with three barges in the Houston Ship Channel in Galveston Bay, Texas. Over 700,000 gallons of petroleum product were released into the bay from one of the Apex barges. The spilled petroleum was a processed product known as a vacuum reformate that contained unusually high concentrations of the toxic polynuclear aromatic hydrocarbon (PAH), benzo[a]pyrene (BaP). The purpose of this study was to assess the effects of the spilled petroleum on the fish of Galveston Bay and to compare results obtained from more typical monitoring methods (i.e., PAH tissue residue concentrations) and a recently developed technique for detecting PAH metabolites in fish bile. Measuring the concentrations of biliary PAH metabolites in fish is a sensitive method that can provide an improved estimation of PAH exposure, early indications of habitat deterioration, and a clear association between pollutant source and resultant exposure. Data of this nature is beneficial for informed management and regulatory decisions.

Field crews were on Galveston Bay collecting fish as part of the Galveston Bay National Estuary Program (GBNEP) monitoring study the week following the oil spill. One of the designated stations in this study, Todd's Dump (or Eagle Point), is located within two miles of the Apex barge oil spill and was sampled on August 3, 1990, one week after the spill. Fish were collected using gill nets at the north/northwest end of Redfish Island and over the oyster reef at Todd's Dump. A prominent oil slick was observed in waters surrounding Redfish Island; whereas, no obvious slick was evident in waters over the oyster reef. Additionally, follow up studies resampled the Todd's Dump area for fish approximately four and sixteen weeks after the spill. The fish captured were analyzed for PAH metabolites in bile and PAH residue in liver and muscle tissues. The PAH metabolites analyzed were naphthalenes, phenanthrenes, and BaPs.

Fish rapidly metabolize lipophillic PAH to more polar and excretable metabolites. A number of polar metabolites formed by the enzymatic transformation of PAH in fish livers are excreted into bile and urine. Biliary PAH metabolites were analyzed using a non-radiometric technique employing HPLC and fluorescence detection. The advantages of this technique include the ease with which samples are collected and stored, the minimal sample preparation required, its sensitivity, its low cost, and that it is an *in vivo* measurement. Field studies have documented elevated concentrations of PAH metabolites in the bile of fish collected near hydrocarbon contaminated sediments and downstream from an oil spill. An increased incidence of idiopathic hepatic lesions and reduced ovarian maturation has been correlated with high concentrations of biliary PAH metabolites in fish.

The analysis of fish collected near Redfish Island, one week after the spill, revealed the highest biliary concentrations of PAH metabolites ever reported for fish. The mean concentration of naphthalene, phenanthrene and benzo[a]pyrene

metabolites was 4,200,000, 1,900,000, and 11,000 ng/g wet weight, respectively. Fish captured over the oyster reef, in waters that were not obviously oiled, had metabolite concentrations of 1,100,000 (naphthalene), 540,000 (phenanthrene), and 3,900 (BaP) ng/g. The high concentration of BaP metabolites is of particular concern since many of these compounds are highly carcinogenic and reflect the high concentration of BaP in the spilled petroleum. The mean biliary concentrations of PAH metabolites in fish captured four weeks after the spill were lower than those observed one week after the spill, but were still elevated (naphthalene = 900,000, phenanthrene = 290,000, and BaP = 2400 ng/g); whereas, fish collected sixteen weeks after the spill had significantly lower concentrations of PAH metabolites in their bile (naphthalene = 240,000, phenanthrene = 70,000, and BaP = 630 ng/g).

In contrast to results of the bilary analysis, the concentration of PAH residues in the tissues of fish captured one week after the spill are low to nondetected. Significant concentrations of PAH are seldom detected in fish, even when the adjacent environment contains high concentrations of PAH, because fish rapidly metabolize PAH to derivatives not detected by routine analytical techniques for monitoring hydrocarbon exposure. Evaluating the effects of the Apex oil spill only on the concentration of PAH residue in fish tissues would suggest no significant evidence of exposure. However, metabolite data indicates that the fish near Todd's Dump were exposed to high concentrations of PAH.

Preprint 1

Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990)

Thomas J. Jackson, Terry L. Wade, Thosmas J. McDonald, Dan L. Wilkinson, and James M. Brooks

Manuscript 00646

Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990)

Thomas J. Jackson, Terry L. Wade, Thomas J. McDonald, Dan L. Wilkinson and James M. Brooks

Geochemical and Environmental Research Group,
College of Geosciences and Maritime Studies,
Texas A & M University,
College Station, Texas, U.S.A. 77845

ABSTRACT

Polynuclear aromatic hydrocarbon (PAH) contaminant concentrations in 870 composite oyster samples from coastal and estuarine areas of the Gulf of Mexico analyzed as part of National Oceanic and Atmospheric Administration's (NOAA's) National Status and Trends (NS&T) Mussel Watch Program exhibit a lognormal distribution. There are two major populations in the data. The cumulative frequency function was used to deconvolute the data distribution into two probability density functions and calculate summary statistics for each population. The first population consists of sites with lower PAH concentration probably due to background contamination (i.e., stormwater runoff, atmospheric deposition). The second population are sites with higher concentrations of PAHs associated with local point sources of PAH input (i.e., small oil spills, etc.). The temporal pattern for the mean concentration of the populations from the Gulf of Mexico is consistent with large-scale climatic factors such as the El Niño cycles which affect the precipitation regime.

INTRODUCTION

Oysters and other bivalve molluscs have been used for monitoring contaminants in the environment (Farrington, *et al.*, 1983). Oysters are sentinel organisms which concentrate contaminants from the marine environment, yet do not readily metabolize contaminants such as polynuclear aromatic hydrocarbons (PAHs) (Farrington and Quinn, 1973). PAHs enter the near-coastal environment through a number of mechanisms (e.g. runoff, discharge of industrial waste or sewage, natural or industrial combustion processes, natural oil seepages, and spills of petroleum or petroleum products).

The contaminants found in oysters reflect the current contaminant burden of an ecosystem. The concentration of a contaminant in an oyster is the difference between uptake and excretion of that contaminant. Galveston Bay oysters transplanted from a "high" level site to a "low" level site and *vice versa* come to a new equilibrium concentration, for trace organic contaminants such as PAHs, within approximately one month (Sericano and Wade, unpublished data).

To assess the spatial and temporal variation of contaminant levels of coastal and estuarine environments, the National Oceanic and Atmospheric Administration (NOAA) instituted the National Status and Trends (NS&T) Mussel Watch Program under its Program for Marine Environmental

Quality (O'Connor, 1990). The sample sites were selected to characterize the overall concentration of contaminants in coastal and estuarine ecosystems away from known point-sources of contamination.

The focus of this paper is to examine the distribution of the PAH contaminant concentrations in oysters collected from the Gulf of Mexico as part of NOAA's NS&T Mussel Watch Program, and determine the environmental factors controlling the concentration of PAHs.

METHODS

Sample Collection

Oysters (*Crassostrea virginica*) were collected from three stations at each site, during the winter of each year (1986 - 1990). The number of sites per year varied from 48 to 68. In some years not all sites had three stations due to the low abundance of oysters at a specific site (Table 1). Sample sites give coverage of the Gulf of Mexico coastal and estuarine areas from southern-most Texas to southern-most Florida (Figure 1). Individual stations at each site are generally from 100 to 1,000 meters apart. An analysis at each station represents a composite of twenty individual oysters. Each year, the field sampling returned to as many sites as possible. In some instances it was necessary to relocate or abandon an established oyster site

due to lack of suitable size bivalves (Wilkinson, *et al.*, 1991). The locations and designators for the oyster sites are found in Wilkinson, *et al.* (1991), Sericano *et al.* (1990) and Wade *et al.* (1990).

Tissue Extraction

The tissue extraction used was adapted from a method developed by MacLeod, *et al.* (1985). Approximately 15 grams of wet tissue were used for the PAH analysis. After the addition of internal standards (surrogates) and 50 grams of anhydrous Na_2SO_4, the tissue was extracted three times with dichloromethane using a tissuemizer. A 20 ml sample was removed from the total solvent volume and concentrated to one ml for lipid percentage determination. The 280 ml of remaining solvent was concentrated to approximately 20 ml in a flat-bottomed flask equipped with a three-ball Synder column condenser. The tissue extract was then transferred to a Kuderna-Danish tubes heated in a water bath (60°C) to concentrate the extracts to a final volume of two milliliters. During concentration, the dichloromethane was exchanged for hexane.

The tissue extracts were fractionated by alumina:silica (80-100 mesh) open column chromatography. The silica gel was activated at 170°C for 12 hours and partially deactivated with 3% distilled water (v/w). Twenty grams of silica gel were slurry-packed in dichloromethane over ten

grams of alumina. Alumina was activated at 400°C for four hours and partially deactivated with 1% distilled water (v/w). The dichloromethane was replaced with pentane by elution. The extract was then applied to the top of the column. The extract was sequentially eluted from the column with 50 ml of pentane (aliphatic fraction) and 200 ml of 1:1 pentane:dichloromethane (aromatic fraction). The aromatic fraction was further purified by HPLC to remove the lipids. The lipids were removed by size exclusion using dichloromethane as an isocratic mobile phase (7 ml/min) and two 22.5 x 250 mm Phenogel 100 columns (Krahn, *et al.*, 1988). The purified aromatic fraction was collected from 1.5 minutes prior to the elution of 4,4'-dibromofluorobiphenyl to 2 minutes after the elution of perylene. The retention times of the two marker peaks were checked prior to the beginning and at the end of a set of ten samples. The purified aromatic fraction was concentrated to 1 ml using Kuderna-Danish tubes heated in a water bath at 60°C.

Quality assurance for each set of ten samples included a procedural blank, matrix spike, duplicate, and tissue standard reference material (NIST-SRM 1974) which were carried through the entire analytical scheme. Internal standards (surrogates) were added to the samples prior to extraction and were used for quantitation. The surrogates were d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene. Surrogates were added at a

concentration similar to that expected for the analytes of interest. To monitor the recovery of the surrogates, chromatography internal standards d_{10}-fluorene and d_{12}-benzo(a)pyrene were added just prior to GC-MS analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)

PAHs were separated and quantified by GC-MS (HP5980-GC interfaced to a HP5970-MSD). The samples were injected in the splitless mode on to a 30 m x 0.25 mm (0.32 um film thickness) DB-5 fused silica capillary column (J&W Scientific Inc.) at an initial temperature of 60°C and temperature programmed at 12°C/min to 300°C and held at the final temperature for 6 minutes. The mass spectral data were acquired using selected ions for each of the PAH analytes. The GC-MS was calibrated and linearity determined by injection of a standard containing all analytes at five concentrations ranging from 0.01 ng/ul to 1 ng/ul. Sample component concentrations were calculated from the average response factor for each analyte. Analyte identifications were based on correct retention time of the quantitation ion (molecular ion) for the specific analyte and confirmed by the ratio of quantitation ion to confirmation ion.

Calibration check samples were run with each set of samples (beginning, middle, and end), with no more than six hours between calibration checks. The calibration check must maintain an average response factor within 10% for all

analytes, with no one analyte greater than ±25% of the known concentration. A laboratory reference sample (oil spiked solution) was also analyzed with each set of samples to confirm GC-MS system performance and calibration.

RESULTS and DISCUSSION

Oyster site Variations

During the first five years of this study a total of 870 composited oyster samples have been analyzed for PAHs. The tPAH (total NS&T PAHs) is the sum of the eighteen aromatic hydrocarbon analytes, as measured in Year I, with concentrations greater than 20 ng/g dry weight (Table 2); this was the reporting limit for Year I data (Wade, *et al.*, 1988). The median PAH concentration at a site is used as a measure of the best indicator of the concentration. The median is a more stable (or "resistant") estimator of the typical value than the mean for data which may contain outliers (Hensel, 1990).

The data in Table 3 presents the spatial and temporal variation for the median tPAH concentration in the coastal and estuarine areas of the Gulf of Mexico. The sites are separated into Bay groups (Wilson, *et al.*, 1992) for data comparison. The variability for each Bay group is the standard deviation as computed from the interquatrile range (IQR) for the five years of data (Hensel, 1990). In Texas,

Corpus Christi (CCBH, CCNB, CCIC & ABHI) and Galveston Bays (GBCR, GBOB, GBTD, GBYC, GBSC & GBHR) are near industrial and population centers and exhibit high median concentrations of tPAH and large variability in concentration compared to Matagorda (ESBD, MBGP, MBLR, MBCB, MBTP & MBDI) and Aransas Bays (ABLR, CBCR & MBAR) which exhibit low median concentrations of tPAH and small variability in concentration. The highest median tPAH concentration for a Bay Group in Texas is the Brazos River (BRCL & BRFS), which carries the runoff from agriculture and wastewater discharge from industrial point-sources (NOAA, 1985). For the entire coastal and estuarine area of the Gulf of Mexico (Table 3), the highest median tPAH concentration for a Bay Group is near Panama City, Florida (PCLO, PCMP & SAWB), which is close to a paper mill (NOAA, 1985; Wilkinson *et al.*, 1991).

There are fifteen sites (LMSB, ABLR, CBCR, MBAR, SAPP, ESSP, ESBD, MBGP, MBCB, MBTP, CLCL, LBMP, TBCB, CBBI & RBHC) with low concentration of tPAH (< 100 ng/g) and little variation in the observed values (e.g., Figure 2). There are also six sites (GBSC, BBMB, MSBB, CBJB, PCMP & SAWB), of the seventy-eight different sites, where high concentrations of tPAH (> 1000 ng/g) are observed. Four sites (CCIC, PBPH, PBIB & PCMP) exhibited a decrease in the tPAH each year during the first five years of this study. Many sites exhibited a cyclic variation with time. At Choctawhatchee

Bay off Santa Rosa (CBSR, Figure 3), the order of magnitude increase in concentration of tPAH in Years II and III is probably due to relocation of the collection site to an area containing wood pilings, which if treated with creosote are a source of PAHs. The decrease in Years IV and V probably reflects relocation of the collection stations to an oyster reef away from wood pilings. Due to prolonged freshwater conditions in San Antonio Bay during 1988 and 1989 (Years III and IV), the oyster reefs experienced a die-off resulting in no oysters being taken from SAPP, SAMP and ESSP.

Cumulative Frequency Model

Bar Graphs (Wade, *et al.*, 1990), or crossplots (Wade and Sericano, 1989) of data comparing one year's data versus another have been used to display the general trend for tPAH data (Wade and Sericano, 1989; Wade, *et al.*, 1990; Wade, *et al.*, 1991). These data presentations easily visualize the variation in concentration for a particular site. In this report the cumulative frequency function is used to examine the heterogeneous distribution of PAHs in Gulf of Mexico oysters (Mackay and Paterson, 1984). This approach has the advantage of examining the Gulf of Mexico as a single environmental system, determining the percentage of sites exposed to a particular threshold concentration, and providing information for environmental evaluation.

The distribution of the PAH data in Table 3 is best described by a lognormal distribution; i.e. the distribution of data is skewed to low concentrations and has a fraction which extends to high concentrations (Figure 4). O'Connor (1990) used the lognormal distribution, typical of environmental data, to define "high" concentrations as those whose logarithmic value is more than the mean plus one standard deviation of the logarithms for all concentrations. The tPAH data in Figure 4 is further skewed in that analytes with concentrations less than 20 ng/g are not included in the sum of eighteen 2 - 5 ring aromatic hydrocarbon analytes in Table 2, i.e., the data has been censored. For Years I - III, only censored data was available, whereas for Years IV and V both censored and uncensored data was available. A regression analysis of the censored (tPAH) data versus uncensored data for the sum of all analytes (T-PAH) in Table 2 from Years IV and V yields the best fit line as $y = 153.0 + 0.9834\ x$ (r^2=0.9989); where y = uncensored data, and x = censored data. Using the best fit line from the Year IV and V data, the censored data for the cumulative frequency data was corrected to be the same as the uncensored cumulative frequency data.

Distribution functions are useful measures of environmental quality data in that changes with time can be ascertained without being influenced by "outliers". For the cumulative distribution plot, the data is sorted from the

lowest value to the highest, similar to rank transformation (Conover and Iman, 1981). Each observation is 1/n fraction of the data set, where n is the number of samples in the data set. The sum of the fraction of samples less than the concentration is plotted against the concentration. From this plot the median can be determined, since it is defined as the 50th percentile. The interquatrile range (IQR) is used as a measure of variability. The IQR is the 75th percentile minus the 25th percentile and equals 1.35 times the standard deviation for a normal distribution (Hensel, 1990).

To begin the examination of the distribution of the PAH concentration data, the logarithm of the sum of all PAH analytes (T-PAH) for Year V data was plotted as a cumulative frequency distribution. The 50th percentile was 250 ppb and the standard deviation as determined from the IRQ was 218. The log of the data versus fraction of the samples was plotted and compared with a lognormal distribution (Figure 5). The shape of the cumulative frequency curve (i.e., the positive deviation from the lognormal model) for the T-PAH data suggests two overlapping lognormal distributions. Making the assumption that there is a 2.5% overlap for the two distributions, the mean and standard deviation were computed for each data set, or population (Table 4). The cumulative frequency distribution from the two population model data compare well with the actual T-PAH

data (Figure 6). Other increments of overlap were computed, but did not compare as well with the actual data for Year V.

The implication of the two populations in the data is that there are two primary mechanisms accounting for the distribution of T-PAH concentration in the Year V data. The sites with lower concentration PAHs are probably due to low level background inputs from stormwater runoff, atmospheric deposition and sewage effluents, etc. (NOAA, 1985). The sites with higher concentration PAHs are probably due to local point-sources of PAH contamination (i.e., small spills). From the lognormal cumulative frequency function two probability density functions were derived, the relative proportion of the two populations were estimated to be 0.9 for population one and 0.25 for population two. Comparison of the cumulative frequency distribution derived from the sum of the two probability density functions, in the above proportions, with the actual data for the cumulative frequency distribution (Figure 7) indicates a good correlation.

Since historical NS&T data (Table 3) is censored data (Wade, *et al.*, 1988; Wade and Sericano, 1989; Wade, *et al.*, 1990), the cumulative frequency distribution of this censored (tPAH) data was corrected using the best-fit-line from the data for Years IV and V. Data below the reporting limit were extrapolated (Hensel, 1990; Mackay and Paterson, 1984). The summary statistics for the corrected data using

the two population model for Years I to Year V data (Table 5) were calculated using the data from 0-80% for the original cumulative frequency distribution for Population 1 and from 77.5-100% of the original cumulative frequency distribution for population 2 (Table 6).

The summary statistics for the first five years of measuring PAH contaminants in the Gulf of Mexico for NOAA's NS&T Mussel Watch Program (Table 5) show variation in the means for both populations, indicating temporal change in the total Gulf of Mexico data, with the highest values found in Years III and IV. The higher mean concentrations of PAHs in Years III and IV and the lower abundance in Years I, II and V pattern is probably related to large-scale climatic factors such as the El Niño cycles (Philander, 1989) which affects the precipitation regime (Wilson, *et al.*, 1992). Examination of the PAH data for individual sites, as discussed above, does not show this pattern.

The cumulative frequency data for Years I to V gives the percentage of sites whose PAH concentration is less than a particular concentration (Table 6). As an example, using 1,000 ppb as an arbitrary concentration, 89% of the sites for Years I and II are less than this concentration, while Year III had 80%, Year IV 83% and Year V had 87%. Alternatively, the cumulative frequency data can be used to calculate the percentage of sites exposed to a concentration in excess of a particular threshold.

The cumulative frequency distribution was used in this study as an environmental evaluation tool to examine the heterogeneous distribution of total PAH contaminants in Gulf of Mexico oysters from coastal and estuarine areas collected during the winters of 1986 - 1990. The PAH concentration exhibits a lognormal distribution with two major populations in the data for each year. The two populations were deconvoluted into probability density functions and summary statistics for each population were calculated. The lower PAH concentrations are probably related to chronic inputs. Many of these low PAH concentration sites show little variability from year to year, supporting the contention that the PAH contamination is on a continual basis. The higher concentration PAHs are probably associated with local point-sources of PAH contamination or spills. Most of the high concentration sites (> 1000 ng/g dry tissue) show large variability from year to year, supporting the contention that PAH contamination for these sites is on an episodic basis. In addition, 20% of Gulf of Mexico sites in Year III were exposed to a PAH threshold concentration of greater than 1000 ng/g of dry oyster tissue. Whereas, in Years I and II only 11% of the Gulf of Mexico sites had concentrations greater than 1000 ng/g of total NS&T PAHs. The changes in the mean concentration of the two populations between years display a cyclic pattern which is probably due to large-scale climatic factors such as the El Niño cycles which affects the precipitation regime (Wilson, *et al.*,

1992). The cyclic pattern was obtained by examining the Gulf of Mexico as a single heterogeneous system, since the PAH concentration data for individual sites does not clearly show this pattern.

ACKNOWLEGEMENTS

Funding for this research was supported by the National Oceanic and Atmospheric Administration, contract number 50-DGNC-5-00262 (National Status and Trends Mussel Watch Program), through the Texas A & M Research Foundation, Texas A & M University.

REFERENCES

Conover, W.J. & Iman, R.L. (1981). Rank Transformations as a Bridge between Parametric and Nonparametric Statistics. *The American Statistician*, **35**, 124-129.

Farrington, J.W. & Quinn, J.G., (1973). Petroleum hydrocarbons in Narragansett Bay. I. Survey of hydrocarbons in sediments and clams (*Mercenaria mercenaria*). *Estuarine and Coastal Mar. Sci.*, **1**, 71-79.

Farrington, J.W., Goldberg, E.D., Risebrough, R.W., Martin, J.H. & Bowen, V.T. (1983). US 'Mussel Watch' 1976-1978: An overview of the trace metal, DDE, PCB, hydrocarbon and artificial radionuclide data. *Environ. Sci. Technol.*, **17**, 490-6.

Hensel, D.R. (1990). Less than obvious. Statistical treatment of data below the detection limit. *Environ. Sci. Technol.*, **24**, 1766-1774.

Krahn, M.M., Moore, L.K., Bogar, R.G., Wigren, C.A., Chan, S-L. & Brown, D.W. (1988). High-performance liquid chromatography method for isolating organic contaminants from tissue and sediment extracts. *J. Chromatogr.*, **437**, 161-175.

Mackay, D. & Paterson, S. (1984). Spatial concentration distributions. *Environ. Sci. Technol.*, **18**, 207A-214A.

MacLeod, W.D., Brown, D.W., Friedman, A.J., Burrows, D.G., Maynes, O., Pearce, R.W., Wigren, C.A. & Bogar, R.W. (1985). Standard analytical procedures of the NOAA National Analytical Facility 1985-1986. Extractable Toxic Organic Compounds, 2nd Ed. U.S. Department of Commerce, NOAA/NMFS. NOAA Tech. Memo NMFS F/NWC-92.

NOAA (1985). Gulf of Mexico Coastal and Ocean Zones Strategic Assessment: Data Atlas, United States Department of Commerce, National Oceanic and Atmospheric Administration. pp. 4.0-5.32.

O'Connor, T.P. (1990). Coastal Environmental Quality in the United States, 1990. Chemical Contamination in Sediment and Tissues. A Special NOAA 20th Anniversary Report. 34 pp.

Philander, G. (1980). El Niño and La Niña. *American Scientist*, 77, 451-459.

Sericano, J.L., Wade, T.L., Atlas, E.L. & Brooks, J.M. (1990). Historical Perspective on the Environmental Bioavailability of DDT and Its Derivatives to Gulf of Mexico Oysters. *Environ. Sci. Technol.*, 77, 1541-1548.

Wade, T.L., Atlas, E.L., Brooks, J.M., Kennicutt II, M.C., Fox, R.G., Sericano, J.L., Garcia-Romero, B. & Defreitas, D.A. (1988). NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediments and oysters. *Estuaries*, 11, 171-179.

Wade, T.L. & Sericano, J.L. (1989). Trends in Organic Contaminant Distribution in Oysters form the Gulf of Mexico. *Oceans'89 Proceedings*, pp. 585-589.

Wade, T.L., Sericano, J.L., Garcia-Romero, B., Brooks, J.M. & Presley, B.J. (1990). Gulf Coast NOAA National Status & Trends Mussel Watch: The First Four Years. *Proc. Mar. Tech. Soc.*, 274-280.

Wade, T.L., Brooks, J.M., Kennicutt II, M.C., Denoux, G.J. & Jackson, T.J. (1991). Oysters as Biomonitors of Oil in the Ocean. *Proceedings of the 23rd Annual Offshore Technology Conference*, OTC 6529, pp. 275-280.

Wilkinson, D.L., Brooks, J.M. & Fay, R.R. (1991). NOAA Status and Trends: Mussel Watch Program - Field Sampling and Logistics Report - Year VI. GERG Technical Report 91-046, U.S. Department of Commerce, National Oceanic & Atmospheric Administration, Ocean Assessment Division.

Wilson, E.A., Powell, E.N., Wade, T.L., Taylor, R.J. Presley, B.J., and Brooks, J.M. (1992). Spatial and temporal distributions of body burden and disease in Gulf of Mexico oyster populations: The role of local and large-scale climatic controls. *Helgol. Meeresunters.* (in press).

Table 1: National Status and Trends Oysters
Gulf of Mexico Sampling Program - Summary of Sampling

	1986	1987	1988	1989	1990
Year	I	II	III	IV	V
Number of Sites	49	48	65	62	68
Number of Samples	142	144	195	186	203

Table 2: National Status and Trends Oysters
Polynuclear Aromatic Hydrocarbon Analytes

* = Analytes used in tPAH summation

Low Molecular Weight Aromatic Hydrocarbons

* Biphenyl	* Acenaphthene
* Naphthalene	Acenaphthylene
* 1-methylnaphthalene	* Fluorene
* 2-methylnaphthalene	* Phenanthrene
* 2,6-dimethylnaphthalene	* Anthracene
1,6,7-trimethylnaphthalene	* 1-methylphenanthrene

High Molecular Weight Aromatic Hydrocarbons

* Fluoranthene	* Benzo(a)pyrene
* Pyrene	* Benzo(e)pyrene
* Benz(a)anthracene	* Perylene
* Chrysene	* Dibenz[a,h]anthracene
Indeno[1,2,3-cd]pyrene	Benzo(g,h,i)perylene

Table 3a: Total NS&T PAH concentration in Oysters (Texas)
Median concentration in ng/g of tPAH

#	Site Code	V 1990	IV 1989	III 1988	II 1987	I 1986	Bay Group Median
1	LMSB	22	20	30	20	25	
52	LMPI			3380			30 ± 58
78	LMAC	120					
53	CCBH	1530		1600			
2	CCNB	161	264	598	434	45	565 ± 725
3	CCIC	137	430	848		1140	
54	ABHI			1870			
4	ABLR	20	20	20	21	20	
5	CBCR	88		20	20	22	20 ± 1
6	MBAR	20	20	20	20	21	
7	SAPP	26			51	45	
8	SAMP				49	93	25 ± 23
9	ESSP	20			21	20	
10	ESBD	21	70	21			
12	MBGP		20	86	56	20	
11	MBLR	96	348		59	90	45 ± 48
56	MBCB	20		56			
13	MBTP	20	20	56	20	20	
55	MBDI			53			
14	MBEM	201	200	23	22	78	138 ± 119
72	BRCL	761	60				
57	BRFS	955	1670	682			792 ± 792
18	GBCR	370	1170	525	478	1070	
58	GBOB	315	593	543			
16	GBTD	25	44	20	112	149	259 ± 606
15	GBYC	247	132	207	568	1030	
59	GBSC	1290	1350	3100			
17	GBHR	20	119	34	20	31	

Manuscript 00646

Table 3b: Total NS&T PAH concentration in Oysters (Louisiana, Mississippi, Alabama) Median concentration in ng/g of tPAH

#	Site Code	V 1990	IV 1989	III 1988	II 1987	I 1986	Bay Group Median
Louisiana							
19	SLBB	108	154	169	26	247	154 ± 72
20	CLSJ	180	228	102	57	376	220 ± 218
60	CLLC	404	726	20			
21	JHJH	88	72	20	84	43	44 ± 50
22	VBSP	189	31	20	118	79	79 ± 108
24	ABOB	20	28	192	115	32	22 ± 42
25	CLCL	20	54	20	20	20	
26	TBLB	20	49	306	37	20	40 ± 162
27	TBLF	101	50	83	20	25	
61	BBTB			20			
28	BBSD	963	5480	44	25	57	963 ± 1020
29	BBMB	1080	1380	1460	1150	822	
65	MRTP	212	310	1410			391 ± 582
64	MRPL	403	330	695			
31	BSSI	185	71	484	68	177	181 ± 134
30	BSBG	45	202	213	118	265	
32	LBMP	20	84	89	26	20	39 ± 59
62	LBNO			81			
Mississippi							
33	MSPC	103	300	175	319	99	
34	MSBB	1210	893	1500	4310	1600	322 ± 654
35	MSPB	59	306	776	300	246	
Alabama							
36	MBCP	20	90	288	137	31	
66	MBHI	767	554	1110			295 ± 740
79	MBDR	1520					

Table 3c: Total NS&T PAH concentration in Oysters (Florida)
Median concentration in ng/g of tPAH

#	Site Code	V 1990	IV 1989	III 1988	II 1987	I 1986	Bay Group Median
67	PBPH	168	369	842			
37	PBIB		21	204	250	406	**197 ± 198**
80	PBSP	130					
73	CBJB	1680	8590				
39	CBSP	225	355	703	543	428	**429 ± 1140**
38	CBSR	69	21	2540	2470	208	
74	PCLO	98	229				
68	PCMP	1210	2690	4750			**1800 ± 1590**
40	SAWB	1150	2090	1990	1970	11800	
41	APDB	20	24	2800	20	20	**57 ± 530**
42	APCP	269	1110	740	20	109	
75	AESP	33	74				**64 ± 103**
69	SRWP			119			
43	CKBP	20	74	24	68	22	**46 ± 103**
76	TBNP	269	394				
47	TBMK	101	170	20	49	372	
44	TBPB	20	217	286	68	95	
70	TBOT	112	357	212			**126 ± 165**
77	TBKA	252	834				
45	TBHB			552	2150	460	
46	TBCB	20	65	94	22	20	
48	CBBI	20	83	31	43	20	**51 ± 180**
71	CBFM	69	546	272			
49	NBNB	87	203	253	108	228	**72 ± 129**
50	RBHC	20	77	67	20	47	
51	EVFU	47	68	257	20	112	**68 ± 125**

Table 4: Two Population Lognormal Distribution Model						
Year V - T-PAH data (2.5% overlap)						
		Percentile		STD=		STD of
Set	25%	50%	75%	IRQ/1.35	Log-mean	Log-data
1	135	214	320	137	2.3308	0.2783
2	801	1210	1530	544	3.0810	0.2093

Table 5: Two Population Lognormal Distribution Model

Corrected tPAH data - ng/g dry weight

Year	Median Total Data	Population 1 Mean (LOG)	Population 1 STD (LOG)	Population 2 Mean (LOG)	Population 2 STD (LOG)
I	229	197 (2.2945)	108 (0.2298)	1075 (3.0314)	714 (0.2772)
II	208	186 (2.2695)	87 (0.1967)	1150 (3.0599)	1100 (0.3811)
III	345	259 (2.4133)	216 (0.3435)	1910 (3.2808)	1190 (0.2618)
IV	352	269 (2.4298)	174 (0.2500)	1350 (3.1316)	1190 (0.3039)
V	270	212 (2.3263)	131 (0.2639)	1170 (3.0689)	637 (0.2435)

Table 6: NS&T Concentration Distribution Data (Cumulative Frequency)

Corrected tPAH data - ng/g dry weight

	1990 Year V	1989 Year IV	1988 Year III	1987 Year II	1986 Year I
10%	110	171	110	110	110
20%	140	200	153	140	140
30%	164	226	206	162	169
40%	212	269	259	186	197
50%	270	352	345	208	229
60%	318	435	445	258	286
70%	397	519	832	370	378
80%	597	869	1030	480	557
90%	1290	1440	2090	1300	1180
95%	1670	2840	3020	2300	1750
98%	1920	5630	4550	3740	2450

Figure Captions

Figure 1: Location of NS&T Mussel Watch Sites in the Gulf of Mexico (Sericano, *et al.*, 1990).

Figure 2: Total NS&T PAH concentration distribution during the first five years for all three stations; Caillou Lake in Louisiana (Site 25 - clcl).

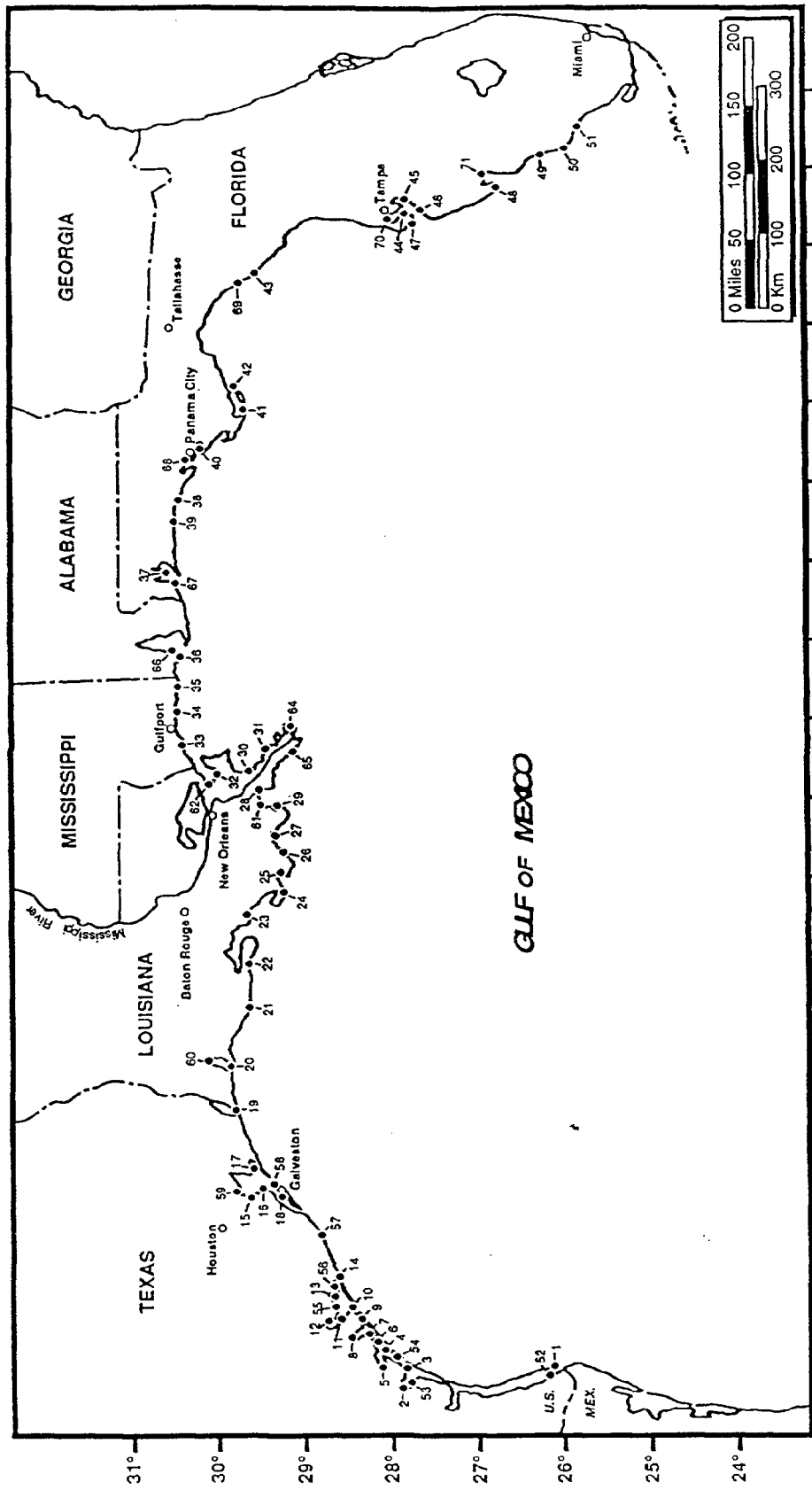
Figure 3: Total NS&T PAH concentration distribution during the first five years for all three stations; Choctawatchee Bay off Santa Rosa (Site 38 - CBSR).

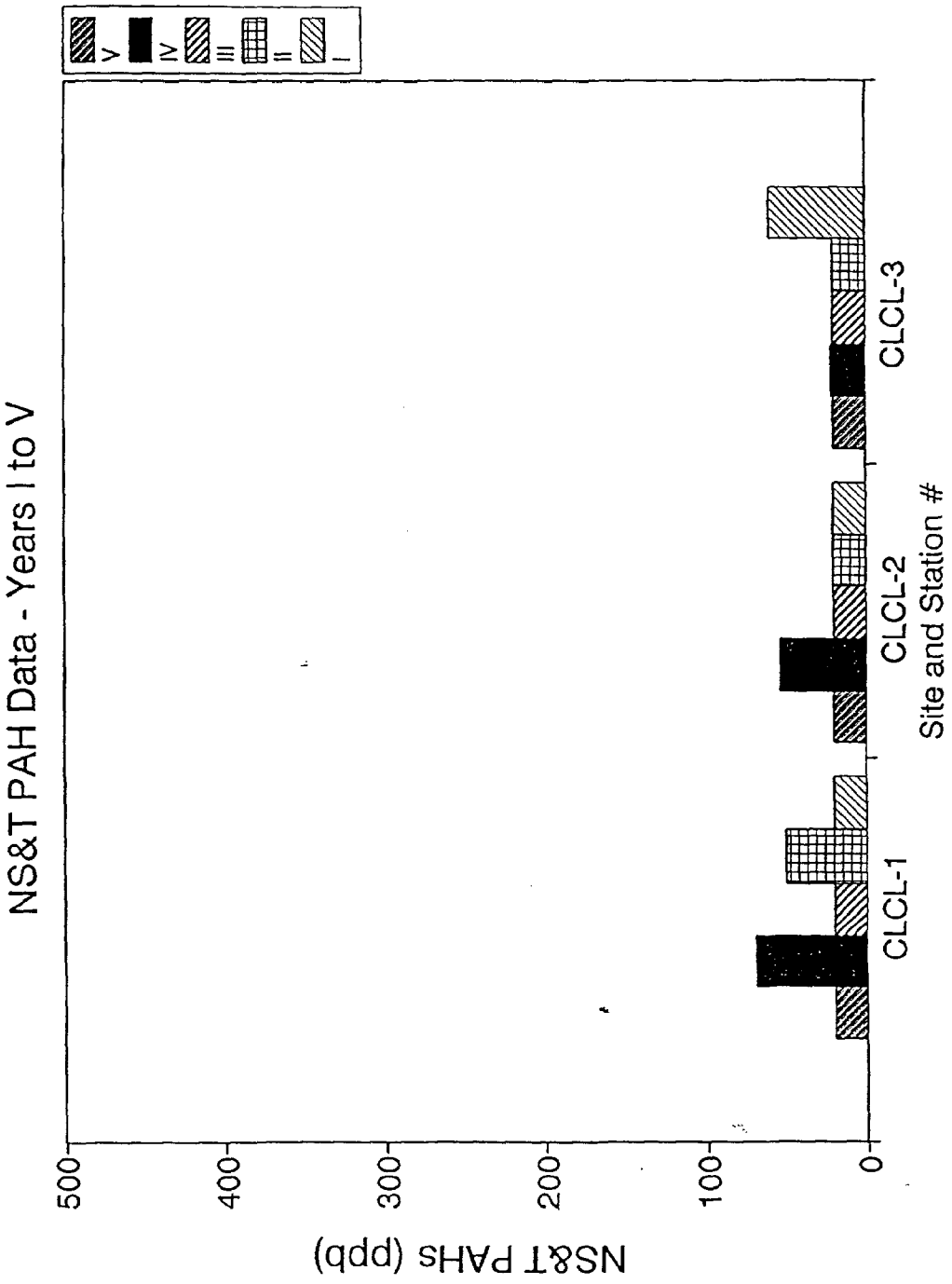
Figure 4: Frequency distribution of the median total NS&T PAH (tPAH) concentration in the Gulf of Mexico during the first five years of the program.

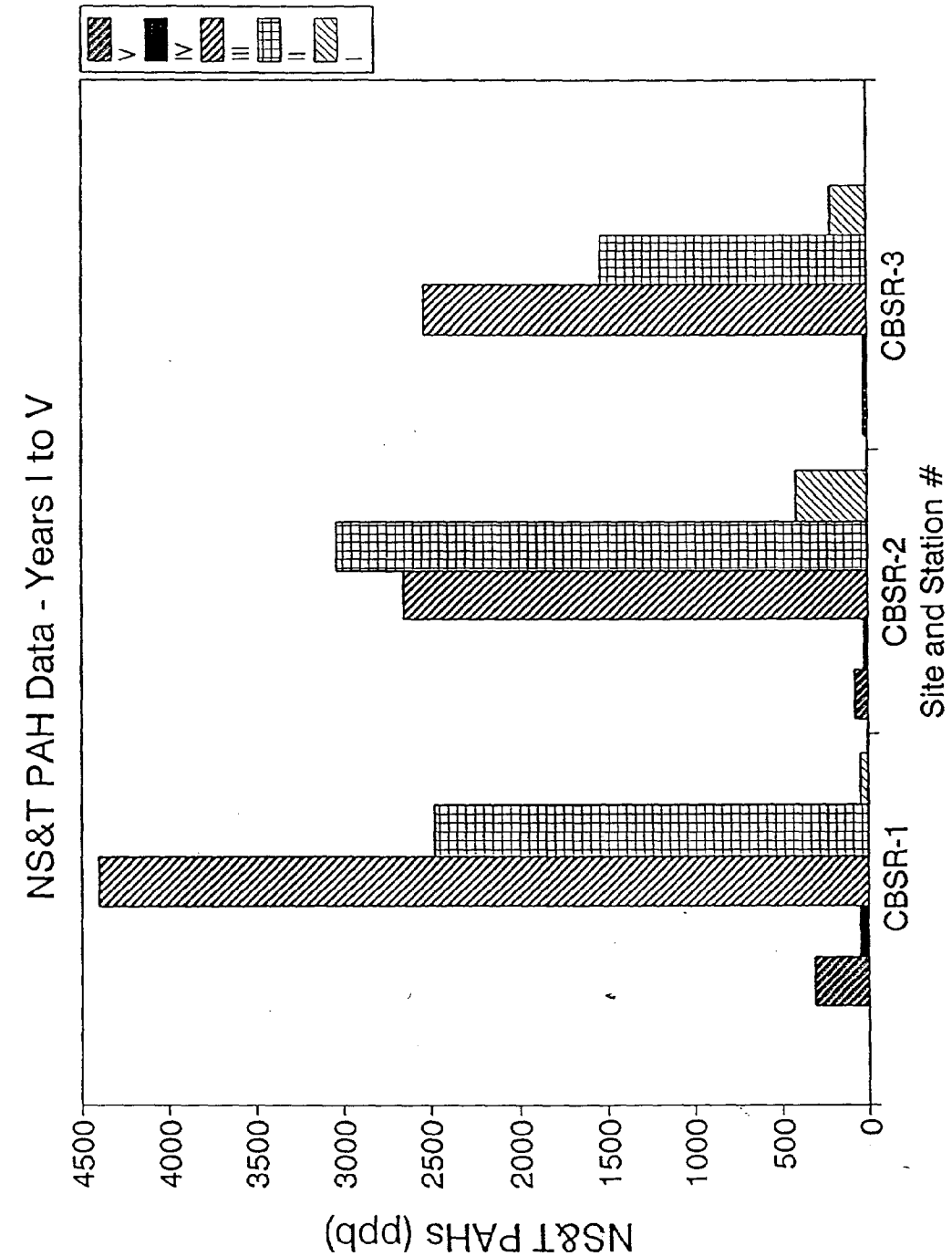
Figure 5: Plot of the cumulative frequency distribution for Year V total NS&T PAH (tPAH) concentration, compared to the gaussian curve and its cumulative frequency distribution generated from a lognormal model with a mean of 250 ppb and standard deviation of 218.

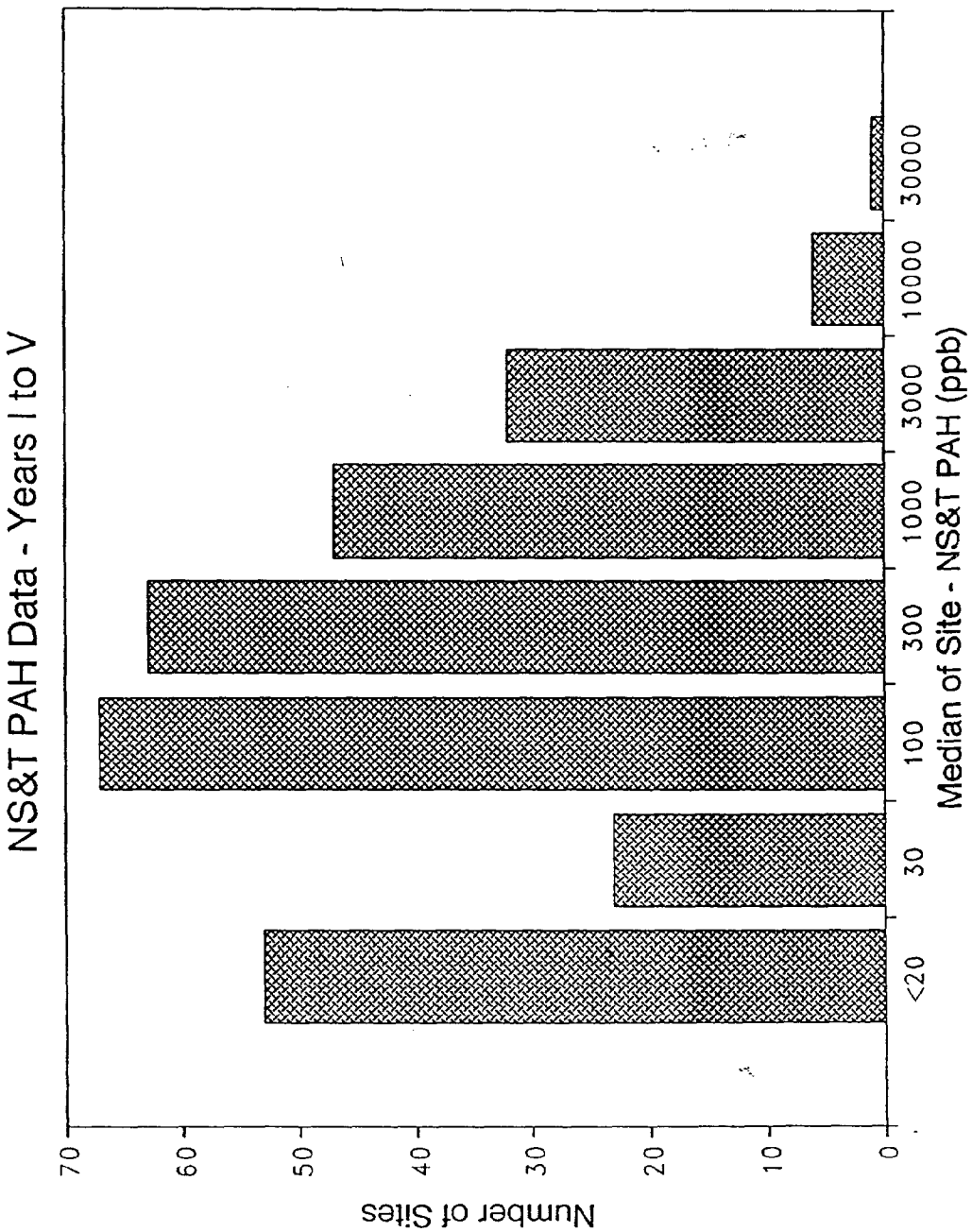
Figure 6: Plot of the cumulative frequency distribution for Year V NS&T PAH (tPAH) concentration, compared to the gaussian curves and their cumulative frequency distributions generated from a two population lognormal model with a mean of 214 ppb for Population 1 and a mean of 1205 ppb for Population 2.

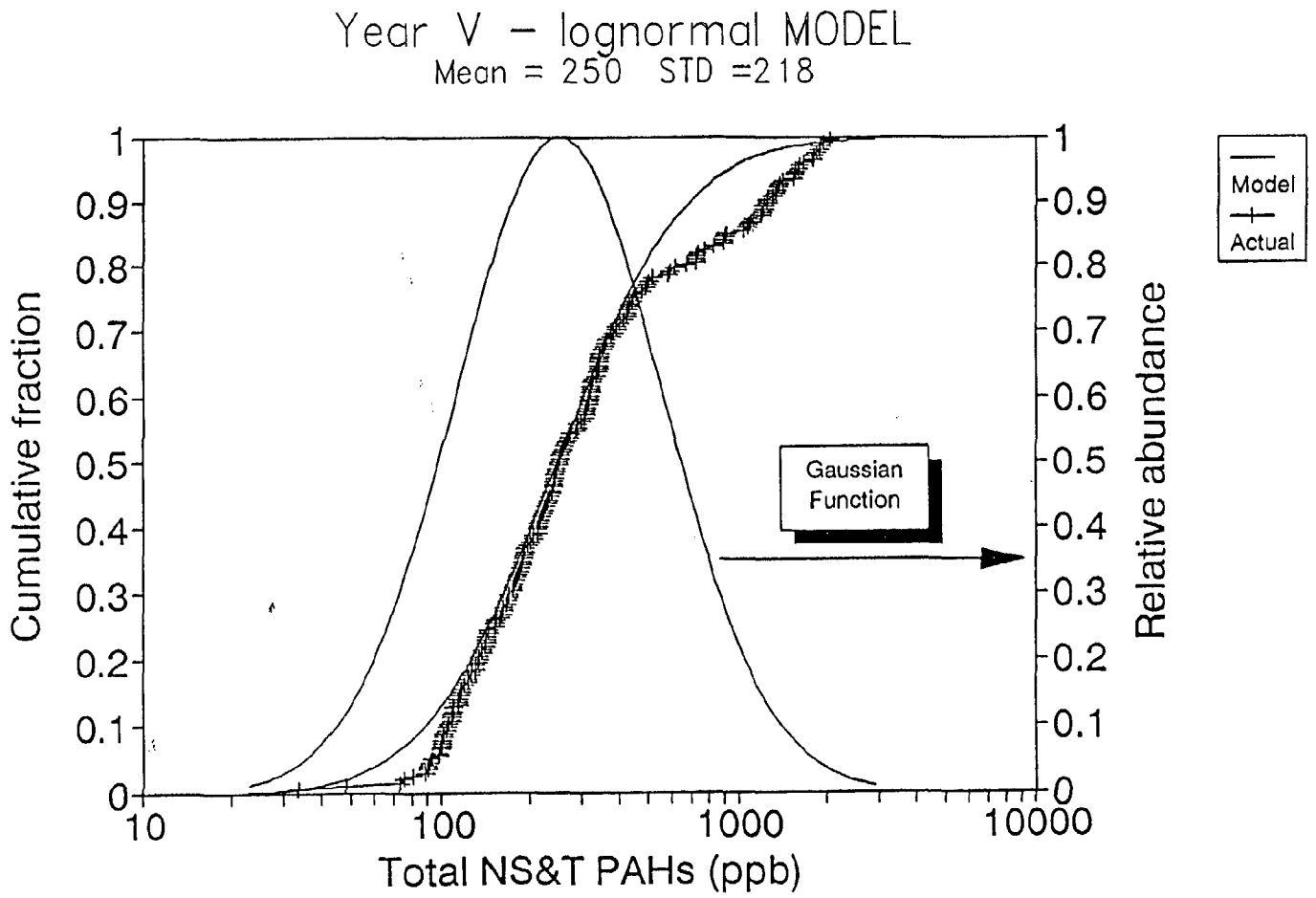
Figure 7: Comparison of the cumulative frequency distributions for the actual Year V total NS&T PAH (tPAH) concentration data and the cumulative frequency distribution generated from the two population model.

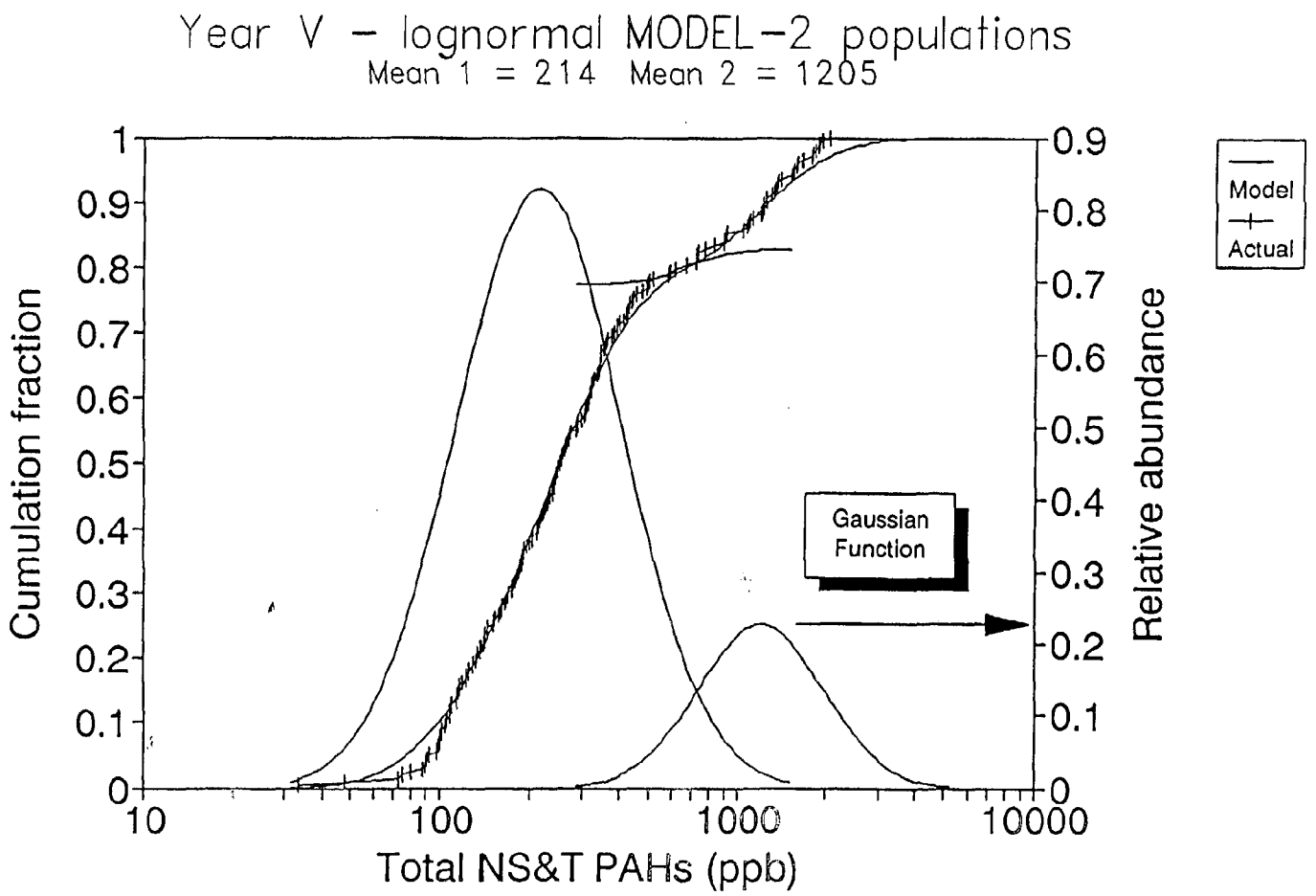

GEORGIA
FLORIDA
ALABAMA
MISSISSIPPI
LOUISIANA
TEXAS
GULF OF MEXICO
Tallahassee
Panama City
Tampa
Miami
Gulfport
New Orleans
Baton Rouge
Houston
Galveston
Mississippi River
U.S.
MEX.
0 Miles 50 100 150 200
0 Km 100 200 300
97°
96°
95°
94°
93°
92°
91°
90°
89°
88°
87°
86°
85°
84°
83°
82°
81°
31°
30°
29°
28°
27°
26°
25°
24°

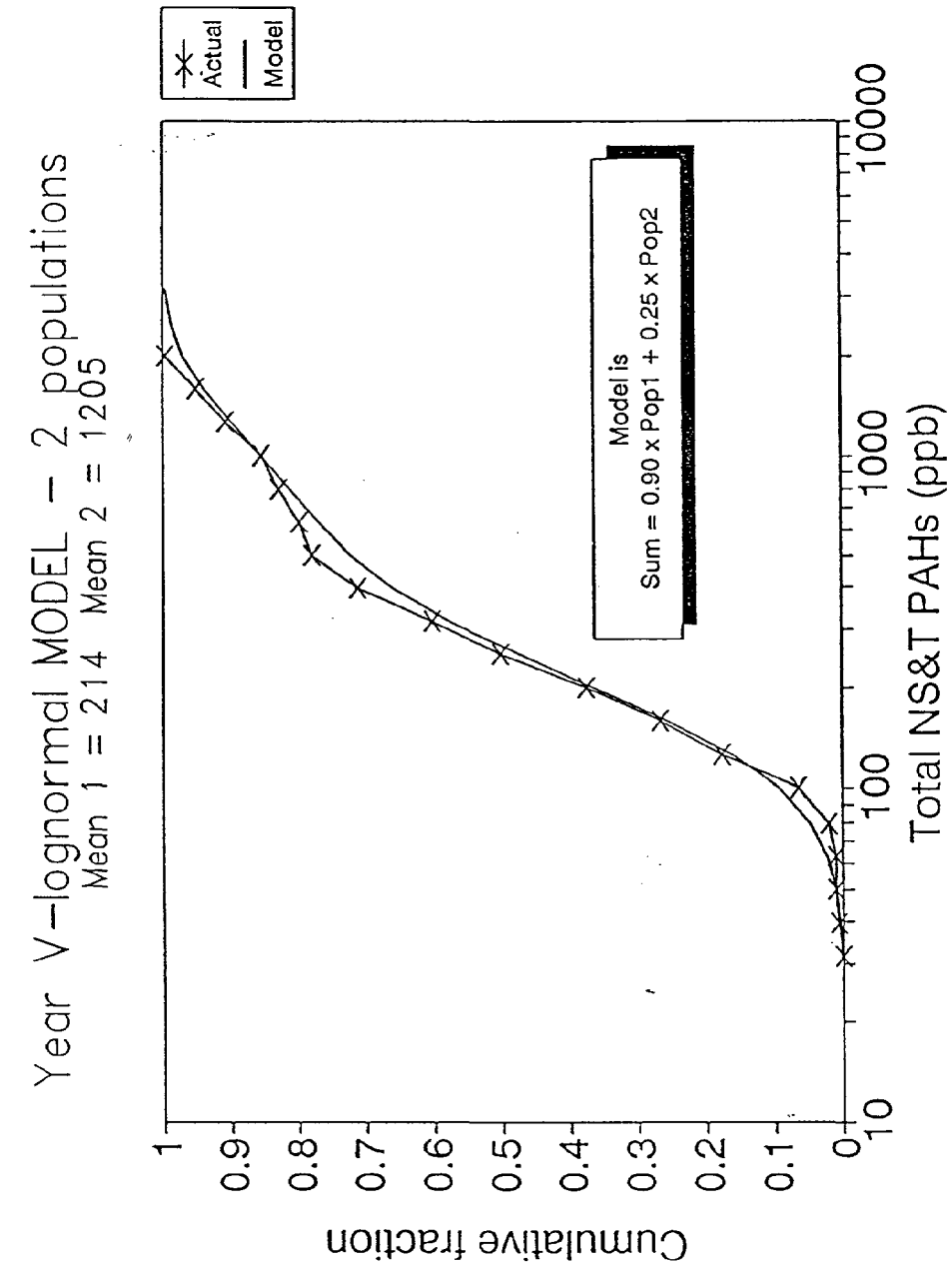

NS&T PAH Data - Years I to V
NS&T PAHs (ppb)
500
400
300
200
100
0
CLCL-1
CLCL-2
CLCL-3
Site and Station #


NS&T PAH Data - Years I to V
NS&T PAHs (ppb)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
CBSR-1
CBSR-2
CBSR-3
Site and Station #


NS&T PAH Data - Years I to V
Number of Sites
0
10
20
30
40
50
60
70
<20
30
100
300
1000
3000
10000
30000
Median of Site - NS&T PAH (ppb)


Year V – lognormal MODEL
Mean = 250 STD =218
Cumulative fraction
Relative abundance
Total NS&T PAHs (ppb)
10
100
1000
10000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Model
Actual
Gaussian Function


Year V - lognormal MODEL-2 populations
Mean 1 = 214 Mean 2 = 1205
Cumulation fraction
Relative abundance
Total NS&T PAHs (ppb)
10
100
1000
10000
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Model
Actual
Gaussian Function


Year V-lognormal MODEL - 2 populations
Mean 1 = 214 Mean 2 = 1205
Actual
Model
Model is
Sum = 0.90 x Pop1 + 0.25 x Pop2
Cumulative fraction
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10
100
1000
10000
Total NS&T PAHs (ppb)

Preprint 2

Sources of Local Variation in Polynuclear Aromatic Hydrocarbon and Pesticide Body Burden

Matthew S. Ellis, Kwang-Sik Choi, Terry L. Wade, Eric N. Powell, Thomas J. Jackson, Donald H. Lewis

ABSTRACT

The sources of local (intrapopulation) variation in PAH body burden among adjacent oysters on a reef in Galveston Bay were examined. Both eggs and sperm contain significantly more PAH than somatic tissue. The quantity of gonadal material was the most important correlate of PAH body burden. Sex was an important secondary determinant. Body burden of males was correlated with general indicators of health such as digestive gland atrophy; body burden of females was not. The evidence suggests that the most important factor determining variation in PAH body burden within an oyster population during any single sampling period is the frequency of spawning and how soon collection occurred after the most recent spawn. Analysis of eggs and sperm for PAHs and pesticides revealed that eggs and sperm were enriched in all PAHs relative to somatic tissue. Eggs, but not sperm, were enriched in chlorinated compounds (e.g. chlordane, DDE, DDD). Both eggs and sperm were enriched in total PCBs relative to somatic tissue. Oysters may lose 50% or more of their total body burden of certain PAHs and pesticides in a single spawn.

INTRODUCTION

Bivalve molluscs have frequently been used as indicator organisms in studies monitoring levels of contaminants in the environment. These organisms are utilized because of their ability to accumulate and concentrate both metal and organic contaminants enabling them to serve as long-term integrators of their environment (Phillips, 1977). One such program is the NOAA Status and Trends (NS&T) Program ("Mussel Watch") designed to monitor changes in environmental quality along the Atlantic, Pacific, and Gulf coasts of the United States by measuring levels of chemical contaminants in fish, bivalves, and sediments and identifying biological responses to those contaminants (e.g. Wilson et al., in press, 1990; Sericano et al., 1990; Presley et al., 1990).

Unfortunately, many biological and environmental factors affect the rate and extent of bioaccumulation besides contaminant availability. Biological factors include differential growth rate (Cunningham and Tripp, 1975; Boyden, 1977), reproductive stage (Cunningham and Tripp, 1975; Frazier, 1975; Martincić et al., 1984), stress and disease (Shuster and Pringle, 1969; Sindermann, 1983; Moore et al., 1989). These biological factors make spatial and temporal comparisons designed to evaluate the status and trends of contaminant loading more difficult. The NOAA Status and Trends Program has proven to be no exception.

In the Gulf of Mexico, the mollusc used for monitoring by NOAA is the oyster Crassostrea virginica. Analysis of the first 4 yr of NS&T data has shown that the body burden of polynuclear aromatic hydrocarbons (PAHs) and pesticides in oysters is correlated with latitude in the Gulf of Mexico. Contaminant body burdens average higher at higher latitudes. Wilson et al. (1990) suggested that the latitudinal temperature gradient in the Gulf produced variation in reproductive effort and that this variation in

reproductive effort affected PAH body burden sufficiently to override the effect of local variation in contaminant loading. Wilson et al. (in press), in a more thorough analysis, showed that PAH body burden responds to climate change and that biological factors are the likely intermediaries between climate's effect on temperature and freshwater inflow and the final body burden of PAHs.

Two likely intermediaries are spawning and disease. Spawning has frequently been forwarded as an important route of depuration (Marcus and Stokes, 1985; Jovanovich and Marion, 1987; Cossa, 1989) because lipid loss peaks at this time (Chu et al., 1990). Parasites and pathogens are less frequently implicated (Khan, 1987), but parasites and pathogens should have an effect; if for no other reason, they frequently reduce spawning frequency or the number of gametes per spawn (Akberali and Trueman, 1985; Ford and Figueras, 1988; Barber et al., 1988). In oysters, both spawning frequency and disease are significantly affected by temperature and salinity (Hofmann et al., in press, submitted; Soniat and Gauthier, 1989) and thus could serve as important intermediaries by which variation in climate might affect contaminant body burden.

Climate exerts its influence over large geographic scales. Biological parameters capable of responding to climate change and, thus, affecting contaminant body burden on a large geographic scale should certainly do so as well on a local scale. Accordingly, spawning frequency and disease should be important sources of local (within population) variability in contaminant body burden. Monitoring programs typically sample infrequently (NS&T samples once per year) so that the basis for within-sample variability is an important consideration. Accordingly, the primary purpose of this study was to examine sources of local variability in PAH body burden at any sampling period. Some analyses of pesticides were also conducted.

Unfortunately, the variables likely of most importance in determining local variability in body burden, spawning frequency and the time since the last spawn, are variables that cannot be readily measured even in a temporally-intensive sampling program because continuous (or dribble) spawning is a frequent condition at latitudes south of Chesapeake Bay, including the entire Gulf of Mexico (Hofmann et al., in press). Consequently, more readily measured variables must be used as surrogates for the more desirable variables. Thus, we examined a series of indices related to reproductive state, including stage of reproduction and the quantity of gonadal material present, and a series of indices related to health, namely digestive gland atrophy, condition and P. marinus infection intensity. P. marinus, an endoparasitic protozoan, is responsible for high mortality (typically > 50%) in market-sized oysters in the Gulf each year (Hofstetter, 1977; Osburn et al., 1985; Ray, 1987) and is known to delay reproduction (White et al., 1988; Wilson et al., 1988). Digestive gland atrophy is a putatively pathogenic condition (e.g. Marigómez et al., 1990; Moore et al., 1989) common in Gulf coast oysters (Gauthier et al., 1990).

METHODS

Within-population differences in body burden.

Oysters were collected in September from Confederate Reef in the West Bay extension of Galveston Bay. Confederate Reef oysters normally have a relatively high PAH body burden in comparison to the Gulf-wide mean (Sericano et al., 1990; Wade et al., 1988). September is near the end of the spawning season; most individuals should have spawned at least twice over the 4 previous months. The oysters were placed on ice and returned to the laboratory. Maximum length and wet weight were determined. The condition of each meat was rated on a semiquantitative scale from 1, very good, to 9, very

poor, according to Quick and Mackin (1971). A small section of gonadal tissue was taken and fixed in Davidson's fixative (Fig. 28 in NOAA, 1983). A small section of mantle tissue was removed for determination of P. marinus infection following Ray (1966). The remaining tissue was placed in a precombusted mason jar with a teflon-lined screw cap and frozen for PAH analyses.

P. marinus infection intensity was rated on the 0 (uninfected) to 5 (highly infected) point scale of Mackin (1962) as modified by Craig et al. (1989). Tissue samples were embedded in paraffin, sectioned at 6 μm and stained in Harris' hematoxylin and picro/Navy eosin (Preece, 1972). Reproductive stage was rated on a scale of 1 (sexually undifferentiated) to 8 (spawned out) slightly expanded from Ford and Figueras (1988) by GERG (1990) (Table 1). Digestive gland atrophy was rated semiquantitatively from 0 (no atrophy) to 4 (extreme atrophy) as described by Gauthier et al., (1990) (Table 2).

The analytical procedures used for PAHs and pesticides were based on the NOAA's NS&T techniques for organic compounds (MacLeod et al. 1985) with some modification by Wade et al. (1988). These methods have been detailed elsewhere (Wade et al., 1988; Wade and Sericano, 1989; Sericano et al., 1990; GERG, 1990) and only a brief overview will be given here.

Samples were extracted with methylene chloride after drying with Na_2SO_4. The samples were then purified by silica/alumina column chromatography. In order to remove lipids, a high-performance liquid chromatography separation was performed. Purified extracts were then analyzed by gas chromatography with a mass spectrometry detector, GC/MS/SIM for PAHs and GC-ECD for pesticides. All concentrations are reported as ng of analyte per gram dry weight of sample, or ppb. Concentrations in the procedural blanks were, in all cases, below reporting levels for each individual analyte. The accuracy

and precision of these methods have been established by several intercalibration exercises overseen by the U.S. National Institute of Standards and Technology.

Oyster gonadal tissue surrounds much of the body mass and, thus, is difficult to excise cleanly and weigh (Kennedy and Battle, 1964; Morales-Alamo and Mann, 1989). Thus, a quantitative gonadal index based on gonad weight, as is frequently used in invertebrates and fish, is not available. Accordingly, a polyclonal rabbit anti-oyster egg antibody was used to quantify the amount of egg protein present (Choi et al., in press). A single radial immunodiffusion assay (Mancini et al., 1965; Garvey et al., 1977) was performed to quantitate egg protein using 1.5% agarose in barbitone buffer (0.01 M sodium barbital, 0.0022 M barbital, 0.01% sodium azide as preservative, pH 8.6). Two ml of the rabbit serum containing anti-oyster antibody was mixed in 18 ml of the agarose gel and cast on a 10 X 10 cm glass plate. Four mm diameter wells were made on the plate using a gel puncher and 20 µl of oyster egg standard (0.05 mg ml^{-1} to 3.2 mg ml^{-1}) or the sample were placed in the wells and incubated in a humid chamber for 48 hr at room temperature. After incubation, the plate was pressed, dried, stained with 0.5% (w/v) Coomassie Brilliant Blue, and destained with 50% EtOH and 10% acetic acid. Diameters of the precipitation rings were measured to the nearest 0.1 mm. A standard curve was constructed by plotting concentration of the egg standard against the diameter squared of the precipitation rings and the concentration of each sample was read from the curve.

Removal of the body section for histological analysis biases both the total PAH concentration and the gonadal quantity as measured by us. Sericano et al. (in press b) showed that the effect of this bias on PAH content is an expected 10 to 20% reduction in measured body burden. For gonadal quantity,

the percent reduction can be expected to be considerably higher. Readers are cautioned not to accept the reported measures of gonadal quantity as true measures of completely intact oysters. However, as most oysters were similar in size, the bias introduced in both measures would be equivalent over all samples and thus not compromise the data analysis.

Body burden of eggs and sperm.

In July, 1991 additional oysters were obtained from Galveston Bay for examining the relative PAH and pesticide content of eggs, sperm and the remaining body tissues. Most oysters were 7 to 12 cm long and exhibited fully-developed gonads. Oysters were shucked and their sex determined by microscope slide smear.

The contaminant content of the gametes, which is the only tissue component lost during spawning, may be dissimilar from the remaining gonadal tissue. Therefore, the eggs and sperm were isolated from the remaining gonadal and somatic mass. The body of each oyster was separated from other somatic tissues. The remainder including gill, mantle, adductor muscle, and labial palps were stored at -20°C for PAH and pesticide analysis. Gonads containing eggs or sperm were excised from the visceral mass using scissors and forceps. Gonads were placed on a petri dish and phosphate buffered saline (0.15 M NaCl, 0.003 M KCl, 0.01 M phosphate buffer, pH 7.4) (PBS) was added. Eggs or sperm were extracted by squeezing the gonads with a rubber-headed syringe piston. The egg extract was then filtered through a 100 µm nylon mesh screen; the sperm extract was filtered through a 30 µm nylon mesh screen.

Oyster egg filtrates were washed 4 times by resuspending the filtrates into 30 ml of PBS and centrifuging at 700 xg for 10 min. During each washing, tissue debris and other impurities sedimented on the egg pellets were removed by pasteur pipette. After the final washing, the egg pellets were resuspended

into an equal volume of PBS. Five ml of the resuspension was transferred to a 15-ml centrifuge tube, 7 ml PBS added to resuspend the eggs, and the suspension centrifuged at 500 xg for 15 min. Any remaining tissue debris layered on the egg pellet was removed using a pasteur pipette. Egg pellets from 10 to 20 oysters were pooled in a 50-ml centrifuge tube and sedimented by centrifugation (700 xg for 15 min). Oyster egg pellets were then resuspended into an equal volume of PBS. A 60% Percoll solution (4:6 PBS/100% Percoll) (100% Percoll is 9:1 Percoll stock: 10X PBS) was prepared. Five ml egg suspension was mixed with 35 ml 60% Percoll and centrifuged at 900 xg for 20 min. Oyster eggs formed an aggregate at the top of the centrifuge tube after centrifugation. Purified eggs were harvested from the tube and washed twice by centrifuging at 700 xg for 10 min.

Oyster sperm filtrates were washed 4 times with PBS by centrifuging 700 xg for 15 min. Tissue debris found at the top of the oyster sperm pellet was removed using a pasteur pipette during each washing step. After the final washing, the sperm extracts were resuspended into an equal volume of PBS. 70% Percoll was prepared and 35 ml 70% Percoll was mixed with 5 ml sperm suspension and centrifuged at 900 xg for 20 min. Oyster sperm was found at the bottom of the centrifuge tube and other impurities found at the top of the Percoll as a float. Purified oyster sperm was pooled from 20 to 30 oysters and washed twice with PBS by centrifuging at 800 xg for 15 min.

Because an involved procedure of this sort could lend to significant contamination, each solution was subjected to PAH analysis. No solutions were found to be significantly contaminated.

RESULTS

Within-population differences in PAH body burden.

Forty oysters were analyzed, 30 females and 10 males. We present the means and ranges of the variables measured in Table 3. The mean length for the group was 8.0 cm, wet weight 9.61 g, condition code 4.3 (fair plus), P. marinus infection intensity 1.33 (light plus), and digestive gland atrophy 2.1 (about half atrophied). The sample contained individuals covering nearly the entire range of condition codes, two-thirds of the range of possible P. marinus infection intensities, six of eight possible gonadal states and all stages of digestive gland atrophy. The variability in this data set is typical of single collections of oysters in the Gulf of Mexico region (Wilson et al., 1990).

By sex, the lengths of females and males were fairly close (7.9 cm vs. 8.1 cm), however females were heavier than males (9.9 vs. 8.6 g). The weight difference is considerable since females are actually 0.2 cm shorter on average. Condition code for both sexes was also fairly close, 4.6 for males vs. 4.2 for females, as was digestive gland atrophy, 1.9 for males and 2.2 for females. P. marinus infection intensity differed substantially with males at 0.77 and females at 1.67. Most animals were nearly ready to spawn or spawning. Reproductive stage was similar: 5.3 and 5.6 for males and females, respectively. When measured quantitatively, the 30 females averaged 6.29 mg eggs per female (equivalent to about 4.8×10^5 fully-developed eggs per female). As a section of gonad was removed for histology, these values underestimate female fecundity.

Although we explored the entire suite of PAHs per NOAA's Status and Trends protocol (GERG, 1990), we only report data for the 5 most important PAHs: fluoranthene, phenanthrene, pyrene, naphthalene, and chrysene. Males

and females had similar body burdens except for fluoranthene where females had about one-third more. Means for both sexes ranged from 12.0 ng g dry wt^{-1} for phenanthrene to 49.0 ng g dry wt^{-1} for fluoranthene.

A Spearman's rank analysis showed that many of the biological variables were correlated as might be expected. Accordingly, prior to considering their relationship with the PAHs, the relationships among the biological variables themselves must be understood. Because of the many significant correlations among them, we chose to identify the best 3-variable model explaining variation for each of the important biological variables, as detailed in Tables 4 to 6. Because gonadal quantity was measured in only 30 of the 40 individuals and only in females, we examined the data with and without this variable included. The variables examined were length, wet weight, P. marinus infection intensity, digestive gland atrophy, sex, condition code, gonadal stage and gonadal quantity.

The important correlations were ones (a) between sex and P. marinus infection intensity, males had lighter infections, and (b) between gonadal stage, condition code and digestive gland atrophy. Among the females, only the relationship between gonadal stage and condition code remained significant. Among the males, digestive gland atrophy was correlated with P. marinus infection intensity. Inasmuch as the two sexes were distinctive in the relationships among biological attributes, we will consider the sexes separately in most of the remaining analyses.

Considering both sexes together, condition code and sex were the most important variables correlating with the PAHs (Table 7). Among the females, gonadal quantity had a significant effect in 3 of 5 cases (Table 8): fluoranthene, pyrene and chrysene. Each of the contaminant's concentrations was higher in females having more eggs. Digestive gland atrophy was also a

significant correlate of chrysene. Female oysters having a higher degree of atrophy had more chrysene. If gonadal quantity was removed, few significant correlations remained. Among the males, digestive gland atrophy was significantly correlated in 3 of 5 cases (Table 9). PAH concentration was lower in male oysters characterized by a greater degree of digestive gland atrophy. Condition code was significant in 2 of 5 cases; higher condition code (less healthy) occurred with higher PAH concentration.

Body burden of eggs and sperm.

Samples of pure eggs and sperm, collected from oysters taken earlier in the spawning season than those supporting the previous data, had significantly higher PAH levels than somatic tissue for all 5 PAHs (Table 10). A factor of 5 difference was typical. Total PCBs were concentrated in eggs and sperm by a factor of about 5 over the somatic tissue. The chlorinated compounds like lindane, chlordane, dieldrin and DDT (plus breakdown products) were concentrated in eggs by about 4 times, but tended to be equivalent to or lower than the somatic tissue in sperm.

DISCUSSION

Spawning as a route of depuration.

Our data suggest that reproduction is an important depuration route for oysters; the frequency of reproduction is the most important determinant of body burden, under equivalent exposure levels. Sex and health are important secondary determinants of body burden because both affect reproductive state and the frequency of reproduction. The three following observations support these two conclusions.

(1) Both eggs and sperm contain significantly more PAH and pesticide than somatic tissue. The concentration factor is sufficient to conclude that

over half of the PAH body burden, and somewhat less of the pesticide body burden, could be in gonadal tissue prior to spawning. Eggs and sperm had PAH concentrations 5 times higher than somatic tissue, 3 to 4 times higher for pesticides, and the gonadal tissue can account for 25% of animal dry weight prior to spawning (Choi et al., in press; Klinck et al., 1992).

(2) The quantity of gonadal material was the most important correlate of PAH body burden and much more important than, for example, gonadal stage. Less gonadal material indicates recent spawning since these oysters were collected well into the spawning season; all had certainly spawned at least once prior to collection.

(3) Sex was an important determinant of body burden. Not only did PAH concentrations differ in some cases, and dramatically so for some pesticides, but the factors correlating with body burden also differed among the sexes. Health-related factors were much more important in males. Factors decreasing health probably also decrease spawning frequency. The most important correlate occurred with digestive gland atrophy; however in males, digestive gland atrophy was highly inversely correlated with P. marinus infection intensity, so the two parameters behaved similarly in explaining the variation in PAH body burden among oysters taken from the same site. PAHs were lower with lower P. marinus infection intensity and P. marinus is known to slow reproduction in oysters (Wilson et al., 1988; White et al., 1988).

Reproduction, health and body burden.

The importance of reproduction in molluscs in controlling or affecting body burden is open to disagreement. Mix et al. (1982) and DiSalvo et al. (1975) found PAHs no more concentrated in Mytilus edulis gonadal material than somatic tissue (purified eggs were not measured), but noticed a significant drop in body burden during the spawning season. Sericano et al. (in press)

found that the central body region including the gonad contained proportionately more PAH in oysters. Lee et al. (1972), Fortner and Sick (1985) and Solbakken et al. (1982), as examples, found the hepatopancreas to be an important depot for PAHs in bivalves, however gonadal material, and in particular, gametes, were not separately measured. In scallops where gonads can be separated from the somatic tissue by dissection, Friocourt et al. (1985) found gonadal material enriched in PAHs over muscle but not digestive gland tissue. Rossi and Anderson (1977) observed spawning to be an important depuration route in a polychaete Neanthes arenaceodentata.

If spawning is an important route of depuration, then factors affecting spawning frequency and how recently the last spawn occurred prior to collection will affect body burden. The biological variables measured as surrogates of spawning frequency are gonadal quantity and gonadal stage, P. marinus infection intensity, and some general indicators of health. Few of these were correlated among themselves, so that most serve as separate, somewhat unique, indicators of the many factors that might affect spawning frequency and how recently the last spawn occurred prior to collection. Each has its own history, in some cases not necessarily related to spawning frequency, so that each is only a poor surrogate for the desired variable, but we emphasize that these are variables that can normally be easily measured in oyster individuals whereas spawning time and frequency cannot. Nevertheless, under these conditions, only the strongest relationships might be expected to generate a signal of sufficient intensity to be observed as a significant correlation.

Correlations were found, indicating the importance of reproductive state and health on body burden. The amount of variation explained among individuals in their PAH body burdens was generally low; however, this

probably emphasizes the previous point, that each of the measured variables are themselves relatively poor indictors of how recently and how frequently each animal had spawned. Stegeman and Teal (1973) emphasized the importance of the total exposure history of any individual organism in determining body burden. One aspect of this exposure history is the time since the last significant depuration event due to spawning.

Hydrocarbons can be taken up by feeding as well as in the dissolved phase (e.g. McElroy et al., 1989) and can affect filtration rate (Axiak et al., 1988; Barszcz et al., 1978). PAHs can also affect the digestive gland (Nott and Moore, 1987). Theoretically, digestive gland atrophy should be related to nutritional state. Digestive gland atrophy was correlated weakly with higher PAHs in females and more strongly with lower PAHs in males. One possible explanation for these divergent results is the strong correlation of digestive gland atrophy and P. marinus infection intensity in males. In any case, no unambiguous effect of digestive gland atrophy could be discerned.

Our data clearly support the importance of reproduction, at least in oysters, during the summer and fall. We suggest that the weak evidence for the importance of reproduction in most time series of contaminant body burden generally stems from 3 factors: collection of animals out of spawning season when little gonadal material is present, failure to analyze purified gametes which are the primary vehicle of depuration during spawning, and the poor understanding of the dynamics of uptake after spawning. We suggest that the timing of the last spawning event prior to sampling – animals recover their body burden within a month or less after a depuration event (Sericano et al., in press a) – and the degree of gonadal development (e.g. Hofmann et al., in press) are important variables affecting PAH body burden in oysters.

Lowe and Pipe (1987) and Moore et al. (1989) observed gonadal resorption at high PAH concentrations. We observed no such effect in our analyses, however body burdens were lower.

Variation between compounds.

Fluoranthene, pyrene and chrysene were very similar in their response to the biological variables; naphthalene and phenanthrene formed a second group quite different from the other three. Certainly, uptake, storage and depuration must be relatively similar within these two groups but different between them. Phenanthrene and naphthalene are lower molecular weight, more water soluble compounds and equilibrate faster with the environment (Pruell et al., 1986, Sericano et al., in prep.). They might lose the signal imposed by spawning events faster than the larger three PAHs examined. Phenanthrene and naphthalene supported fewer significant correlations, none with reproduction, despite their enrichment in eggs and sperm, but were correlated with general measures of health, like condition. Possibly such general measures include factors controlling the equilibrium state of these PAHs. These analyses again suggest that an important variable controlling PAH body burden is the time between the most recent spawning and collection.

Nasci and Fossato (1982) noted that female gonadal material was enriched in total DDTs but male gonadal material was not in Mytilus galloprovincialis. Total PCBs were enriched in both female and male gonadal tissue. We observed the same phenomenon in oysters. Unlike PAHs and PCBs, sperm do not concentrate DDTs. The biochemical basis for this observation remains unclear.

Reproduction and the latitudinal gradient in body burden.

The data suggest one explanation for the latitudinal gradient in PAH and pesticide body burden observed in the Gulf of Mexico (Wilson et al., 1990) and

the relationship of PAH body burden and climate change (Wilson et al., in press). Slight variations in temperature, as affected by climate change, or varying average temperature across latitudes will vary the reproductive season, the annual reproductive effort, and the frequency of spawning in oysters (Hofmann et al., in press, submitted). Small changes in temperature produce large changes in reproductive effort. As a result, body burdens will vary even under similar exposure levels and this variability may be considerable if a substantial fraction of the body burden is lost in spawning.

Wilson et al. (1990) found the latitudinal gradient in PAH body burden to be stronger than the latitudinal gradient in pesticide body burden. We found gonadal material concentrated much more highly in PAHs than pesticides and some pesticides are not concentrated in male gonadal material at all. Our data would suggest that temperature, and therefore latitude, should have a much greater impact on PAHs through reproduction than on pesticides, in agreement with the findings of Wilson et al. (1990). Taken together, our data and those of Wilson et al. (1990, in press) suggest that interpretation of the results of monitoring studies such as the Status and Trends program using bivalves requires that close attention be paid to the reproductive state and health of the sampled populations.

ACKNOWLEDGMENTS

This research was supported by a grant from the Center for Energy and Minerals Resources, Texas A&M University (TAMU), an institutional grant NA89-AA-D-SG139 to TAMU by the National Sea Grant College Program, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce, grant 50-DGNC-5-00262 from the U.S. Department of Commerce, NOAA, Ocean Assessments Division, and computer funds from the TAMU College of Geosciences and Maritime Studies Research Development Fund. We appreciate this support.

REFERENCES

AKBERALI, H.B., AND E.R. TRUEMAN. 1985 Effects of environmental stress on marine bivalve molluscs. Adv. Mar. Biol. 22:101-198.

AXIAK, V., J.J. GEORGE, AND M.N. MOORE. 1988. Petroleum hydrocarbons in the marine bivalve Venus verrucosa: accumulation and cellular responses. Mar. Biol. (Berl.) 97:225-230.

BARBER, B.J., S.E. FORD, AND H.H. HASKIN. 1988. Effects of the parasite MSX (Haplosporidium nelsoni) on oyster (Crassostrea virginica) energy metabolism. I. Condition index and relative fecundity. J. Shellfish Res. 7:25-31.

BARSZCZ, C., P.P. YEVICH, L.R. BROWN, J.D. YARBROUGH, AND C.D. MINCHEW. 1978. Chronic effects of three crude oils on oysters suspended in estuarine ponds. J. Environ. Pathol. Toxicol. 1:879-896.

BOYDEN, C.R. 1977. Effect of size upon metal content of shellfish. J. Mar. Biol. Assoc. U. K. 57:675-714.

CHOI, K-S., D.H. LEWIS, E.N. POWELL, AND S.M. RAY. in press. Quantitative measurement of reproductive output in the American oyster, Crassostrea virginica, from a population in Galveston Bay, Texas, using an enzyme-linked immunosorbent assay (ELISA). Aquaculture.

CHU, F-L.E., K.L. WEBB, AND J. CHEN. 1990. Seasonal changes of lipids and fatty acids in oyster tissues (Crassostrea virginica) and estuarine particulate matter. Comp. Biochem. Physiol. A Comp. Physiol. 95:385-391.

COSSA, D. 1989. A review of the use of Mytilus spp. as quantitative indicators of cadmium and mercury contamination in coastal waters. Oceanol. Acta 12:417-432.

CRAIG, A., E.N. POWELL, R.R. FAY, AND J.M. BROOKS. 1989. Distribution of Perkinsus marinus in Gulf coast oyster populations. Estuaries 12:82-91.

CUNNINGHAM, P.A., AND M.R. TRIPP. 1975. Factors affecting the accumulation and removal of mercury from tissues of the American oyster Crassostrea virginica. Mar. Biol. (Berl.) 31:311-319.

DISALVO, L.H., H.E. GUARD, AND L. HUNTER. 1975. Tissue hydrocarbon burden of mussels as potential monitor of environmental hydrocarbon insult. Environ. Sci. & Technol. 9:247-251.

FORD, S.E., AND A.J. FIGUERAS. 1988. Effects of sublethal infection by the parasite Haplosporidium nelsoni (MSX) on gametogenesis, spawning, and sex ratios of oysters in Delaware Bay, USA. Dis. Aquat. Org. 4:121-133.

FORTNER, A.R., AND L.V. SICK. 1985. Simultaneous accumulations of naphthalene, a PCB mixture, and benzo(a)pyrene, by the oyster, Crassostrea virginica. Bull. Environ. Contam. Toxicol. 34:256-264.

FRAZIER, J.M. 1975. The dynamics of metals in the American oyster, Crassostrea virginica. I. Seasonal effects. Chesapeake Sci. 16:162-171.

FRIOCOURT, M.P., G. BODENNEC, AND F. BERTHOU. 1985. Determination of polyaromatic hydrocarbons in scallops (Pecten maximus) by UV fluorescence and HPLC combined with UV and fluorescence detectors. Bull. Environ. Contam. Toxicol. 34:228-238.

GARVEY, J.S., CREMER, N.E., AND D.H. SUSSDORF. 1977. Methods in immunology a laboratory text for instruction and research. The Benjamin/Cummings Publishing Company, Reading, Massachusetts, 545 p.

GAUTHIER, J.D., SONIAT, T.M., AND J.S. ROGERS. 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. J. World Aquacult. Soc. 21:168-200.

GERG. 1990. NOAA Status and Trends Mussel Watch Program analytical methods. U.S. Department of Commerce, NOAA, Ocean Assessment Division report, Geochemical and Environmental Research Group, Texas A&M University.

HOFMANN, E.E., J.M. KLINCK, E.N. POWELL, S. BOYLES, AND M.S. ELLIS. submitted. Modeling oyster populations II. Adult size and reproductive effort.

HOFMANN, E.E., E.N. POWELL, J.M. KLINCK, AND E.A. WILSON. in press. Modeling oyster populations III. Critical feeding periods, growth and reproduction. J. Shellfish Res.

HOFSTETTER, R.P. 1977. Trends in population levels of the American oyster Crassostrea virginica Gmelin on public reefs in Galveston Bay, Texas. Tex. Parks Wildl. Dept. Tech. Ser. 24:1-90.

JOVANOVICH, M.C., AND K.R. MARION. 1987. Seasonal variation in uptake and depuration of anthracene by the brackish water clam Rangia cuneata. Mar. Biol. (Berl.) 95:395-403.

KENNEDY, A.V. AND H.I. BATTLE. 1964. Cyclic changes in the gonad of the American oyster, Crassostrea virginica (Gmelin). Can. J. Zool. 42:305-321.

KHAN, R.A. 1987. Effects of chronic exposure to petroleum hydrocarbons on two species of marine fish infected with a hemoprotozoan, Trypanosoma murmanensis. Can. J. Zool. 65:2703-2709.

KLINCK, J.M., E.N. POWELL, E.E. HOFMANN, E.A. WILSON, AND S.M. RAY. 1992. Modeling oyster populations: the effect of density and food supply on production. Proc. Adv. Mar. Technol. Conf. 5:85-195.

LEE, R.F., R. SAUERHEBER, AND A.A. BENSON. 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel Mytilus edulis. Science (Wash. D.C.) 177:344-346.

LOWE, D.M., AND R.K. PIPE. 1987. Mortality and quantitative aspects of storage cell utilization in mussels, Mytilus edulis, following exposure to diesel oil hydrocarbons. Mar. Environ. Res. 22:243-251.

MACKIN, J.G. 1962. Oyster disease caused by Dermocystidium marinum and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Tex. 7:132-229.

MACLEOD, W.D., D.W. BROWN, A.J. FRIEDMAN, D.G. BURROWS, O. MAYNES, R.W. PEARCE, C.A. WIGREN, AND R.G. BOGAR. 1985. Standard analytical procedures of the NOAA National Analytical Facility 1985-1986. Extractable toxic organic compounds. 2nd edition, NOAA Tech. Memo. NMFS F/NWC-92.

MANCINI, G. A.O. CARBONARA, AND J.H. HEREMANS. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235-254.

MARCUS, J.M., AND T.P. STOKES. 1985. Polynuclear aromatic hydrocarbons in oyster tissue around three coastal marinas. Bull. Environ. Contam. Toxicol. 35:835-844.

MARIGÓMEZ, J.A., V. SÁEZ, M.P. CAJARAVILLE, AND E. ANGULO. 1990. A planimetric study of the mean epithelial thickness (MET) of the molluscan digestive gland over the tidal cycle and under environmental stress conditions. Helgol. Meeresunters., 44.81-94.

MARTINICIĆ, D., H.W. NÜRNBERG, M. STOEPPLER, AND M. BRANICA. 1984. Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Sea). Mar. Biol. (Berl.), 81:177-188.

MCELROY, A.E., J.W. FARRINGTON, AND J.M. TEAL. 1989. Bioavailability of polycyclic aromatic hydrocarbons in the aquatic environment, p. 1-39. In U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida.

MIX, M.C., S.J. HEMINGWAY, AND R.L. SCHAFFER. 1982. Benzo(a)pyrene concentrations in somatic and gonad tissues of bay mussels, Mytilus edulis. Bull. Environ. Contam. Toxicol., 28:46-51.

MOORE, M.N., D.R. LIVINGSTONE, AND J. WIDDOWS. 1989. Hydrocarbons in marine mollusks: biological effects and ecological consequences, p. 291-329. In U. Varanasi (ed.), Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, Florida.

MORALES-ALAMO, R. & R. MANN, 1989: Anatomical features in histological sections of Crassostrea virginica (Gmelin, 1791) as an aid in measurements of gonad area for reproductive assessment. J. Shellfish Res., 8: 71-82.

NASCI, C., AND V.U. FOSSATO. 1982. Studies on physiology of mussels and their ability in accumulating hydrocarbons and chlorinated hydrocarbons. Environ. Technol. Lett., 3:273-280.

NOAA. 1983. Histological techniques for marine bivalve mollusks. NOAA Tech. Mem. NMFS-F/NEC-25, 97 p.

NOTT, J.A., AND M.N. MOORE. 1987. Effects of polycyclic aromatic hydrocarbons on molluscan lysosomes and endoplasmic reticulum. Histochem. J., 19:357-368.

OSBURN, H.R., G.E. SAUL, AND C.L. HAMILTON. 1985. Trends in Texas commercial fisheries landings, 1977-1984. Tex. Parks Wildl. Dept. Coastal Fish. Branch Mgmt. Data Ser., 84:1-90.

PHILLIPS, D.J.H. 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments - a review. Environ. Pollut., 13:281-317.

PREECE, A. 1972. A manual for histologic technicians. Little, Brown and Company, Boston, Massachusetts, 428 p.

PRESLEY, B.J., R.J. TAYLOR, AND P.N. BOOTHE. 1990. Trace metals in Gulf of Mexico oysters. Sci. Total Environ., 98:551-593.

PRUELL, R.J., J.L. LAKE, W.R. DAVIS, AND J.G. QUINN. 1986. Uptake and depuration of organic contaminants by blue mussels (Mytilus edulis) exposed to environmentally contaminated sediment. Mar. Biol. (Berl.), 91:497-507.

QUICK JR., J.A., AND J.G. MACKIN. 1971. Oyster parasitism by Labyrinthomyxa marina in Florida. Fla. Dept. Nat. Resour. Mar. Res. Lab. Prof. Pap. Ser., 13:1-55.

RAY, S.M. 1966. A review of the culture methods for detecting Dermocystidium marinum, with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc., 54:55-69.

RAY, S.M. 1987. Salinity requirements of the American oyster, Crassostrea virginica. p. E1-E28. In A.J. Mueller and G.A. Matthews (eds.), Freshwater inflow needs of the Matagorda Bay system with focus on penaeid shrimp. NOAA Tech. Mem. NMFS-SEFC-189.

ROSSI, S.S., AND J.W. ANDERSON. 1977. Accumulation and release of fuel-oil-derived diaromatic hydrocarbons by the polychaete Neanthes arenaceodentata. Mar. Biol. (Berl.), 39:51-55.

SERICANO, J.L., E.L. ATLAS, T.L. WADE, AND J.M. BROOKS. 1990. NOAA's status and trends mussel watch program: chlorinated pesticides and PCB's in oysters (Crassostrea virginica) and sediments from the Gulf of Mexico, 1986-1987. Mar. Environ. Res., 29:161-203.

SERICANO, J.L., T.L. WADE, AND J.M. BROOKS. in press a. The usefulness of transplanted oysters in biomonitoring studies. Proc. Coastal Society 12th International Conf., San Antonio, Texas.

SERICANO, J.L., T.L. WADE, E.N. POWELL, AND J.M. BROOKS. in press b. Concurrent chemical and histological analyses: are they compatible? Chem. Ecol.

SHUSTER JR., C.N., AND B.H. PRINGLE. 1969. Trace metal accumulation by the American eastern oyster, Crassostrea virginica. Proc. Natl. Shellfish. Assoc., 59:91-103.

SINDERMANN, C.J. 1983. An examination of some relationships between pollution and disease. Rapp. P-V. Réun. Cons. Int. Explor. Mer, 182:37-43.

SOLBAKKEN, J.E., F.M.H. JEFFREY, A.H. KNAP, AND K.H. PALMORK. 1982. Accumulation and elimination of [9-^{14}C]phenanthrene in the calico clam (Macrocallista maculata). Bull. Environ. Contam. Toxicol., 28:530-534.

SONIAT, T.M., AND J.D. GAUTHIER. 1989. The prevalence and intensity of Perkinsus marinus from the mid northern Gulf of Mexico, with comments on the relationship of the oyster parasite to temperature and salinity. Tulane Stud. Zool. Bot., 27:21-27.

STEGEMAN, J.J., AND J.M. TEAL. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster Crassostrea virginica. Mar. Biol. (Berl.), 22:37-44.

WADE, T.L., E.L. ATLAS, J.M. BROOKS, M.C. KENNICUTT II, R.G. FOX, J. SERICANO, B. GARCIA-ROMERO, AND D. DEFREITAS. 1988. NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediments and oysters. Estuaries, 11:171-179.

WADE, T.L., AND J.L. SERICANO. 1989. Trends in organic contaminant distribution in oysters from the Gulf of Mexico. p. 585-589. In Proc. Oceans '89 Conf., Seattle, Washington.

WHITE, M.E., E.N. POWELL, S.M. RAY, E.A. WILSON, AND C.E. ZASTROW. 1988. Metabolic changes induced in oysters (Crassostrea virginica) by the parasitism of Boonea impressa (Gastropoda: Pyramidellidae). Comp. Biochem. Physiol. A Comp. Physiol., 90:279-290.

WILSON, E.A., E.N. POWELL, AND S.M. RAY. 1988. The effect of the ectoparasitic pyramidellid snail, Boonea impressa, on the growth and health of oysters, Crassostrea virginica, under field conditions. U.S. Fish Wildl. Serv. Fish. Bull., 86:553-566.

WILSON, E.A., E.N. POWELL, M.A. CRAIG, T.L. WADE, AND J.M. BROOKS. 1990. The distribution of Perkinsus marinus in Gulf coast oysters: its relationship with temperature, reproduction, and pollutant body burden. Int. Rev. Gesamten Hydrobiol., 75:533-550.

WILSON, E.A., E.N. POWELL, T.L. WADE, R.J. TAYLOR, B.J. PRESLEY, AND J.M. BROOKS. in press. Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: the role of local and large-scale climatic controls. Helgol. Meeresunters.

LEGENDS TO TABLES

Table 1 The scale used for the analysis of gonadal stage (after GERG, 1990).

Table 2 The scale used for digestive gland atrophy.

Table 3 Means and ranges of PAH concentration and the biological parameters measured. ND, not determined.

Table 4 Best 3-variable model for each biological variable for all oysters combined (i.e. both sexes combined) and the amount of variation explained (R^2). Significant partial correlations are shown by asterisks: *, $0.05 < P < 0.1$; **, $0.025 < P < 0.05$; ***, $0.01 < P < 0.025$; ****, $0.001 < P < 0.01$; *****, $0.0001 < P < 0.001$.

Table 5 Best 3-variable model for each biological variable for female oysters and the amount of variation explained (R^2). Analyses were conducted with and without gonadal quantity included. Significant partial correlations are shown by asterisks, as defined in Table 4.

Table 6 Best 3-variable model for each biological variable for male oysters and the amount of variation explained (R^2). Significant partial correlations are shown by asterisks, as defined in Table 4.

Table 7 Best 3-variable model for each PAH for all oysters combined and the amount of variation explained (R^2). Significant partial correlations are shown by asterisks, as defined in Table 4.

Table 8 Best 3-variable model for each PAH for female oysters and the amount of variation explained (R^2). Analyses were conducted with and without gonadal quantity included. Significant partial correlations are shown by asterisks, as defined in Table 4.

Table 9 Best 3-variable model for each PAH for male oysters and the amount of variation explained (R^2). Significant partial correlations are shown by asterisks, as defined in Table 4.

Table 10 PAH concentrations in pooled samples of purified oyster eggs, purified sperm and somatic tissue (in ppb).

Table 11 Pesticide concentrations in pooled samples of purified oyster eggs, purified sperm and somatic tissue (in ppb).

Table 1

Developmental Stage	Assigned Numerical Value	Description
Sexually Undifferentiated	1	Little or no gonadal tissue visible
Early Development	2	Follicles beginning to expand
Mid-Development	3	Follicles expanded and beginning to coalesce; no mature gametes present
Late Development	4	Follicles greatly expanded, coalesced, but considerable connective tissue remaining; some mature gametes present
Fully Developed	5	Most gametes mature; little connective tissue remaining
Spawning	6	Gametes visible in gonoducts
Spawned	7	Reduced number of gametes; some mature gametes still remaining; evidence of renewed reproductive activity
Spawned	8	Few or no gametes visible, gonadal tissue atrophying

Table 2

Assigned Numerical Value	Description
0	normal
1	less than one-half atrophied
2	about one-half atrophied
3	greater than one-half atrophied
4	completely atrophied.

Table 3

	Length (cm)	Condition Code	*P. marinus* Infection Intensity	Wet Weight (g)	Gonadal Stage	Digestive Gland Atrophy	Gonad Quantity (mg dry wt.)	Fluoranethene (ppb)	Phenanthrene (ppb)	Naphthalene (ppb)	Pyrene (ppb)	Chrysene (ppb)
Mean	8.0	4.3	1.45	9.6	5.5	2.1	ND	48.95	11.98	24.60	26.01	21.70
Range	4.8-10.5	2-6	0.-3.33	5.9-20.9	2-7	0-4	ND	13.-104.8	5.2-52.0	14.1-83.8	8.-56.3	5.7-41.1
Mean Male	8.1	4.6	0.77	8.6	5.3	1.8	ND	38.47	14.08	28.84	21.34	21.59
Mean Female	7.9	4.2	1.67	9.9	5.6	2.2	6.29	52.44	11.28	23.19	27.57	21.74

Table 4

Variable	R^2	Explanatory Variable (N=39)
Perkinsus marinus infection intensity	.18	Condition code
		Wet weight
		Sex***
Digestive gland atrophy	.14	Length
		Condition code
		Gonadal stage **
Sex	.21	Length
		Condition code
		P. marinus infection intensity ***
Gonadal stage	.34	Condition code *
		Wet weight ****
		Digestive gland atrophy *
Condition code	.15	Gonadal stage *
		Wet weight **
		Digestive gland atrophy

Table 5

Variable	With Gonadal Quantity (N=23)		Without Gonadal Quantity (N=29)	
	R^2	Explanatory Variable	R^2	Explanatory Variable
Gonadal stage	.54	Length	.47	Condition code
		Condition code**		Wet weight *****
		Wet weight***		Digestive gland atrophy
Condition code	.23	Length	.11	Gonadal stage
		Gonadal state **		Wet weight
		Wet weight ***		Digestive gland atrophy
Perkinsus marinus infection	.22	Length	.06	Condition code
intensity		Gonadal stage		Gonadal stage
		Digestive gland atrophy		Digestive gland atrophy
Digestive gland atrophy	.16	*Perkinsus marinus* infection	.11	Condition code
		intensity		Wet weight
		Length		Gonadal stage
		Wet weight		
Gonadal quantity			.07	*Perkinsus marinus* infection
				intensity
				Wet weight
				Digestive gland atrophy

Table 6

Variable	R^2	Explanatory Variable (N=10)
Gonadal stage	.70	Length
		Wet weight
		Perkinsus marinus infection intensity***
Condition code	.20	Length
		Wet weight
		Perkinsus marinus infection intensity
Perkinsus marinus infection intensity	.74	Length*
		Wet weight**
		Digestive gland atrophy****
Digestive gland atrophy	.80	*Perkinsus marinus* infection intensity****
		Length**
		Wet weight***

Table 7

Variable	R^2	Explanatory Variable
Fluoranthene	.20	Length
		Perkinsus marinus infection intensity
Phenanthrene	.11	Sex**
		Condition code
		Gonadal stage
Naphthalene	.20	Sex
		Condition code***
		Gonadal stage
Pyrene	.19	Sex
		Length
		Perkinsus marinus infection intensity
Chrysene	.14	Sex**
		Perkinsus marinus infection intensity
		Wet Weight
		Sex

Table 8

	With Gonadal Quantity		Without Gonadal Quantity	
Variable	R^2	Explanatory Variable	R^2	Explanatory Variable
Fluoranthene	.37	Condition code	.18	Length
		Wet Weight		Condition code
		Gonadal quantity***		Perkinsus marinus
				infection intensity
Phenanthrene	.18	Perkinsus marinus	.16	Condition code
		infection intensity		Perkinsus marinus
		Digestive gland atrophy		infection intensity
		Gonadal quantity		Digestive gland atrophy
Naphthalene	.21	Length	.27	Length***
		Digestive gland atrophy		Perkinsus marinus
		Gonadal quantity		infection intensity
				Digestive gland atrophy*
Pyrene	.31	Gonadal quantity**	.20	Length
		Digestive gland atrophy		Condition code
		Gonadal stage		Perkinsus marinus
				infection intensity
Chrysene	.51	Length***	.25	Perkinsus marinus
		Digestive gland atrophy**		infection intensity*
		Gonadal quantity*****		Wet weight*
				Digestive gland atrophy

Table 9

Variable	R^2	Explanatory Variable
Fluoranthene	.49	Length
		Gonadal stage
		Digestive gland atrophy*
Phenanthrene	.67	Condition code**
		Gonadal stage
		Digestive gland atrophy
Naphthalene	.73	Condition code***
		Gonadal stage*
		Digestive gland atrophy
Pyrene	.68	Length
		Gonadal stage
		Digestive gland atrophy***
Chrysene	.59	Condition code
		Gonadal stage
		Digestive gland atrophy*

Table 10

	Group A		Group B		Group C		Group D		Group E	
	Eggs	Tissue	Eggs	Tissue	Eggs	Tissue	Sperm	Tissue	Sperm	Tissue
Naphthalene	45.1	9.0	51.9	8.9	42.5	5.9	64.8	12.3	70.5	12.3
Phenanthrene	23.5	2.9	26.9	4.1	29.0	3.4	26.1	5.6	29.9	5.6
Fluoranthene	16.1	2.9	15.8	3.0	17.7	3.2	11.6	3.3	17.6	3.3
Pyrene	20.7	3.7	18.4	3.7	18.2	3.8	13.1	4.0	18.1	4.0
Chrysene	11.5	2.4	12.5	2.0	10.9	2.2	7.2	2.4	16.6	2.4

Table 11

	Group A		Group B		Group C		Group D		Group E	
	Eggs	Tissue	Eggs	Tissue	Eggs	Tissue	Sperm	Tissue	Sperm	Tissue
Lindane	9.4	2.06	5.5	2.2	8.2	1.8	<1.0	2.2	<1.0	2.2
Total BHCs	14.7	5.0	9.5	5.2	14.0	3.9	<1.0	5.2	2.4	5.2
α-chlordane	6.5	3.8	5.0	3.8	5.1	2.4	<1.0	4.5	3.6	4.5
Dieldrin	6.3	2.2	6.1	1.9	5.8	1.7	<1.0	1.8	1.7	1.8
4,4' DDE	32.1	9.1	26.0	8.2	26.7	7.5	4.1	11.9	6.6	11.9
4,4' DDD	12.3	3.7	11.7	3.2	12.5	3.1	<1.0	3.6	3.5	3.6
Total PCBs	132.6	36.5	147.8	33.5	113.0	29.6	114.2	53.8	102.3	53.8

4.0 Chlorinated Hydrocarbons

The concentration of selected chlorinated hydrocarbons has been measured for six years (1986-1991) in oyster samples from 50 to 71 Gulf of Mexico sites as part of the NOAA National Status and Trends (NS&T) Mussel Watch project. The results for pesticides and PCBs as the mean of years 1 to 5 verses year 6 are plotted in Figures 4.1 to 4.19. Oysters are valuable sentinel organisms that reflect contamination of an ecosystem on time scale of months. These sites, removed from known point-sources of contamination, give coverage of U.S. Gulf of Mexico coastal areas from southern-most Texas to southern-most Florida. General overviews of the results of the NOAA's NS&T Program pesticide and PCB data have already been reported (Table 1.1 and Reprint 7).

Total DDT (sum of o-p'DDE + p-p'DDE + o-p'DDD + p-p'DDD + o-p'DDT + p-p'DDT) data for oysters collected along the U.S. Gulf of Mexico coast between 1986 and 1989 is shown in Figure 4.10. Total DDT is the most abundant chlorinated pesticide found in Gulf of Mexico oysters. Most of the DDT is present as metabolites, DDE and DDD. Less than 10% of the total contaminant load in oysters is the parent compound, DDT. The highest total DDT concentrations were encountered in samples near the Brazos River mouth (BRFS) and Galveston Bay (GBSC) in Texas, Mississippi River (MRPL and LPGO) in Louisiana, Mobile Bay (MBHI) in Alabama, and Choctawhatchee (CBSP and PCLO) and St. Andrews Bays (SAWB) in Florida. With few exceptions, total DDT concentrations were consistently low in samples from southern Texas, Louisiana sites to the west of the Mississippi River, and southernmost Florida. The general distribution of total DDT concentrations encountered during 1991 in the Gulf of Mexico was very similar to the distribution for the 1986-1990 sampling period. Total DDT concentrations measured during Year 6 were lower or the same as those encountered in the average of the previous five years of this study at all except five sites. The incorporation of new sampling sites, located closer to supposed contaminating centers, during 1989, 1990, and 1991 added more details to the overall DDT distribution in the northern Gulf of Mexico, but did not greatly affect the regional distribution. In general, the average concentrations measured at those sites were in good agreement with the concentrations previously reported for the surrounding geographical area. A greater fraction of DDT is found as DDE in the general region south of Galveston Bay along the Texas coast. This may be related to an "older" source of DDTs in the South Texas area, but additional data is required to examine this hypothesis.

The concentrations of hexachlorobenzene (HCB, Figure 4.1) were generally low (maximum was 2.10 ng/g). These values are very close to the method detection limit and analytical difficulties make

interpretation of this data problematic. This same problem is found when attempting to interpret the data for lindane, heptachlor, aldrin, heptachlor epoxide and mirex (Figures 4.2, 4.3, 4.4, 4.5 and 4.9).

Chlordane and its breakdown products are represented by α-chlordane (Figure 4.6) and trans-nonachlor (Figure 4.7). The chlordane distribution is similar to the DDT distribution with low concentrations in Southern Texas, highs in Galveston Bay, near the Mississippi River, and in Florida Bays. Details of the chlodane distribution have been reported (Preprint 3). Dieldrin (Figure 4.8) has a distribution similar to chlodane, with the exception that the Florida sites are not as high relative to the Galveston Bay and Mississippi River sites.

Endrin was measured in NS&T oysters for the first time in Year 5. The concentrations ranged from below detection (~1 ng/g) to 30.6 ng/g. The concentrations are lower in southernmost Texas and Florida, with higher concentrations in the northern regions of the Gulf Coast. Endrin concentrations were in general lower in year 6 compared to year 5. Endrin does not appear to have a simlar distribution to other chlorinated hydrocarbons.

The general trend of chlorinated hydrocarbon concentrations is relatively constant at most sites with episodic increases and decreases at selected sites. These episodic changes are probably due to site specific input events. However, Gulf-wide temporal changes have been reported (Reprint 1).

PCBs proved to be ubiquitous contaminants in Gulf of Mexico oysters. PCB congeners were detected in all NS&T samples analyzed (Figure 4.18). PCB concentrations were higher at 18 sites in year 6 compared to the mean of years 1 to 5. As in the case of DDTs, the addition of new locations to the sampling project did not greatly modify the general distribution of average PCB concentrations in Gulf of Mexico oysters described for the first three years of the NS&T project. A possible exception could be Tampa Bay (TBKA), which had average PCB concentrations clearly higher than the surrounding sites. The new site for year 6 (LPGO) has the highest concentration reported.

GERG has recently taken a closer look at the consequence of removing part of the oyster sample that was collected for organic analyses to use in biological testing (Preprint 4). While it would not affect the overall interpretation of NS&T data, it does add a bias into the data. More details are available in the attached Preprint 3. GERG has also recently developed methods for analyses of the most toxic planar PCB congeners and applied these techniques to selected NS&T samples (Preprint 5).

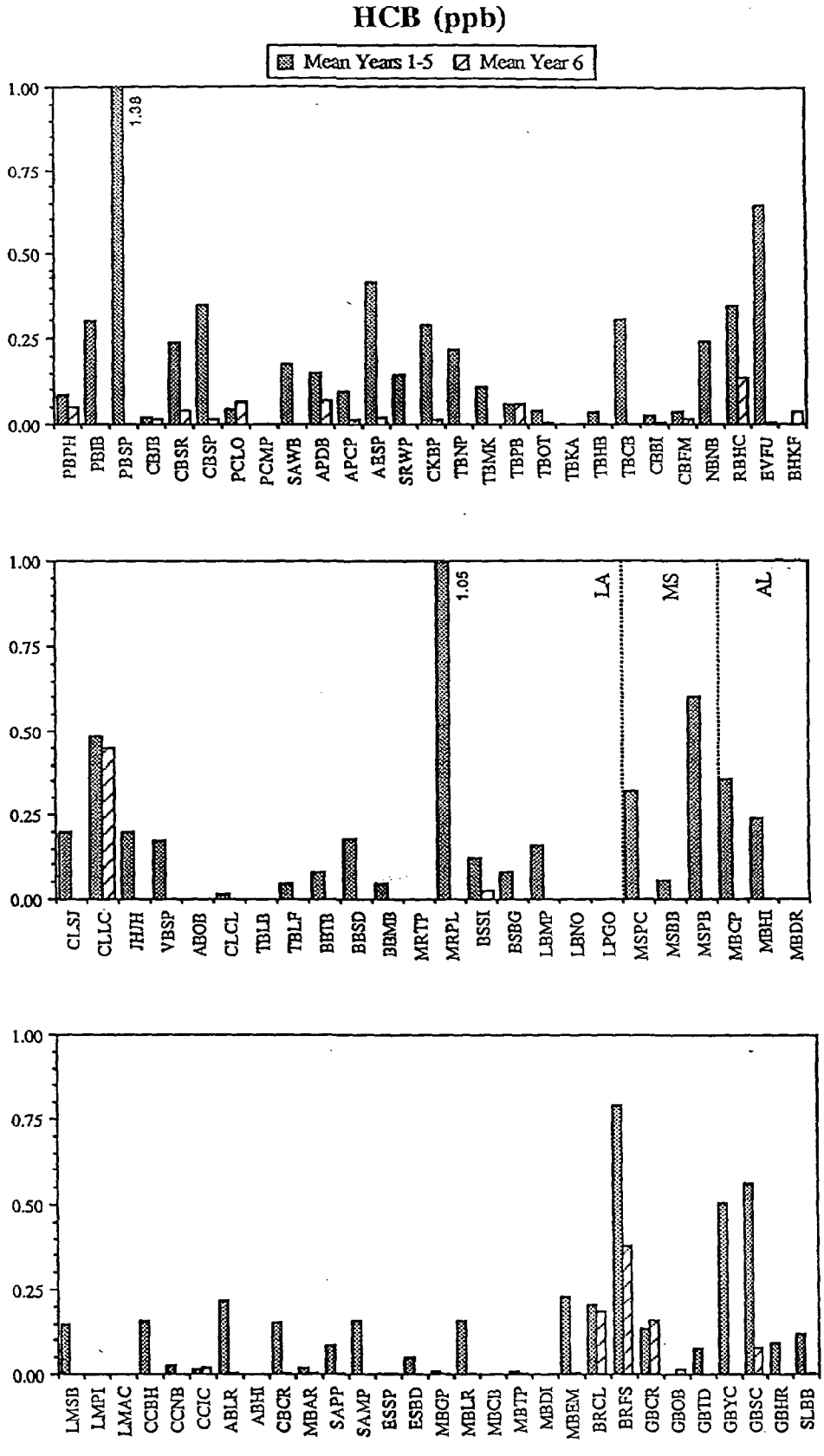


Figure 4.1 Average HCB concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

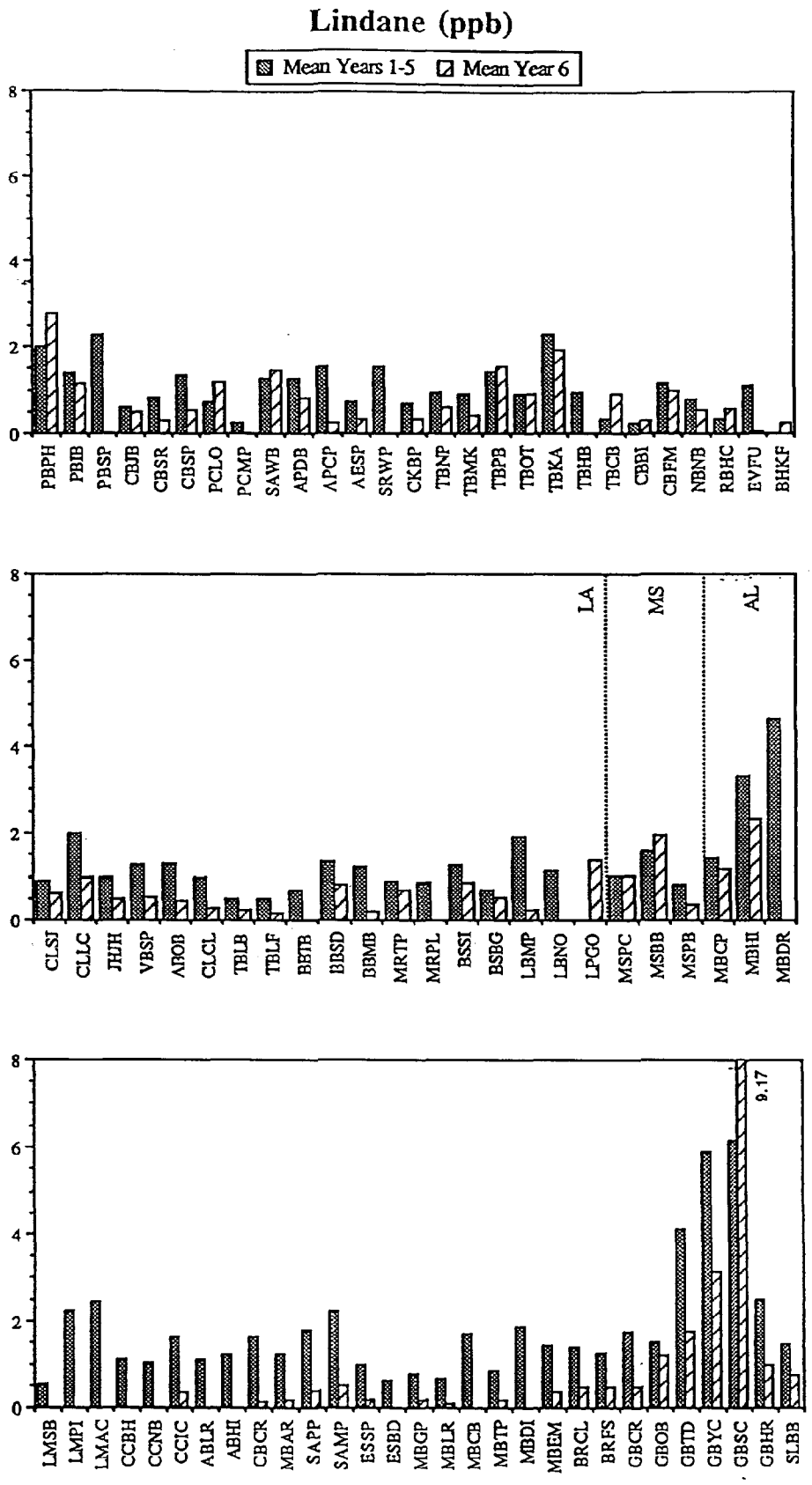


Figure 4.2 Average lindane concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

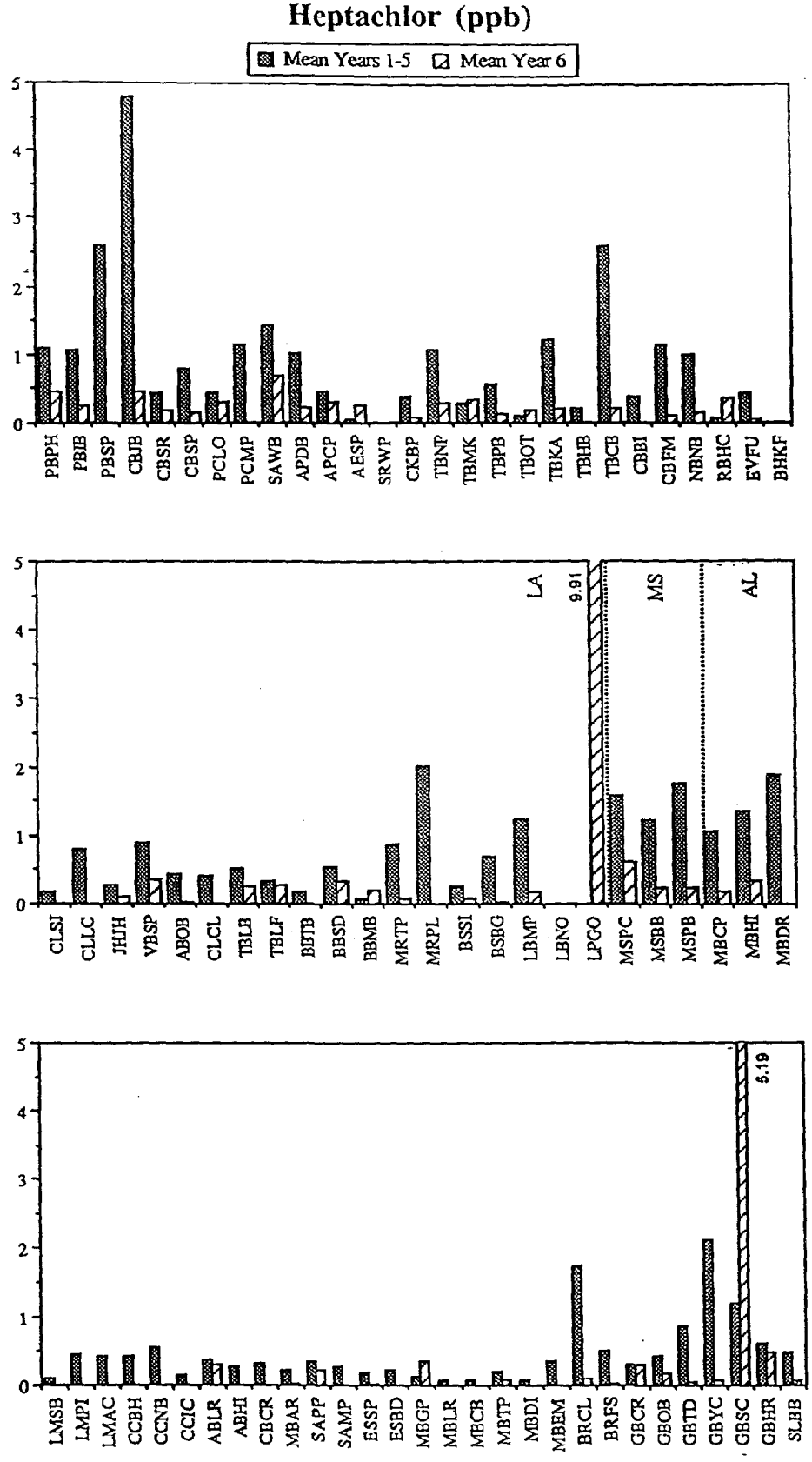


Figure 4.3 Average heptachlor concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

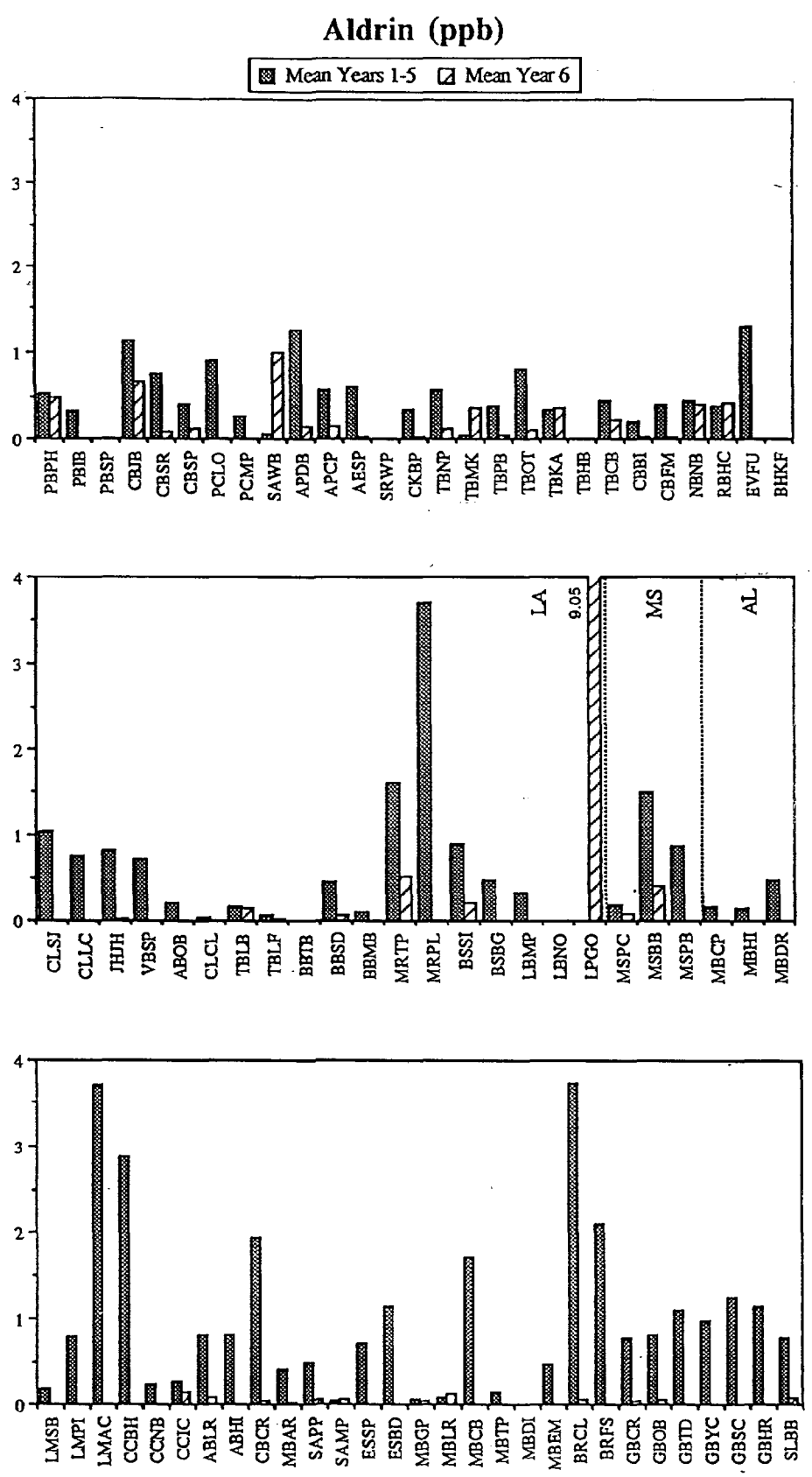


Figure 4.4 Average aldrin concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

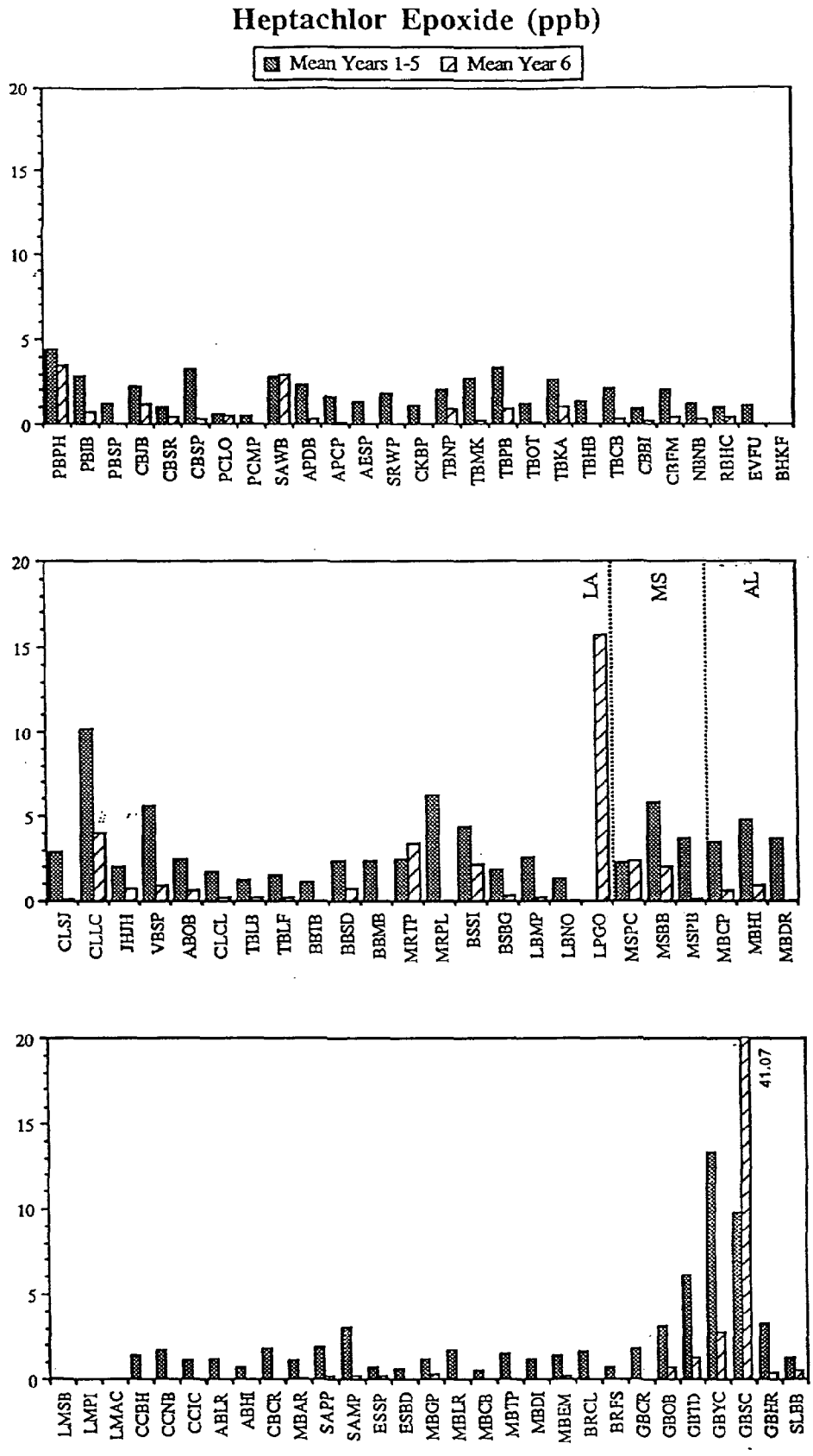


Figure 4.5 Average heptachlor epoxide concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

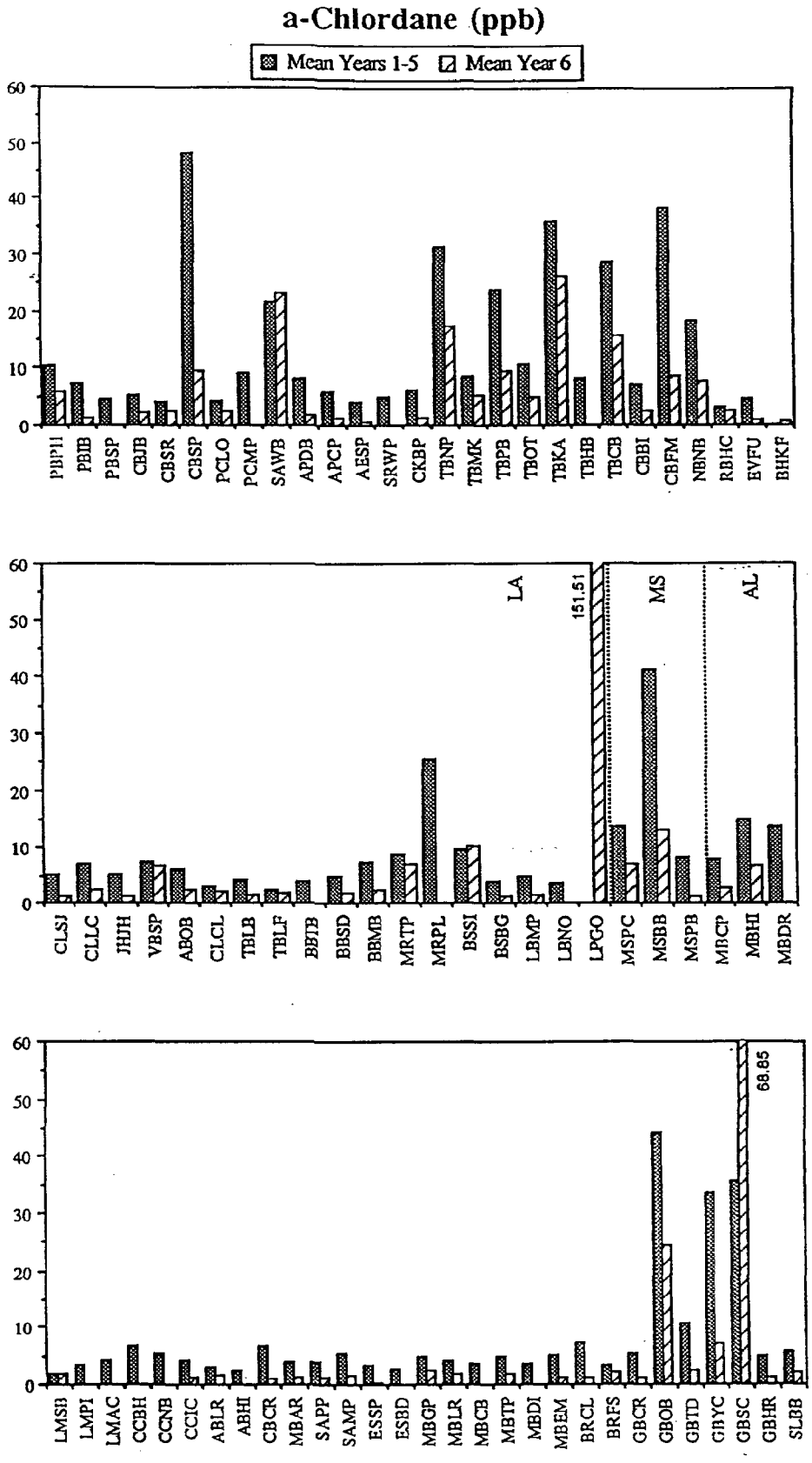


Figure 4.6 Average a-chlordane concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

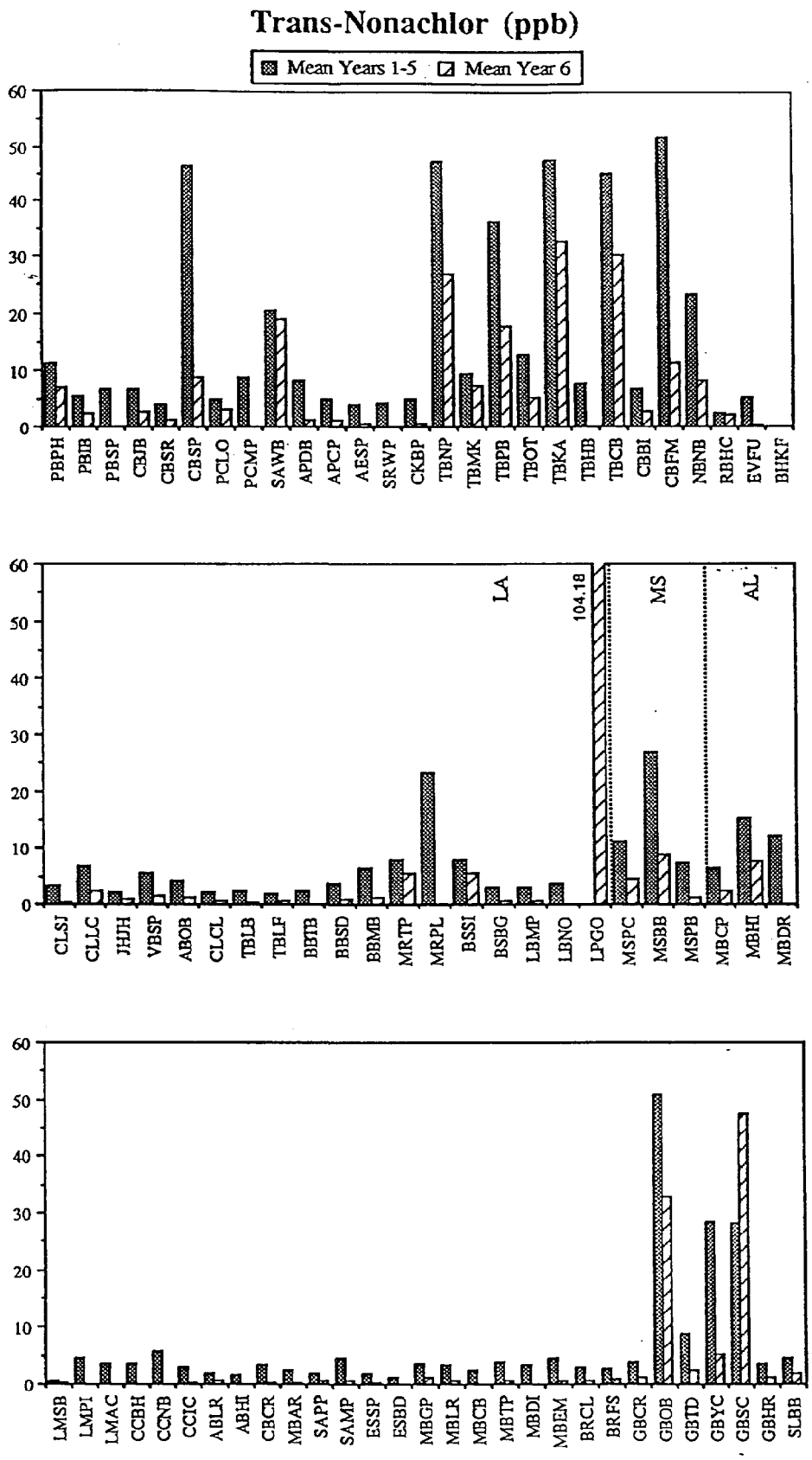


Figure 4.7 Average trans-nonachlor concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

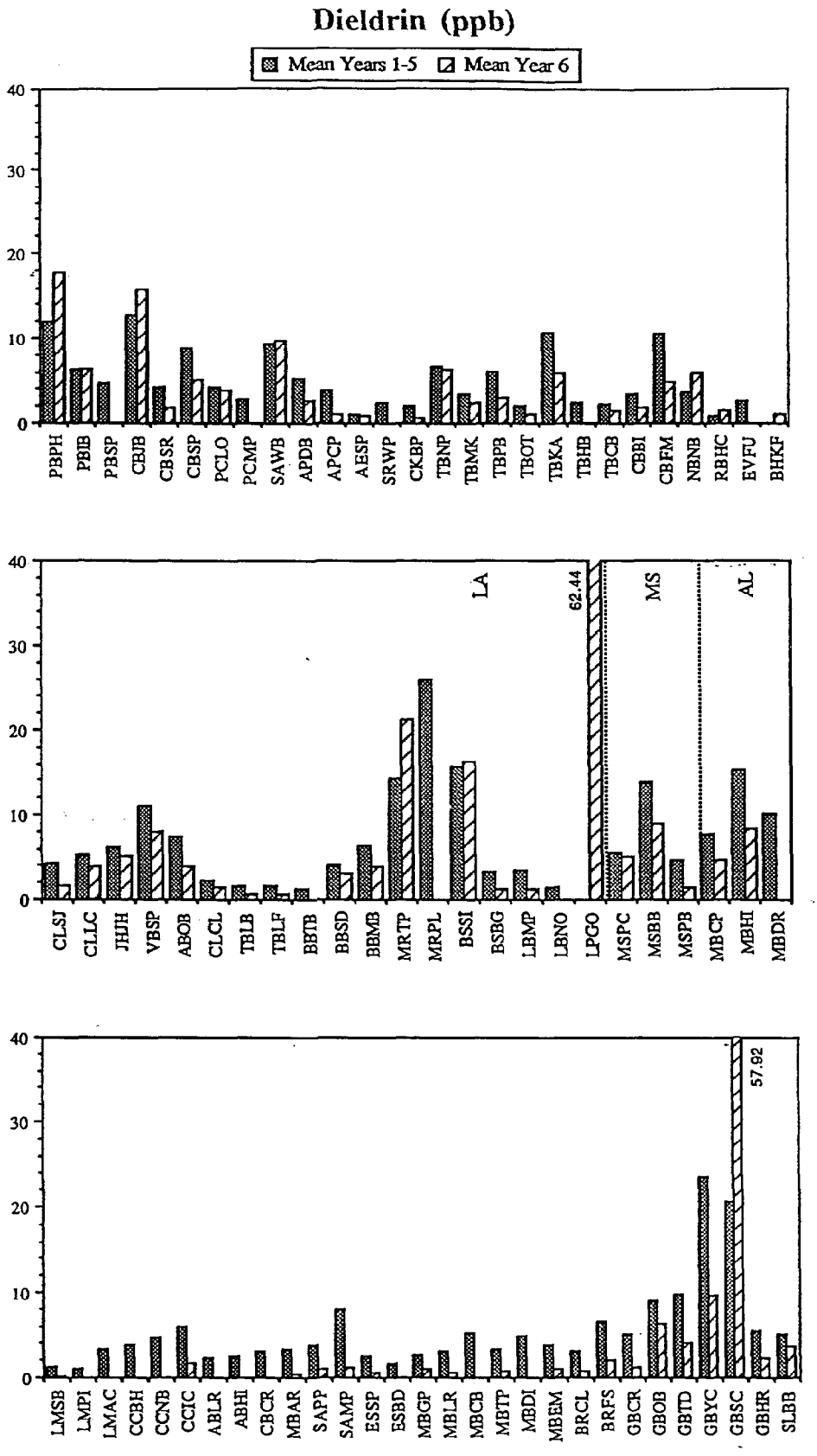


Figure 4.8 Average dieldrin concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

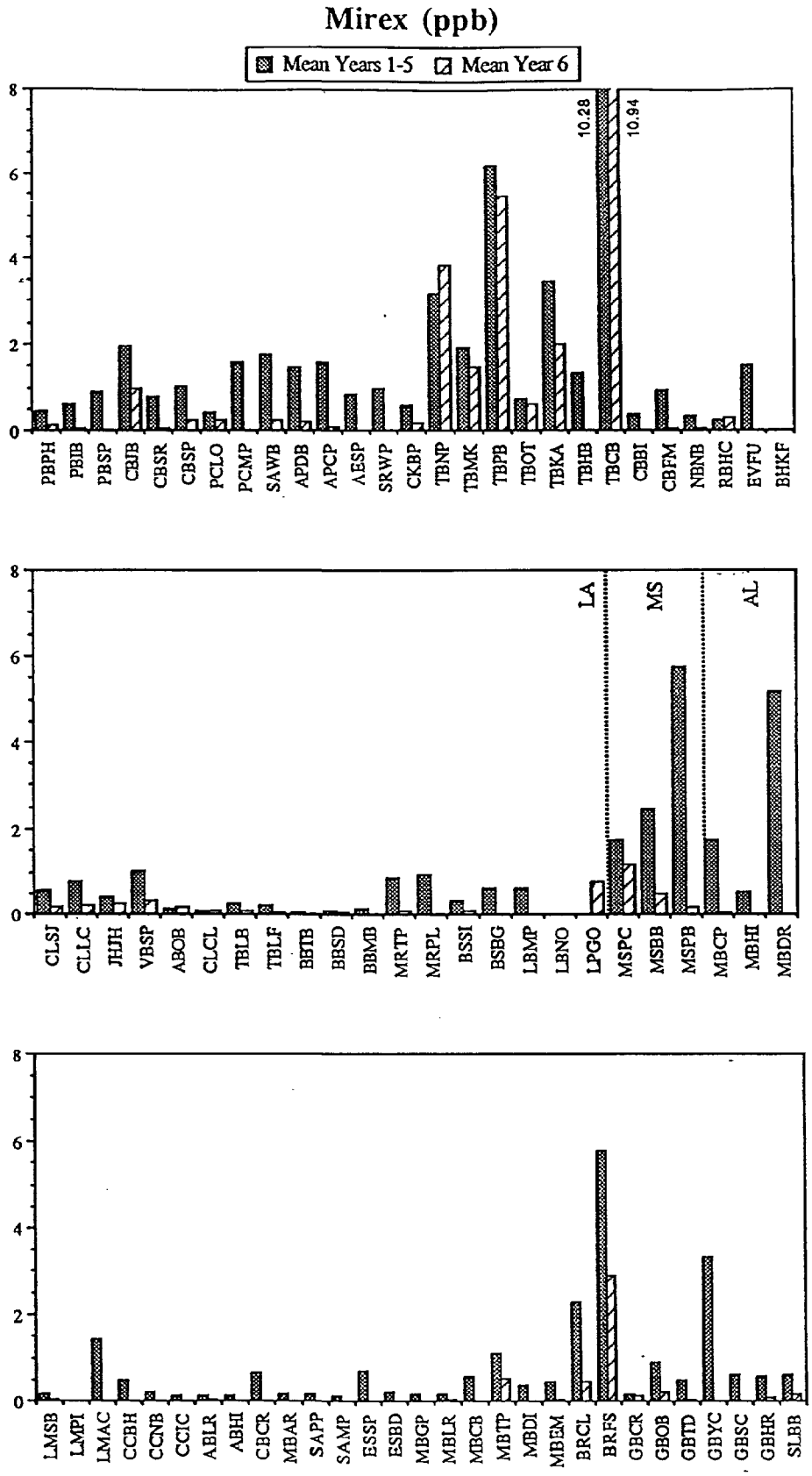


Figure 4.9 Average mirex concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

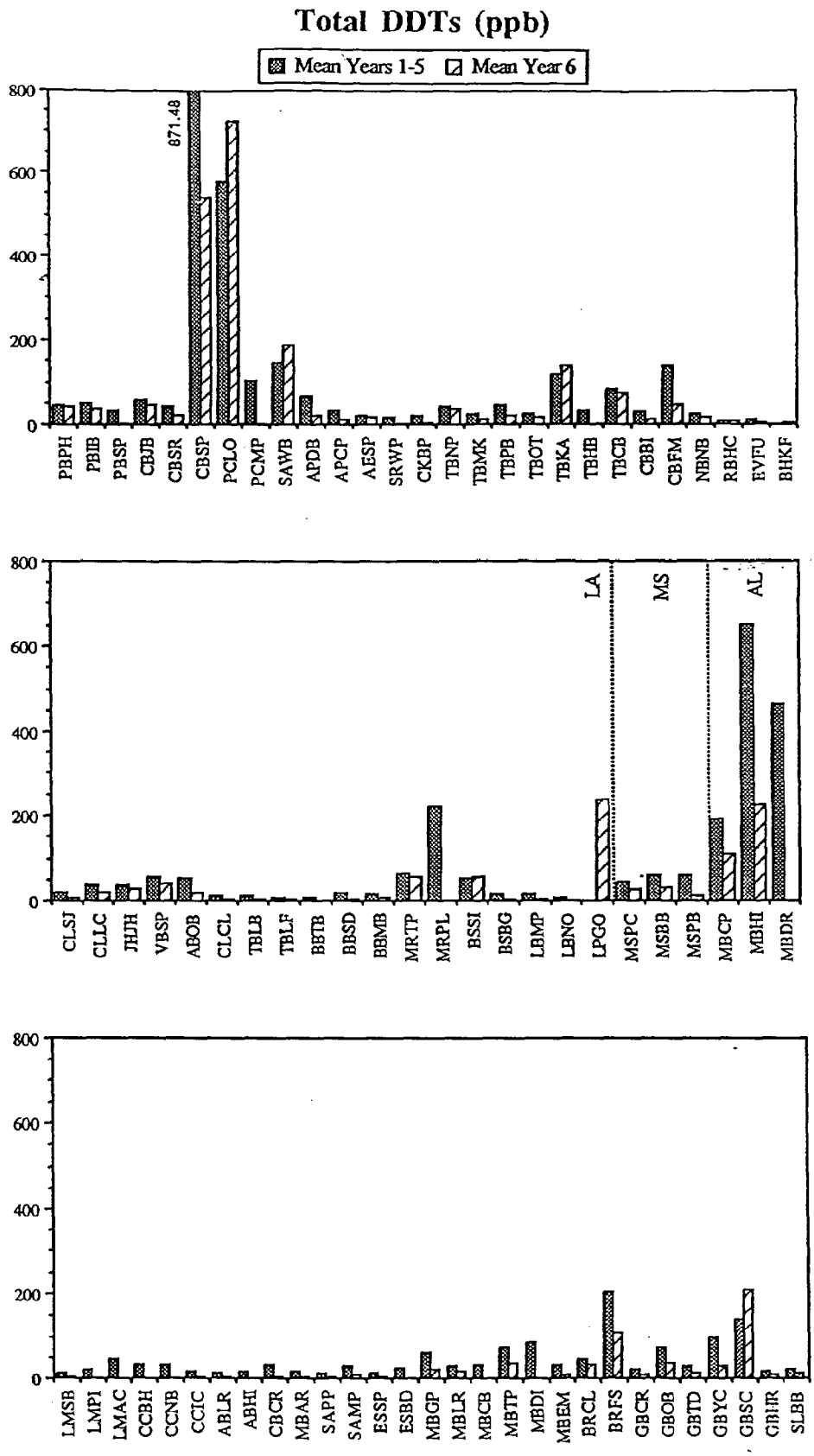


Figure 4.10 Average total DDTs concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

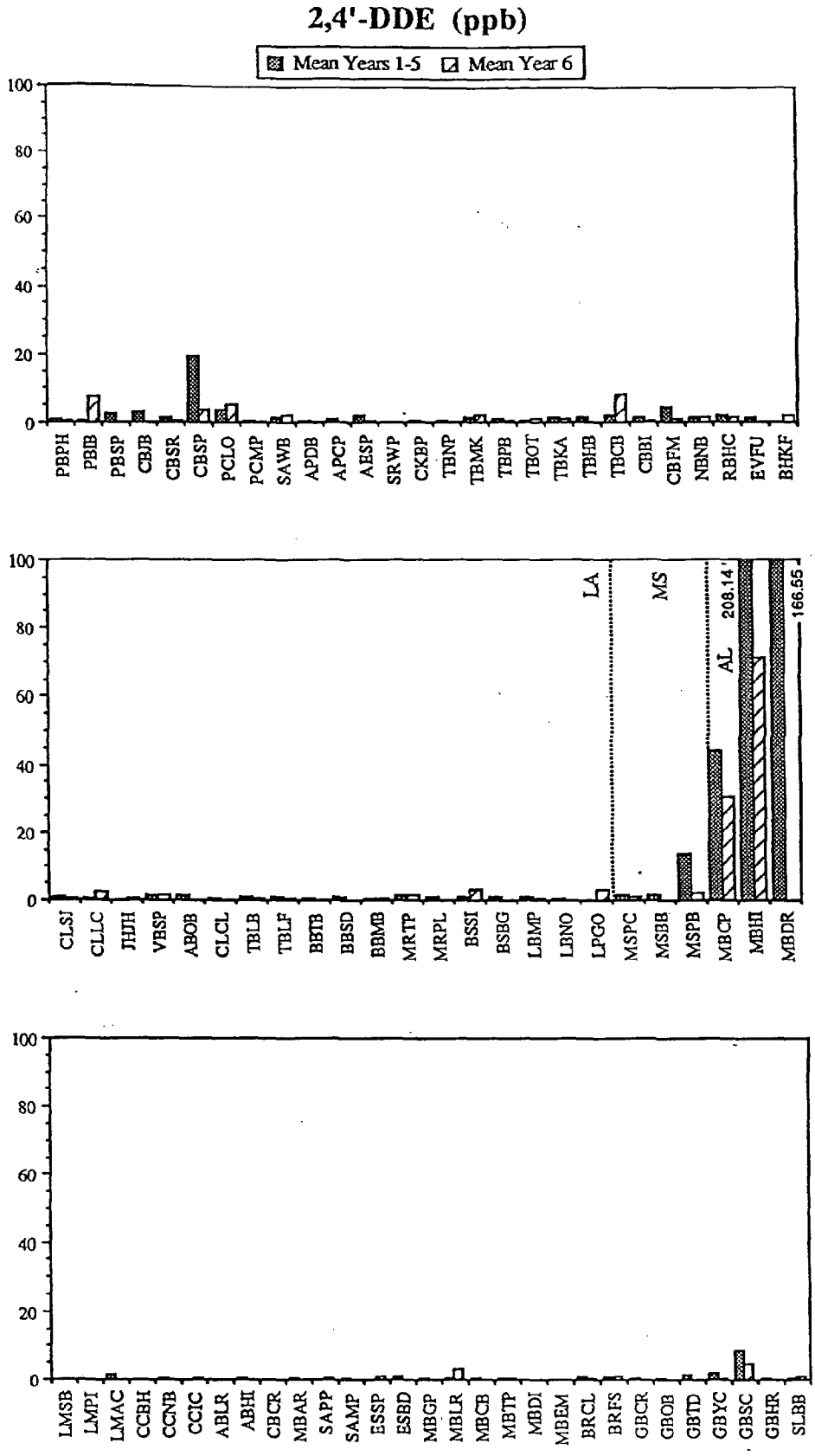


Figure 4.11 Average 2,4' DDE concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

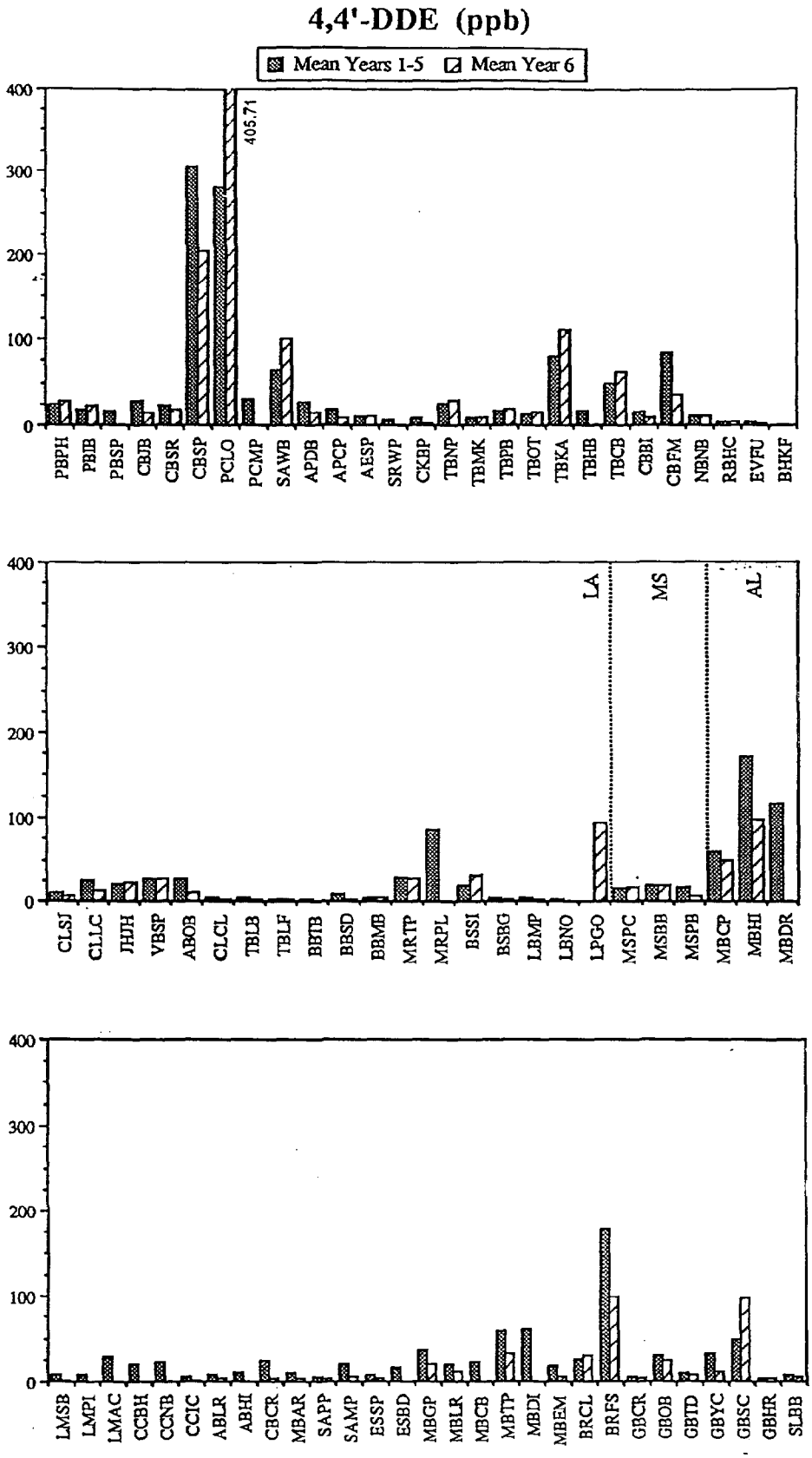


Figure 4.12 Average 4,4' DDE concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

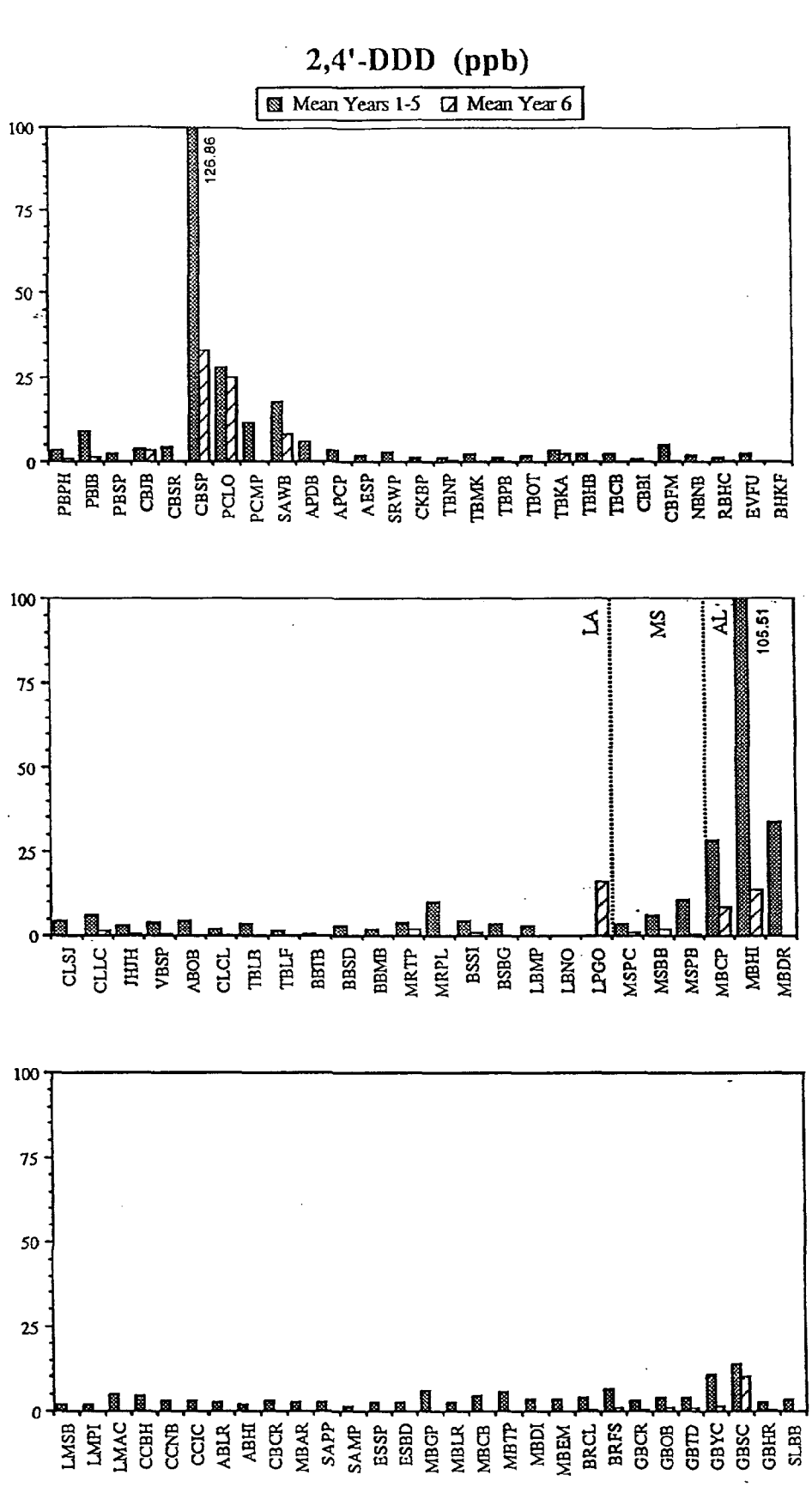


Figure 4.13 Average 2,4' DDD concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

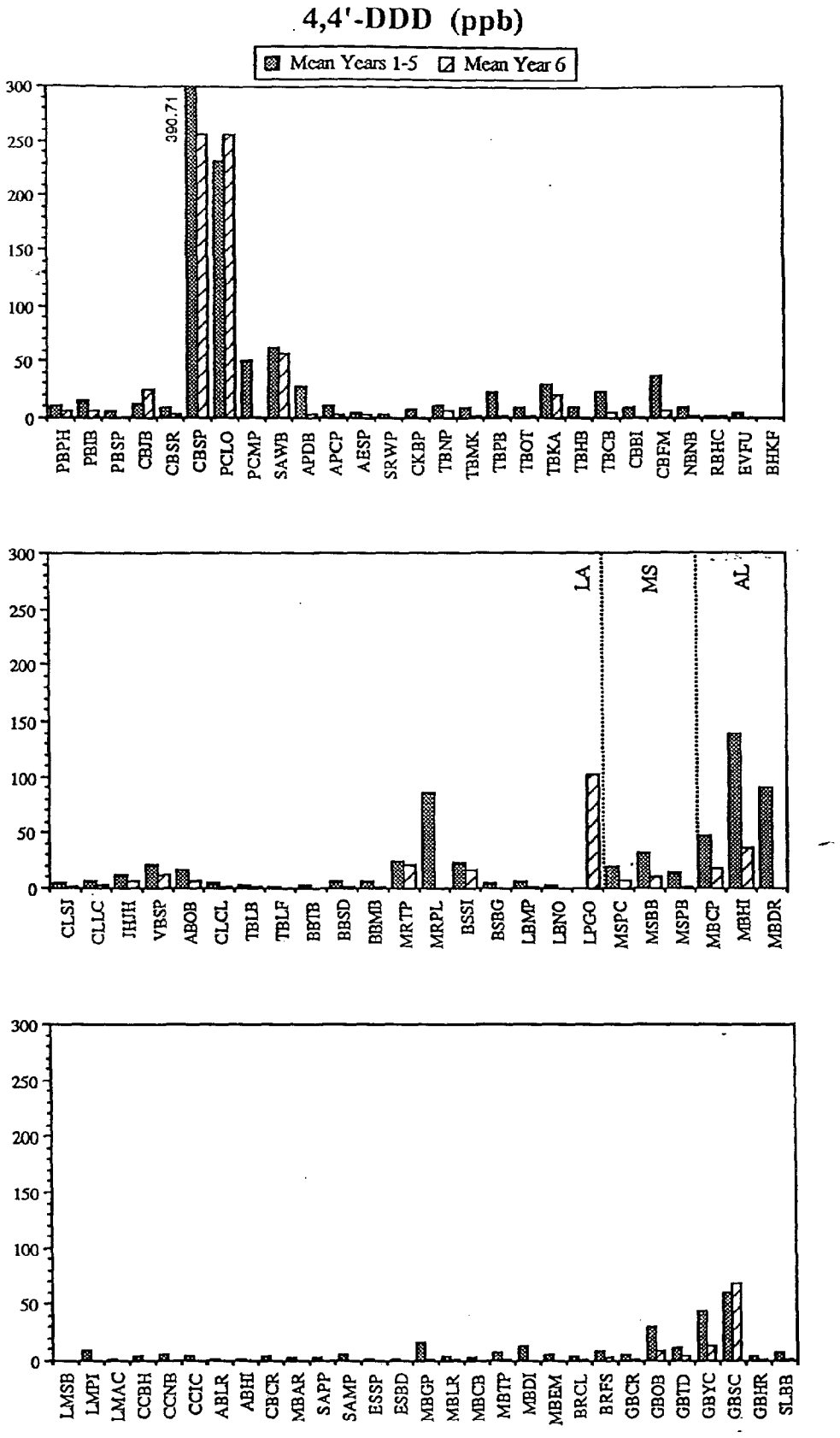


Figure 4.14 Average 4,4' DDD concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

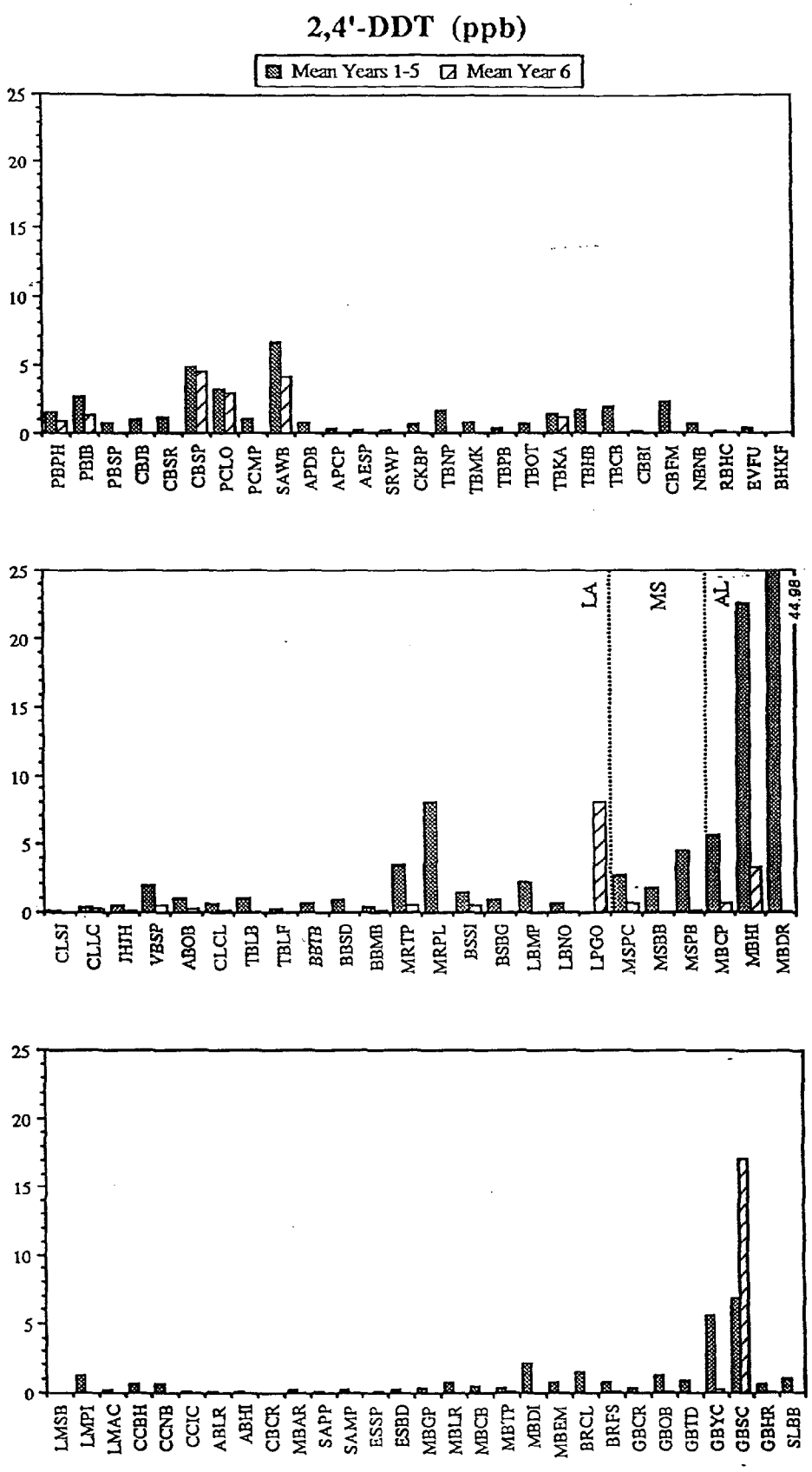


Figure 4.15 Average 2,4' DDT concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

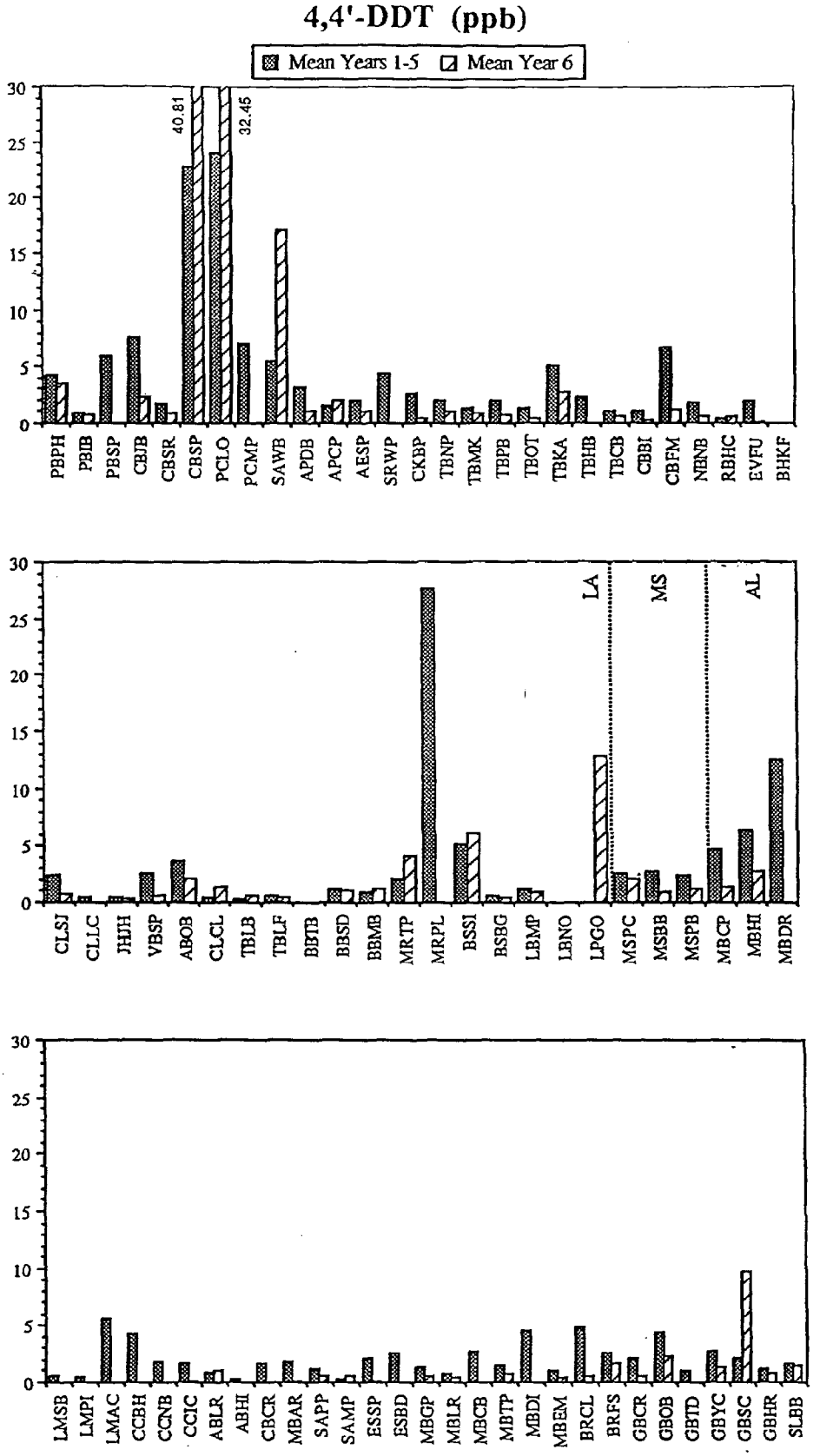


Figure 4.16 Average 4,4' DDT concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

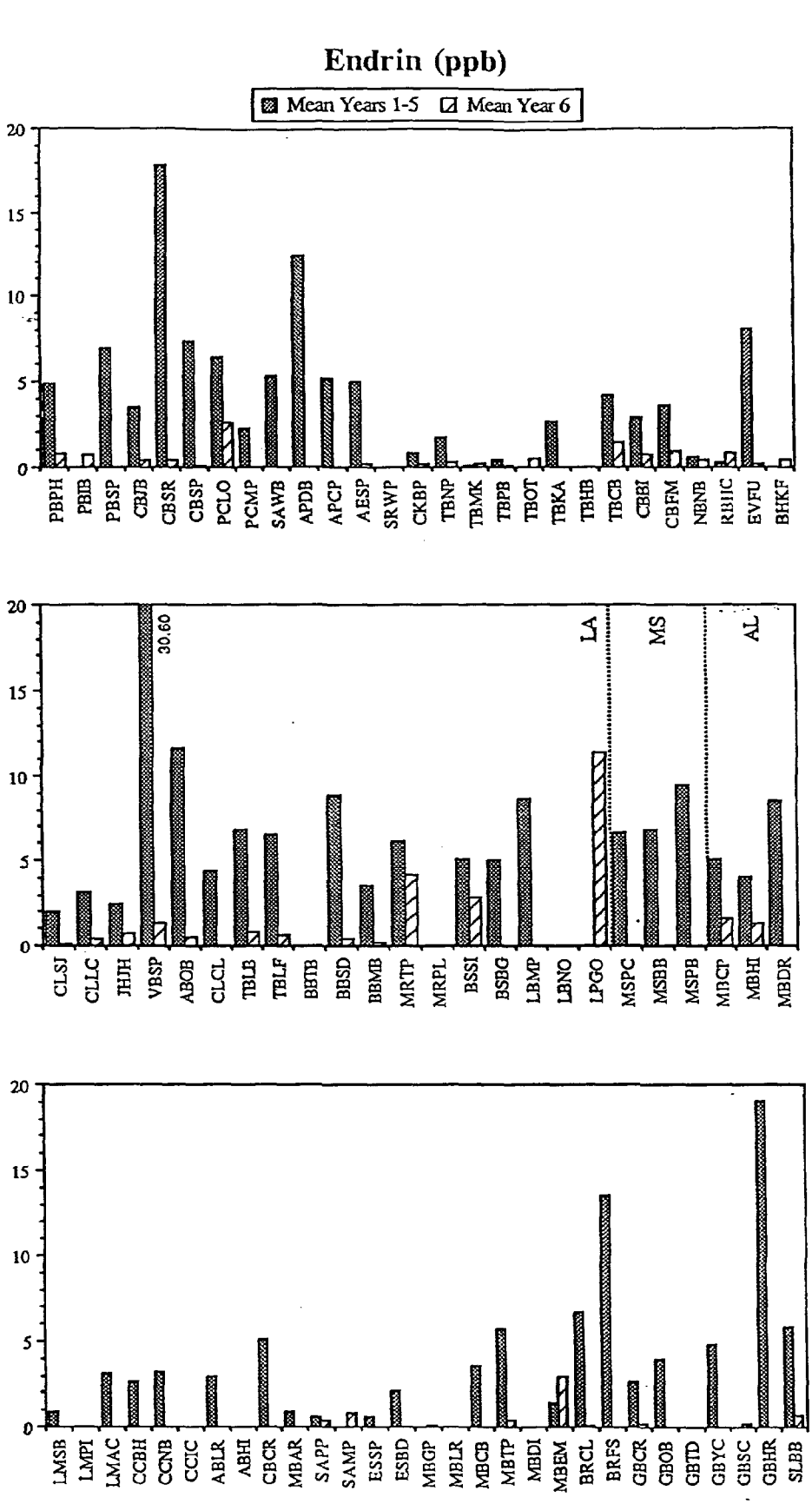


Figure 4.17 Average endrin in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

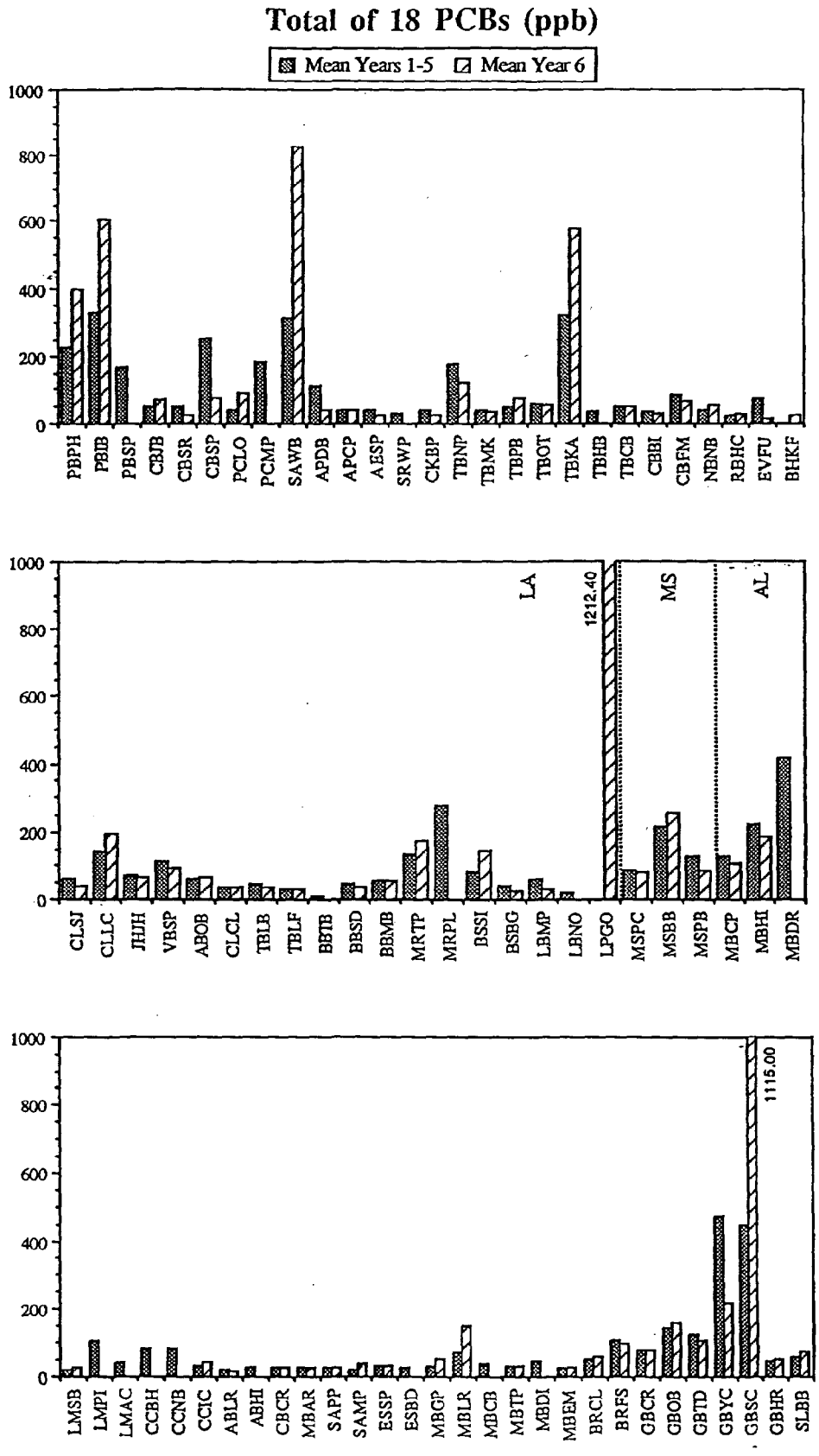


Figure 4.18 Average total of 18 PCB concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6. 4-20

Reprint 7

Chlorinated Hydrocarbons in Gulf of Mexico Oysters: Overview of the First Four Years of the NOAA's National Status and Trends Mussel Watch Program (1986-1989)

J.L. Sericano, T.L. Wade, and J.M. Brooks

Chlorinated Hydrocarbons in Gulf of Mexico Oysters: Overview of the First Four Years of the NOAA's National Status and Trends Mussel Watch Program (1986-1989)

J.L. Sericano, T.L. Wade, J.M. Brooks
Geochemical and Environmental Research Group, College of Geosciences, Texas A&M University, 833 Graham Road, College Station, Texas 77845, U.S.A.

ABSTRACT

During the first four years of the NOAA's National Status and Trends Mussel Watch program selected chlorinated hydrocarbons were analyzed in more than 660 oyster samples from the northern coast of the Gulf of Mexico. Chlordane-related compounds, DDT and its metabolites and PCB congeners were detected at all the locations monitored. Concentrations ranged over two to three orders of magnitude. *Alpha*-chlordane and *trans*-nonachlor comprised more than 90% of the total load of chlordane-related compound in the samples. The bulk of the total DDT burden in oysters corresponded to the degradation products, DDE and DDD, while DDT isomers only accounted for a small fraction of the total load. PCB congeners corresponded mainly to the four-, five- and six-chlorine homologs. After the first four years of this program, the concentration distributions in oysters from the northern Gulf of Mexico is well defined. Temporal trends are not apparent at most sites.

INTRODUCTION

The National Oceanic and Atmospheric Administration's National Status and Trends Mussel Watch (NS&T) Program has been designed to monitor the current status and long-term trends of selected organic and inorganic environmental contaminants; e.g. chlorinated pesticides, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) and trace metals, along the coasts of the U.S.A. by measuring the concentrations of these contaminants in bivalves and sediments over several years. The rationale for the "Mussel Watch" approach using bivalves; e.g. mussels, oysters and clams, has been summarized by

different authors [1-4], and its concept has been applied to several monitoring programs during the last decade [5-10].

An overview of the concentrations of the selected chlorinated hydrocarbons analyzed in oyster samples collected during the first four years of the NOAA's NS&T program in the Gulf of Mexico are presented here. The ultimate goals of this program are to define the geographical distributions of contaminants and determine trends in their concentrations.

MATERIALS AND METHODS

Sampling

Originally, the NS&T sampling program contemplated the collection of bivalve samples from three stations at 51 sites from Gulf of Mexico coastal areas. Distances between stations within each site varied from 100 to 1000 meters. Oyster samples were collected over two- to three-month periods starting in late December or early January. Depending on the water depth, oysters were collected by hand, tongs or dredge. Twenty oysters per site were pooled in precombusted jars and frozen until analysis. During 1986 and 1987, oyster samplings were completed at 49 and 48 sites with totals of 147 and 143 samples, respectively. These sites provided a good coverage of the northern Gulf of Mexico coast from the U.S.A.-Mexico border to southernmost Florida, with an ample variety of different environmental conditions. The sites were selected to avoid known-point source of contaminants. Starting in 1988, new sites were added to the sampling program to obtain more information in areas located closer to suspected sources of contaminants. During 1988 and 1989, oyster samples were collected from 63 and 62 different locations with totals of 189 and 186 samples, respectively. By the end of the fourth sampling period, 76 sites have been visited, 41 of them in all four years (Figure 1 and Table 1).

Table 1. Sampling site locations in the Gulf of Mexico for the NOAA's Status and Trends Mussel Watch Program, 1986-1989.

	Site	General	Specific	State
1	LMSB	Laguna Madre	South Bay	Tx
2	CCNB	Corpus Christi	Nueces Bay	Tx
52	LMPI	Laguna Madre	Port Isabel	Tx
53	CCBH	Corpus Christi	Boat Harbor	Tx
3	CCIC	Corpus Christi	Ingleside Cove	Tx
54	ABHI	Aransas Bay	Harbor Island	Tx
4	ABLR	Aransas Bay	Long Reef	Tx

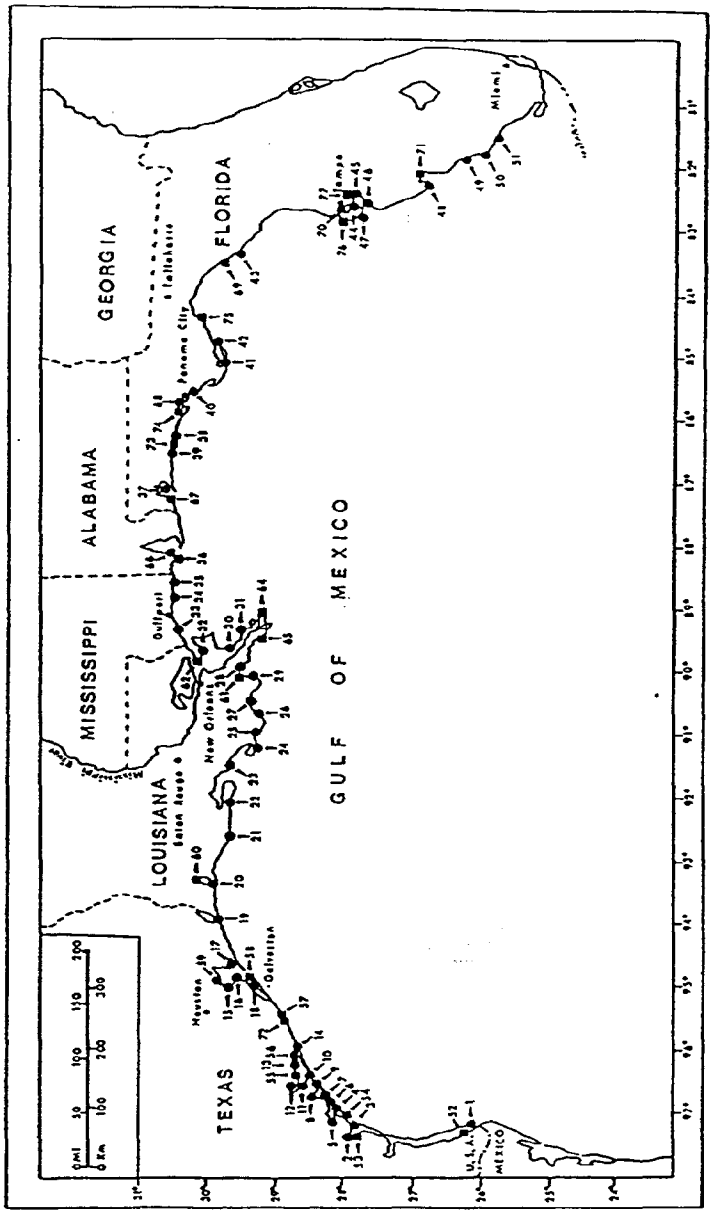


Fig. 1 Gulf of Mexico sampling site locations. Shown are the original sites (●) and the sites added to the sampling program (■)since 1988. See Table 1 for a complete site identification.

Table 1. (Continued)

	Site	General	Specific	State
5	CBCR	Copano Bay	Copano Reef	Tx
6	MBAR	Mesquite Bay	Ayres Reef	Tx
7	SAPP	San Antonio Bay	Panther Pt. Reef	Tx
8	SAMP	San Antonio Bay	Mosquito Point	Tx
9	ESSP	Espiritu Santo	South Past Reef	Tx
10	ESBD	Espiritu Santo	Bill Days Reef	Tx
11	MBLR	Matagorda Bay	Lavaca River	Tx
12	MBGP	Matagorda Bay	Galliniper Point	Tx
56	MBCB	Matagorda Bay	Carancahua Bay	Tx
13	MBTP	Matagorda Bay	Tres Palacios Bay	Tx
55	MBDI	Matagorda Bay	Dog Island	Tx
14	MBEM	Matagorda Bay	East Matagorda	Tx
57	BRFS	Brazos River	Freeport Surfside	Tx
72	BRCL	Brazos River	Cedar Lakes	Tx
15	GBYC	Galveston Bay	Yacht Club	Tx
59	GBSC	Galveston Bay	Ship Channel	Tx
58	GBOB	Galveston Bay	Offats Bayou	Tx
16	GBTD	Galveston Bay	Todd's Dump	Tx
17	GBHR	Galveston Bay	Hanna Reef	Tx
18	GBCR	Galveston Bay	Confederate Reef	Tx
19	SLBB	Sabine Lake	Blue Buck Point	Tx
20	CLSJ	Calcasieu Lake	St. John's Island	La
60	CLLC	Calcasieu Lake	Lake Charles	La
21	JHJH	Joseph Harbor Bayou	Joseph Harbor Bay	La
22	VBSP	Vermillion Bay	Southwest Pass	La
23	ECSP	East Cote Blanche	South Point	La
24	ABOB	Atchafalaya Bay	Oyster Bayou	La
25	CLCL	Caillou Lake	Caillou Lake	La
26	TBLB	Terrebonne Bay	Lake Barre	La
27	TBLF	Terrebonne Bay	Lake Felicity	La
61	BBTB	Barataria Bay	Turtle Bay	La
28	BBSD	Barataria Bay	Bayou St. Denis	La
29	BBMB	Barataria Bay	Middle Bank	La
65	MRTP	Mississippi River	Tiger Pass	La
64	MRPL	Mississippi River	Pass a Loutre	La
30	BSBG	Breton Sound	Bay Garderne	La
31	BSSI	Breton Sound	Sable Island	La
32	LBMP	Lake Borgne	Malheureux Point	La
62	LBNO	Lake Borgne	New Orleans	La
33	MSPC	Mississippi Sound	Pass Christian	Ms
34	MSBB	Mississippi Sound	Biloxi Bay	Ms
35	MSPB	Mississippi Sound	Pascagoula Bay	Ms
36	MBCP	Mobile Bay	Cedar Point Reef	Al
66	MBHI	Mobile Bay	Hollingers Is. Ch.	Al

Table 1. (Continued)

	Site	General	Specific	State
67	PBPH	Pensacola Bay	Public Harbor	Fl
37	PBIB	Pensacola Bay	Indian Bayou	Fl
38	CBSR	Choctawhatchee Bay	Off Santa Rosa	Fl
39	CBSP	Choctawhatchee Bay	Shirk Point	Fl
73	CBJB	Choctawhatchee Bay	Joe's Bayou	Fl
68	PCMP	Panama City	Municipal Pier	Fl
74	PCLO	Panama City	Little Oyster Bay	Fl
40	SAWB	St. Andrew Bay	Watson Bayou	Fl
41	APDB	Apalachicola Bay	Dry Bar	Fl
42	APCP	Apalachicola Bay	Cat Point Bar	Fl
75	AESP	Apalachee Bay	Spring Creek	Fl
69	SRWP	Suwannee River	West Pass	Fl
43	CKBP	Cedar Key	Black Point	Fl
44	TBPB	Tampa Bay	Papys Bayou	Fl
70	TBOT	Tampa Bay	Old Tampa Bay	Fl
45	TBHB	Tampa Bay	Hillsborough Bay	Fl
46	TBCB	Tampa Bay	Cockroach Bay	Fl
76	TBNP	Tampa Bay	Narvaez Park	Fl
77	TBKA	Tampa Bay	P. O'Knight Airport	Fl
47	TBMK	Tampa Bay	Mullet Key Bayou	Fl
48	CBBI	Charlotte Harbor	Bird Island	Fl
71	CBFM	Charlotte Harbor	Fort Meyers	Fl
49	NBNB	Naples Bay	Naples Bay	Fl
50	RBHC	Rookery Bay	Henderson Creek	Fl
51	EVFU	Everglades	Faka Union Bay	Fl

Analytical Procedure

The analytical procedure was adapted from a method developed by MacLeod *et al.* [11] and has been described in more details elsewhere [9,10]. Briefly, approximately 15 g of wet tissue were extracted 3 times with methylene chloride using a homogenizer (Tekmar Tissumizer). Before extraction 4:4 dibromooctafluorobiphenyl (DBOFB) and two PCBs, IUPAC numbers 103 and 198, were added to all samples, blanks and reference materials as internal standards. Tissue extracts were fractionated into two fractions by silica-alumina column chromatography. Pentane and pentane:methylene chloride (50:50) were used as elutants for the first (aliphatic hydrocarbons) and second (chlorinated and polynuclear aromatic hydrocarbons) fractions, respectively. A further clean-up of the second fraction was performed by either Sephadex LH-20 column chromatography [12] (years I, II and III) or high performance liquid chromatography (HPLC) (year IV). Both techniques have produced comparable results. Sample extracts were finally concentrated to a volume of 0.5 ml, in

hexane, for gas chromatographic analysis. Each set of eight to ten samples was accompanied by a complete system blank and spiked blank or reference material, carried through the entire analytical procedure.

Gas chromatography

Chlorinated hydrocarbon concentrations were determined by gas chromatography with an electron capture detector (GC-ECD, ^{63}Ni) using a 30 m DB-5 fused silica capillary column (0.25 um film thickness, 0.25 mm i.d.) as previously described [9,10]. Chlorinated hydrocarbon were quantitated against authentic standards injected at four different concentrations.

Quality control/Quality assurance activities, that included several laboratory inter-calibration exercises with repeated, routine analyses of homogenated samples supplied by the National Institute of Standards and Technology (NIST), formerly National Bureau of Standards (NBS), have been undertaken to ensure that the data produced during the NS&T program is reproducible, accurate and analyst independent. Interim reference materials as well as spiked blanks are also part of our ongoing laboratory QA/QC activity.

RESULTS AND DISCUSSION

Over 660 oyster samples from 76 different sites on the northern Gulf of Mexico coast have been analyzed for selected chlorinated hydrocarbons during the first four years of the NS&T program. In the following sections, the average concentrations of total chlordane-related compounds, i.e., sum of *alpha*-chlordane, *trans*-nonachlor, heptachlor and heptachlor epoxide, total DDT, i.e. the sum of *o-p'*-DDE, *p-p'*-DDE, *o-p'*-DDD, *p-p'*-DDD, *o-p'*-DDT and *p-p'*-DDT, and total PCBs will be discussed. During 1986 and 1987, total PCB concentrations represented the sum of all the measurable PCB congeners in the samples. Starting in 1988, total PCB concentrations in oyster samples were calculated by a regression equation relating the sum of 18 selected PCB congener data, generally the major components of commercial PCB mixtures and among the most commonly observed congeners in environmental samples, and total PCB congener data from the previous years. Analyte mean concentrations for each site represent the average of the mean concentrations encountered during each sampling period.

Chlordane-related compounds

Technical chlordane is a complex mixture formed by more than 140 different components. Recently, 120 of these compounds have been identified [13]. The most abundant constituents of technical chlordane are *alpha*-chlordane, *gamma*-chlordane, heptachlor

and *trans*-nonachlor. Because of the toxicity, potential carcinogenicity and environmental persistence of its components, technical chlordane sales and/or applications in the U.S.A. were suspended after April 15, 1988. The NS&T program included three chlordane-related compounds: *alpha*-chlordane, heptachlor and *trans*-nonachlor. Heptachlor epoxide, a metabolite of heptachlor, was also monitored.

Chlordane-related compounds were generally present in low ng g^{-1} concentrations. Average concentrations, plus 1 standard deviation, ranged from 3.5±0.44 to 120±70 ng g^{-1} (Figure 2).

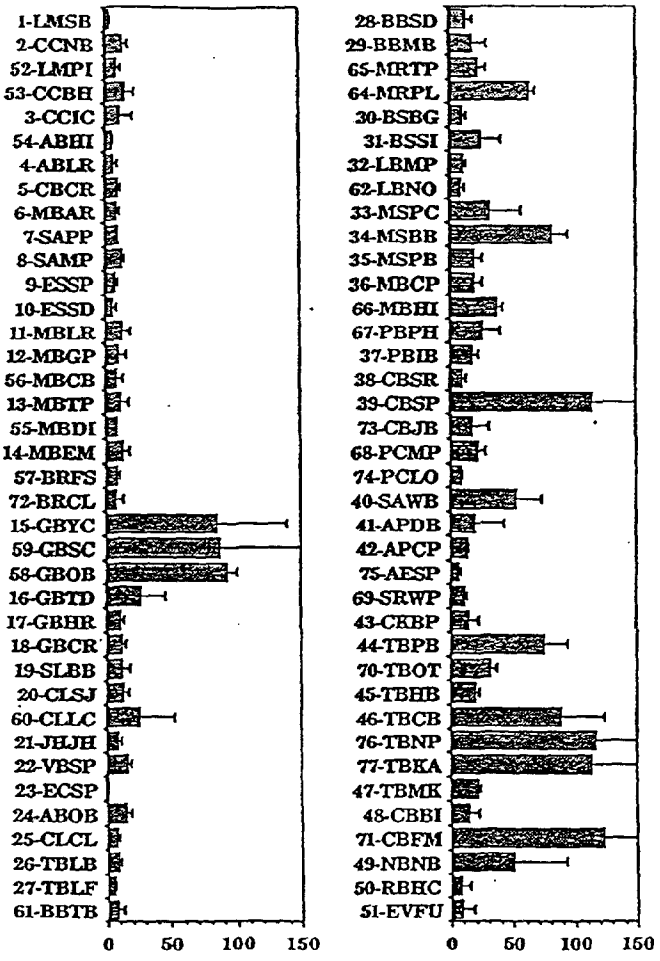


Fig. 2. Average concentrations, in ng g^{-1}, of total chlordane-related compounds in oysters from the Gulf of Mexico.

The highest average concentrations in oyster samples were encountered in samples from sites in Galveston Bay (GBYC, 85±54 ng g^{-1}; GBSC, 87±73 ng g^{-1}; GBOB, 93±7.1 ng g^{-1}), near the mouth of the Mississippi River (MRPL, 64±4.0 ng g^{-1}), Biloxi Bay (MSBB, 82±13 ng g^{-1}), and Choctawhatchee (CBSP, 114±120 ng g^{-1}), Tampa (TBPB, 75±19 ng g^{-1}; TBCB, 88±36 ng g^{-1}; TBNP, 120±66 ng g^{-1}; TBKA, 110±51 ng g^{-1}) and Charlotte Bays (CBFM, 120±70 ng g^{-1}). With the exception of the sites in the Galveston Bay area, average concentrations were lower to the west of the Mississippi River.

Approximately 90% of the total load of chlordane-related compounds encountered in oysters corresponded to the sum of alpha chlordane (45±2.5%) and trans-nonachlor (43±4.2%), two of the most important constituents of technical chlordane [13] (Figure 3). The fact that heptachlor epoxide is dominant over its parent compound indicates environmental degradation of heptachlor.

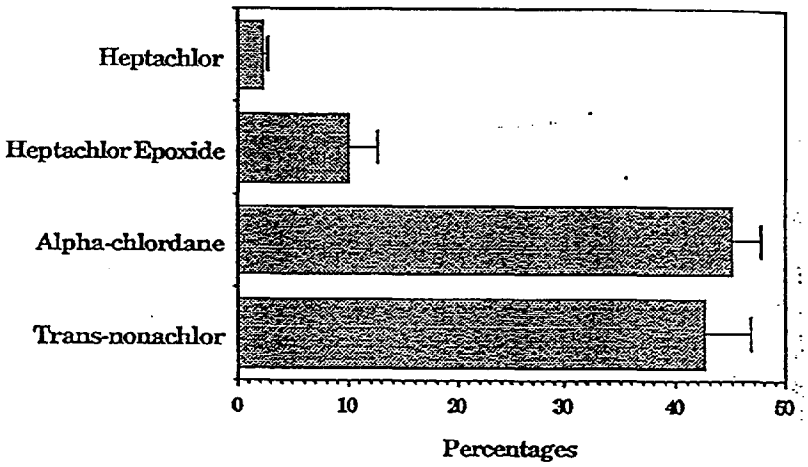


Fig. 3. Average composition of total chlordane-related compounds in oyster samples from the Gulf of Mexico.

A comparison of average concentrations measured in 1989 with the concentrations encountered during the first year of the NS&T program, i.e. 1986, is presented in Figure 4. On this scatter plot, sites with the same concentrations during both sampling years fall on the center line of the graph (intercept = 0; slope = 1). Sites that plot above or below the center line show an increase or a decrease in concentration, respectively, between 1986 and 1989. Also shown are lines that represent a 2-fold increase or decrease

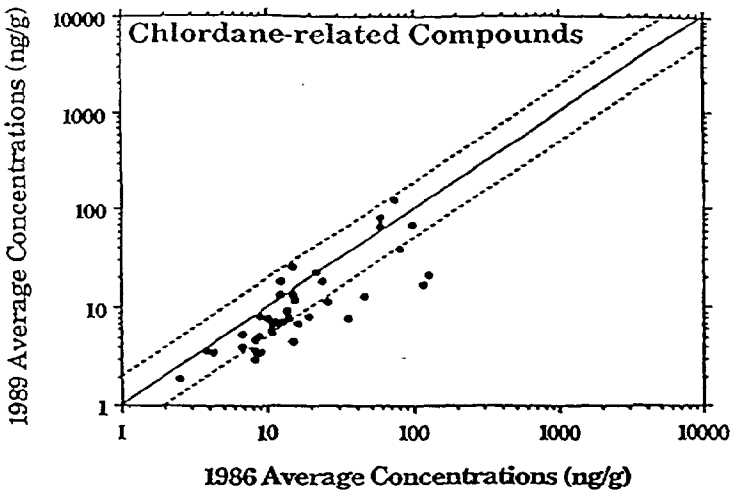


Fig. 4 Oyster total chlordane-related compound concentrations in 1986 versus 1989.

average concentrations encountered in 1986 when compared to data collected four years later. Over 80% of the sites showed a decrease in average concentrations; nearly 30% of the sites had a decrease larger than a 2-fold change. Only 15% of these locations revealed slight increases in their average concentrations.

DDT and metabolites

In spite of its ban in 1972, DDT and its metabolites, DDE and DDD, are still present, in significant concentrations, in the near-shore environments of the U.S.A. DDT and/or its derivatives were detected in every oyster sample analyzed. Figure 5 summarizes the average total DDT concentrations, plus 1 standard deviation, encountered in oyster samples from the Gulf of Mexico. Total DDT concentrations ranged from 6.9±1.9 to 890±440 ng g^{-1}. With the exception of some samples collected from sites in Galveston Bay (GBSC, 170±81 ng g^{-1}) and near the mouth of the Brazos River (BRFS, 220±47 ng g^{-1}), in Texas, and from locations in Tampa (TBKA, 160±32 ng g^{-1}) and Charlotte (CBFM, 170±85 ng g^{-1}) Bays, in Florida, the highest concentrations were encountered in samples collected to the east of the Mississippi River between its mouth and St Andrew Bay, Florida, e.g., MRPL, 280±54 ng g^{-1}; MBCP, 200±64 ng g^{-1}; MBHI, 830±270 ng g^{-1}; CBSP, 890±440 ng g^{-1}, and PCLO, 520±730 ng g^{-1}. With the exceptions mentioned above, the lowest concentrations were generally found along the Texas and southern Florida coasts.

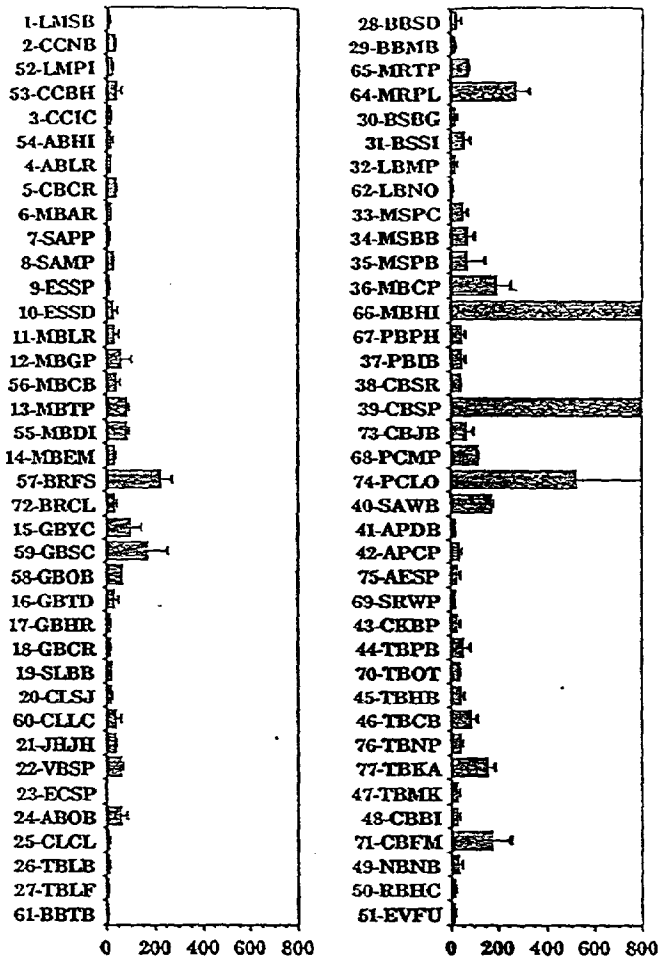


Fig. 5 Average concentrations, in ng g^{-1}, of total DDT in oyster samples from the Gulf of Mexico.

Isomers of the DDT accounted for a small fraction (5.2±3.0%) of the total DDT burden in oysters during this study (Figure 6). Isomers of the DDD and DDE contributed with 50±2.1 and 44±2.3% of the total amount, respectively. Technical DDT contained approximately 75% *p-p'*-DDT, 15% *o-p'*-DDT, 5% *p-p'*-DDE, <05% *o-p'*-DDE, <0.5% *p-p'*-DDD, <0.5% *o-p'*-DDD and <5% unidentified compounds. It is generally accepted that increasing percentages of DDE and/or DDD, which were found as impurities in the

commercial DDT mixture, indicate a decreasing exposure to new inputs of DDT.

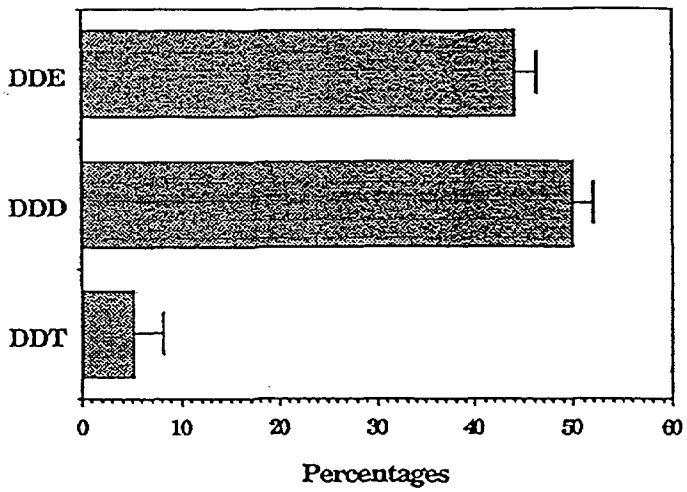


Fig. 6 Average composition of the DDT burden in oysters from the Gulf of Mexico

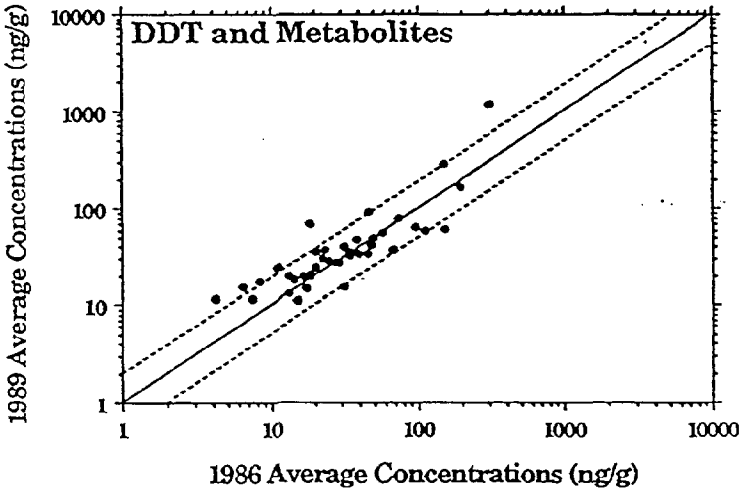


Fig. 7 Oyster total DDT concentrations in 1986 versus 1989.

The average total DDT concentrations encountered at the different sites during 1986 and 1989 are compared on Figure 7. In

general, concentrations were very similar at most of the sites. Only a few sites showed differences in concentrations greater than a 2-fold change. Although it is not possible to visualize a trend in average total DDT concentrations with a four-year data base, mainly due to the long environmental persistency of these compounds, it has been shown that the total DDT concentrations in Gulf of Mexico oysters have decreased since 1969 [10].

Polychlorinated Biphenyls

PCBs have proven to be ubiquitous contaminants in the Gulf of Mexico coastal environment. Average total PCB concentrations, plus 1 standard deviation, are presented in Figure 8.

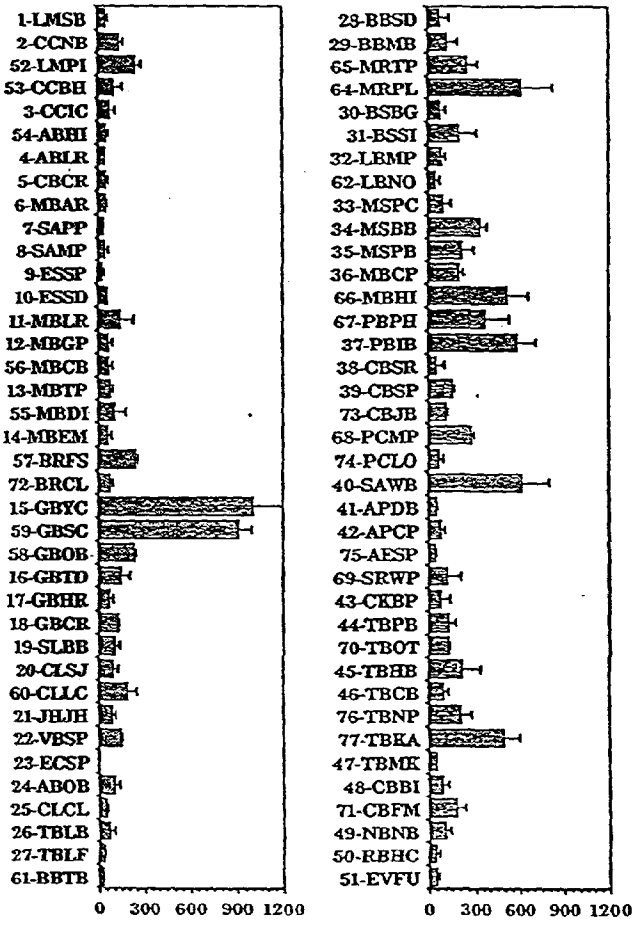


Fig. 8 Average total PCB concentrations in oyster samples from the Gulf of Mexico

PCB congeners were detected in all the oyster samples collected between 1986 and 1989 with average concentrations ranging from 26±17 to 1000±730 ng g^{-1}. The highest concentrations were encountered in samples collected from sites in Galveston Bay (GBYC, 1000±730 ng g^{-1}; GBSC, 910±81 ng g^{-1}), near the mouth of the Mississippi River (MRPL, 620±210 ng g^{-1}), Mobile Bay (MBHI, 520±150 ng g^{-1}) and Pensacola (PBIB, 590±130 ng g^{-1}), St Andrew (SAWB, 620±190 ng g^{-1}) and Tampa (TBKA, 490±110 ng g^{-1}) Bays. Similar to total DDT concentrations, the high concentrations measured in samples from Galveston Bay were the exception to the west of the Mississippi river.

The average composition of PCBs in oysters collected during 1986 and 1987 was largely dominated by pentachlorobiphenyls (46.8%), with some hexa- (22.3%) and tetrachlorobiphenyls (21.0%), and were almost depleted in di- (0.6%), octa- (0.5%) and nonachlorobiphenyls (0.2%) (Figure 9). The same general distribution is found in organisms from different PCB-contaminated locations [14-16]. In general, differential partition of PCB congeners between aqueous and lipids phases as well as stereochemistry appear to significantly affect bioaccumulation [14,17-19]. Maximum PCB uptake by organisms is observed with isomers having four to six chlorine atoms. Congeners with a lower number of chlorines have higher water solubilities and, as a consequence less favorable partition coefficients, while congeners with more than six chlorines have unfavorable steric configurations.

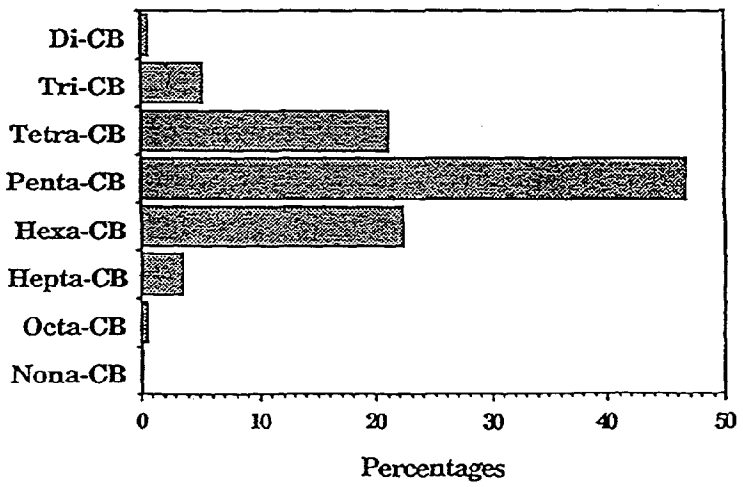


Fig. 9 Fractional composition of PCB homologs in oyster samples from the Gulf of Mexico.

Total PCB concentrations measured in oyster samples in 1989 did not differ significantly from the levels encountered four years earlier (Figure 10). With few exceptions, most of the sites had PCB concentrations in 1986-1989 that fell within the factor-of-two lines.

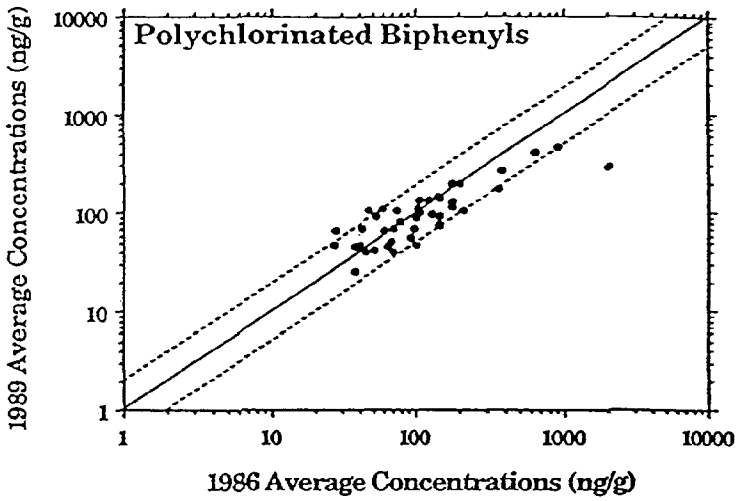


Fig. 10 Oyster total PCB concentrations in 1986 versus 1989.

CONCLUSIONS

After the first four years, the objectives of the NS&T program in the Gulf of Mexico have been partially accomplished. Distributions of chlordane-related compounds, DDT and its metabolites, and PCBs in oysters are well established. Oysters from some locations have consistently had high concentrations of these analytes, e.g. sites located in Galveston and Tampa Bays, near the mouth of the Mississippi River and in different sites along the Florida coastline between the Mississippi River and St Andrew Bay. Temporal trends are not readily apparent for all the contaminants. Continued sampling will help to further establish temporal trends for various analytes at the different sites.

ACKNOWLEDGEMENTS

This work was supported by the National Oceanic and Atmospheric Administration, contract No. 50-DGNC-5-00262, through the Texas A&M Research Foundation, Texas A&M University.

REFERENCES

1. Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, W., Schneider, E. and Gamble, E. The Mussel Watch, Environmental Conservation, Vol 5, 101-125, 1978.

2. Farrington, J.W., Albaiges, J, Burns, K.A., Dunn, B.P., Eaton, P., Laseter, J.L., Parker, P.L. and Wise, S. The International Mussel Watch: Report of a Workshop Sponsored by the Environmental Studies Board Commission on Natural Resources, pp 7-77, National Research Council, Washington DC, 1980.

3. Phillips, D.J.H. Quantitative Biological Indicator, Their Use to Monitor Trace Metals and Organochlorine Pollution, Applied Science, London, 1980.

4. Risebrough, R.W., DeLappe, B.W., Walker II, W., Simoneit, B.T., Grimalt, J., Albaijes, J. and Regueiro, J.A.G. Application of the Mussel Watch Concept in Studies of the Distribution of Hydrocarbons in the Coastal Zone of the Ebro Delta. Marine Pollution Bulletin, Vol. 14, pp. 181-187, 1983.

5. Farrington, J.W., Goldberg, E.D., Risebrough, R.W., Martin, J.H. and Bowen, V.T. U.S. 'Mussel Watch' 1976-1978: An Overview of the Trace Metal, DDE, PCB, Hydrocarbons and Artificial Radionuclide Data. Environmental Science and Technology, Vol. 17, pp. 490-496, 1983.

6. Martin, M. State Mussel Watch: Toxic Surveillance in California, Marine Pollution Bulletin, Vol. 16, pp.140-146, 1985.

7. Tavares, T.M., Rocha, V.C., Porte, C., Barceló, D. and Albaigés, J. Application of the Mussel Watch Concept in Studies of Hydrocarbons, PCBs and DDT in the Brazilian Bay of Todos os Santos (Bahia), Marine Pollution Bulletin, Vol. 19, pp. 575-578, 1988.

8. Wade, T.L., Atlas, E.L., Brooks, J.M., Kennicutt II, M.C., Fox, R.G., Sericano, J.L., Garcia-Romero, B., DeFreitas, D. NOAA Gulf of Mexico Status and Trends Program: Trace Organic Contaminant Distribution in Sediments and Oysters, Estuaries, Vol. 11, pp. 171-179, 1988.

9. Sericano, J.L, Atlas, E.L., Wade, T.L and Brooks, J.M. NOAA's Status and Trends Mussel Watch Program: Chlorinated Pesticides and PCBs in Oysters (*Crassostrea*

virginica) and Sediments from the Gulf of Mexico, 1986-1987. Marine Environmental Research, Vol. 29, pp. 161-203, 1990.

10. Sericano, J.L., Wade, T.L., Atlas, E.L. and Brooks, J.M. Historical Perspective on the Environmental Bioavailability of DDT and its Derivatives to Gulf of Mexico Oysters. Environmental Science and Technology, Vol. 24, pp. 1541-1548, 1990.

11. MacLeod, W.D., Brown, D.W., Friedman, A.J., Burrows, D.G., Maynes, O., Pearce, R.W., Wigren, C.A. and Bogar, R.G. Standard Analytical Procedures of the NOAA National Analytical Facility, 1985-1986.. Extractable Toxic Organic Compounds (2nd Edition), U.S. Department of Commerce, NOAA Technical Memorandum, NMFS F/NWC-92, 1985.

12. Ramos, L and Prohaska, P. Sephadex LH-20 Chromatography of Extracts of Marine Sediments and Biological Samples for the Isolation of Polynuclear Aromatic Hydrocarbons. Journal of Chromatography, Vol. 211, pp. 284-289, 1981.

13. Dearth, M.A. and Hites, R.A. Complete Analysis of Technical Chlordane Using Negative Ionization Mass Spectrometry Environmental Science and Technology, Vol. 25, pp. 245-254, 1991.

14. Shaw, G.R. and Connell, D.W. Relationships Between Stearic Factors and Bioaccumulation of Polychlorinated Biphenyls (PCBs) by the Sea Mullet (*Mugil cephalus* Linnaeus), Chemosphere, Vol. 9, pp. 731-743, 1980.

15. Duinker, J.C., Hillebrand, M.T.J. and Boon, J.P., Organochlorines in Benthic Invertebrates and Sediments from the Dutch Wadden Sea; Identification of Individual PCB Components, Netherlands Journal of Sea Research, Vol 17, pp. 19-38, 1983.

16. Boon, J.P., Van Zantvoort, M.B., Govaert, M.J.M.A. and Duinker, J.C. Organochlorines in Benthic Polychaetes (*Nephtys* spp.) and Sediments from the Southern North Sea. Identification of Individual PCB Components, Netherlands Journal of Sea Research, Vol 19, pp. 93-109, 1985.

17. Matsuo, M. A Thermodynamic Interpretation of bioaccumulation of Aroclor 1254 (PCB) in Fish, Chemosphere, Vol. 9, pp. 671-675, 1980.

18. Shaw, G.R. and Connell, D.W. Physicochemical Properties Controlling Polychlorinated Biphenyls (PCB) Concentrations in Aquatic Organisms, Environmental Science and Technology, Vol. 8, pp.18-23, 1984.

19. Samuelian, J. and O'Connor, J.M. Structure-activity Relationship and Accumulation of PCB Congeners in Estuarine Fishes: A Field Study (Abstract only), Estuaries, Vol. 8, p. 83A, 1985.

Preprint 3

National Status and Trends Mussel Watch Program: Chlordane-Related Compounds in Gulf of Mexico Oysters, 1986-1990

José L. Sericano, Terry L. Wade, James M. Brooks,
Elliot L. Atlas, Roger R. Fay, and Dan L. Wilkinson

National Status and Trends Mussel Watch Program: Chlordane-Related Compounds in Gulf of Mexico Oysters, 1986-1990

José L. Sericano[+*], Terry L. Wade[+], James M. Brooks[+], Elliot L. Atlas[^], Roger R. Fay[+] and Dan L. Wilkinson[+]

[+] Geochemical and Environmental Research Group, Department of Oceanography, Texas A&M University, 833 Graham Road, College Station, Texas 77845, U.S.A.

[^] National Center for Atmospheric Research, P.O. Box 3000, Boulder, Colorado 80307, U.S.A.

Abstract

The National Oceanic and Atmospheric Administration's National Status and Trends (NS&T) Program has been monitoring the chemical contamination in bivalve tissues from the U.S. coastal waters since 1986. *Alpha*-chlordane, *trans*-nonachlor, heptachlor and heptachlor epoxide, components of technical chlordane, are among the chlorinated pesticides measured. The geographical distribution of these chlordane compounds in the oyster samples from the U.S. Gulf of Mexico is well established. For example, highest residue levels, predominantly *alpha*-chlordane and *trans*-nonachlor, were encountered in samples collected near heavily populated areas in contrast with the concentrations measured in predominantly agricultural areas. Data collected during five years of bivalve sampling are used to evaluate temporal trends in residue concentrations at most NS&T sites. Minor decreases can be observed in the concentrations of *alpha*-chlordane and *trans*-nonachlor. Heptachlor and its epoxide concentrations, in contrast, have been increasing since 1987.

Introduction

In 1986, the National Oceanic and Atmospheric Administration (NOAA) initiated the National Status and Trends (NS&T) Mussel Watch Program to assess the extent of coastal marine contamination in the U.S. by measuring selected organic and inorganic contaminants, e.g., chlorinated pesticides, polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) and trace metals, and to identify trends in their concentrations with time. The NS&T Program is an ongoing project which has been collecting bivalve samples, on an annual basis, from over 150 sites along the East, Gulf and West coasts, including the Hawaiian islands. Overviews of the initial NS&T results have been published (1-7).

This report focuses on the chlordane-related compounds, i.e., *alpha*-chlordane, *trans*-nonachlor, heptachlor and heptachlor epoxide, included in the suite of chlorinated pesticides measured for the NS&T program. Technical grade chlordane is a complex mixture of more than 140 different components. Recently, 120 of these compounds have been resolved and identified by high-resolution gas chromatography combined with negative ionization mass spectrometry. *Alpha*-chlordane, *gamma*-chlordane, heptachlor and *trans*-nonachlor are the dominant constituents (8). Since 1946, the total production of chlordane by Velsicol Chem. Co., the major producer in the U.S., is estimated to be over 70,000 tons (8). Because of the toxicity, potential carcinogenecity and environmental persistence of their components and/or metabolites, e.g., heptachlor hepoxide and oxychlordane, the use of chlordane is under federal regulations (9). In 1974, the EPA proposed the cancellation of all uses of chlordane. A year later, the EPA suspended the production of heptachlor and chlordane and limited their uses on most food crops. Its use was permitted only where no other alternative existed and in all home and garden application with the exception of underground termite control. In 1987, Velsicol Chem. Co., in an agreement with the

EPA, voluntarily reduced the sales and distribution of chlordane and all sales and/or uses in the U.S. were suspended after April 15, 1988.

The extensive use of technical chlordane during the last decades, together with their natural persistence, caused a worldwide environmental. This paper examines the geographical distribution and trends in concentrations of chlordane in oyster samples collected from the northern coast of the Gulf of Mexico between 1986 and 1990.

Methods

Sampling. Oyster samples were collected each year over a two- to three-month period starting in late December or early January. Depending on water depth, oysters (twenty per station) were collected by hand, tongs or dredge, pooled in precombusted jars and frozen until analysis.

Bivalve samples were collected from three stations at approximately 50 sites located on the U.S. Gulf of Mexico. Distances between stations within each site varied from 100 to 1000 meters. During 1986 and 1987, oysters collection was completed at 49 and 48 sites with a total of 147 and 143 samples, respectively. Although these sites provided a good coverage of a broad range of different environmental conditions from the U.S.A.-Mexico border to southern Florida, they were specifically selected to avoid known sources of contaminant inputs. Starting in 1988, new sites were added to the sampling program in order to obtain more information from areas located closer to but not at suspected sources of contaminants. During 1988, 1989 and 1990, oyster samples were collected from 63, 62 and 68 sites with totals of 189, 186 and 203 samples, respectively.

Thus, by the end of the fifth year of the NS&T program in the Gulf of Mexico, eighty different sites had been sampled (Figure 1); thirty nine of them in all five years. Sites numbered 1 through 51 are the original locations, sites numbered 52 through 80 are the new sites added to the sampling program since 1988.

Extraction and separation. The analytical procedure used was adapted from a method developed by MacLeod *et al.*(10). Briefly, approximately 15 g of wet tissue were extracted with methylene chloride using a homogenizer (Tekmar Tissumizer). Each set of eight to ten samples was accompanied by a complete system blank and spiked blank or reference material that were carried through the entire analytical procedure. Before extraction, 4:4 dibromooctafluorobiphenyl (DBOFB) and two polychlorinated biphenyls (IUPAC No 103 and 198) were added to all samples, blanks and reference material as internal standards. Tissue extracts were fractionated by silica:alumina column chromatography. The sample extracts were eluted from the column using pentane (f1=aliphatic hydrocarbons) and pentane:methylene chloride (1:1) (f2=chlorinated hydrocarbons and PAHs). The second fractions were further purified to remove lipids by either Sephadex LH-20 column chromatography eluted with a mixture of cyclohexane:methanol:methylene chloride (6:4:3) (11) or high-performance liquid chromatography (12). Finally, the samples extracts were concentrated to a volume of 0.5 to 1 mL, in hexane, for gas chromatographic analysis.

Gas chromatography. *Alpha*-chlordane, *trans*-nonachlor, heptachlor and its epoxide were determined by gas chromatography with an electron capture detector (GC-ECD, ^{63}Ni) using a 30 m DB-5 fused silica capillary column (0.25 mm film thickness, 0.25 mm i.d., J&W Scientific), as previously described (6,7). Quantitation was achieved using authentic standards injected at four different concentrations. The detection limit for each of these compounds, calculated on the basis of 2 g dry weight of oyster tissue sample sizes and 0.2% by volume of the extract injected into the GC-ECD, is 0.25 ng g^{-1}.

Quality Control/Quality Assurance (QA/QC). These activities included several laboratory intercalibration exercises with repeated, routine analysis of homogenated natural samples, supplied by the National Institute of Standards and Technology (NIST), to ensure that the data produced during this program is reproducible, accurate,

and analyst independent. Interim reference material and spiked blanks, analyzed along with each set of samples, are also part of an ongoing laboratory QA/QC program.

Data analysis. For summary and statistical purposes, the reported mean total analyte concentrations include contributions equal to the analytical detection limits for those analytes that were below the limit of detection. Trends in concentrations with time at those locations that were sampled at least four of the five years were statistically evaluated, at alpha=0.05, by linear regression.

Results and discussion

For the last five years, *alpha*-chlordane, *trans*-nonachlor, heptachlor and its metabolite heptachlor epoxide were analyzed in more than 860 oyster samples collected from 80 different sites along the northern coast of the Gulf of Mexico as part of the NS&T Program (Figure 1). A summary of the median and average concentrations as well as ranges and distribution frequencies for each compound and total chlordane, i.e. summed individual analytes, corresponding to the original locations are given in Table I. Table II summarizes the complete data set, i.e. all sites included, from 1988 to 1990.

Except for a few samples collected in 1990, these analytes were detected in every sample analyzed since 1986. Concentrations varied over 1 to 3 orders of magnitude (Table I). In 1986, concentrations for total chlordane ranged from 2.00 to 175 ng g^{-1} with a mean value of 24.1±30.3 ng g^{-1}. During 1987, the overall average concentration for the Gulf of Mexico (29.5±58.5 ng g^{-1}, range 2.12-590 ng g^{-1}) was higher than the average concentration encountered in 1986. As previously discussed for DDT and its metabolites (7), this increase was a consequence of high residue concentrations encountered at site 39 (Choctawhatchee Bay; 288±256 ng g^{-1}). In 1988, the total chlordane average concentration was similar to the concentration measured during the first sampling year (21.7±22.8 ng g^{-1}, range 1.29-132 ng g^{-1}). Further decreases in mean

concentrations were observed in 1989 (16.3±25.0 ng g^{-1}, range 1.37-159 ng g^{-1}) and in 1990 (15.3±14.0 ng g^{-1}, range <1.00-69.4 ng g^{-1}).

The addition of sites closer to suspected sources of contaminants to the sampling program resulted in higher average concentrations and larger ranges (Table II). However, these changes were not as dramatic as expected for sites located near suspected sources of contaminants. In general, the concentrations encountered at the new sites compared well with the existing information obtained from the original 51 sites.

Geographical distribution. At this point of the NS&T program, the distribution of chlordane concentrations in oysters from the U.S. Gulf of Mexico is well established. Overall average concentration in the entire area for the five-year period was 24.1±25.2 ng g^{-1}. The highest residue concentrations were encountered in oysters collected near highly populated urban areas. For example, mean concentrations higher than three times the overall average for the Gulf of Mexico were measured in samples from Galveston Bay, Texas, Mississippi Sound, Mississippi and Choctawhatchee, Tampa and Charlotte Bays, Florida (Figure 2). The existence of higher residue concentrations in these areas, compared to predominantly agricultural coastal areas, is in good agreement with the regulations that have ruled chlordane usage for the last 15 years. After 1975, most of the chlordane use in the U.S. was limited to structural underground termite control; therefore, the higher the population, e.g. more houses, the higher the chlordane concentrations. With the exception of sites in Galveston Bay, the lowest concentrations of total chlordane were encountered in samples collected to the west of the Mississippi River. The addition of the new sites after 1987 (Figure 1), adds to the definition of the distribution of pesticide concentrations for the first two years of this program (6).

Residue composition. *Alpha*-chlordane and *trans*-nonachlor comprised from 38 to 48% and 36 to 49% of the total chlordane load, respectively, in oyster samples

collected between 1986 and 1990. The contribution of heptachlor and its epoxide to the total oyster burden were between 2-9% and 6-17%, respectively. Relative ratios of *alpha*-chlordane, *trans*-nonachlor and heptachlor in Gulf of Mexico oysters were 1.00:0.92:0.05, in 1986; 1.00:0.82:0.04, in 1987; 1.00:0.92:0.05, in 1988; 1.00:1.11:0.09, in 1989; and 1.00:0.98:0.21, in 1990. Ratios for the last three years correspond to the average values between both oyster data sets. Puri *et al.* (13) reported the percent composition of chlordane constituents in four different technical chlordane formulations. The average percent contribution of *alpha*-chlordane, *trans*-nonachlor and heptachlor to the total technical mixtures are 16.5±2.79, 13.4±4.34 and 12.5±2.19%, respectively. Heptachlor epoxide is reported to be present as trace levels. Relative ratios among *alpha*-chlordane, *trans*-nonachlor and heptachlor in the average technical chlordane mixture are 1.00:0.81:0.75. The NS&T data clearly show that *alpha*-chlordane:*trans*-nonachlor ratios for the average technical chlordane mixture and Gulf of Mexico oysters are similar. There is, however, a marked depletion in the relative concentration of heptachlor in oyster samples. This decrease in the relative concentration of heptachlor is commonly reported for biota tissues (13-15). In 1990, however, the higher relative *alpha*-chlordane:heptachlor ratio in oyster tissues indicate a somewhat reduced chemical/biochemical epoxidation of heptachlor, which is confirmed by an increase in the heptachlor: heptachlor epoxide ratio from 1:6, in 1986-88, to 1:2, in 1990. This suggests fresh inputs of technical chlordane mixtures or the related pesticide heptachlor into the coastal marine environment. The increase in the concentrations of heptachlor and its metabolite heptachlor epoxide in oyster tissues during 1990, which are not accompanied by a proportional increase in the concentration of *alpha*-chlordane and *trans*-nonachlor, might point to the second source as the most probable. However, this possibility must be taken with caution until more data, from this or other studies, becomes available.

Temporal trends. A five years database is available to look for trends in chlordane concentrations with time. Although with overlapping standard deviations, the annual average concentrations of total chlordane have been decreasing in oyster samples from the Gulf of Mexico. This tendency is supported by a shift in the percent distribution of concentrations to lower values (Table I and II). For example, in 1986, the total chlordane concentration in oysters from the original sites were largely dominated by concentrations between 10.0 and <100 ng g^{-1} with some values over 100 ng g^{-1} (Table I). In 1990, the total residue concentrations were almost equally distributed between the 1.00-<10.0 and 10.0-<100 ng g^{-1} ranges with 4% of the samples below the quantitation limit. The same analysis can be made for the individual analytes except heptachlor. Heptachlor concentrations have been shifting in the opposite direction since 1987. Again, this suggests that fresh heptachlor has been or is entering the coastal marine environment. The analysis of the samples already collected for the sixth sampling period (1991), and those of the following years, will probably assist in this matter.

Table III indicates the average total chlordane concentrations at each site between 1986 and 1990. Sites listed in this table are shown in geographical order from the U.S.-Mexico border to the southernmost Florida site. In general, the total chlordane concentrations seem to be oscillating around a certain value without showing any trend with time. Statistical analysis of the slopes of the regression lines of concentrations versus time at those sites with at least four years of collected data identified only a thirteen sites with significant decreases. These sites are marked with an asterisk in Table III, . Most of these sites are located along the southern Texas coast from Corpus Christi to Matagorda Bays. Paradoxically, the only site that showed a statistically significant increase in concentration with time, Copano Bay, is located within this area.

Aknowledgements

This research was supported by the National Oceanic and Atmospheric Administration, Contract 50-DGNC-5-00262, through the Texas A&M Research Foundation, Texas A&M University.

Literature Cited

(1) Robertson, A. *Monitoring coastal water environmental quality: The U.S. National Status and Trends Program.* In: G.E. Schweitzer and A.S. Phillips (eds.), Monitoring and Managing Environmental Impact: American and Soviet Perspectives. National Academy Press, 281 pp.

(2) Wade, T.L., Garcia-Romero, B. and Brooks, J.M. *Environ. Sci. Technol.*, **1988**, *22*, 1488-1493.

(3) MacDonald, D.A. *Oceans '89 Conf. Proc.*, **1989**, *2*, 647-651.

(4) O'Connor, T.P., Price, J.E. and Parker, C.A. *Oceans '89 Conf. Proc.*, **1989**, *2*, 569-572.

(5) Presley, B.J., Taylor, R.J. and Boothe, P.N. *Sci. Total Environ.*, **1990**, *97/98*, 551-593..

(6) Sericano, J.L., Atlas, E.L., Wade, T.L. and Brooks, T.L. *Mar. Environ. Res.*, **1990a**, *29*, 161-203.

(7) Sericano, J.L., Wade, T.L., Atlas, E.L.; Brooks, J.M. *Environ. Sci. Technol.*, **1990b**, *24*, 1541-1548.

(8) Dearth, M. A. and Hites, R. A. *Environ. Sci Technol.*, **1991**, *25*, 245-254.

(9) Shigenaka, G. *Chlordane in the Marine Environment of the United States: Review and Results from the National Status and Trends Program.* NOAA Tech. Memo. NOS OMA 55,Seattle, Washington, 1990, 230 pp..

(10) MacLeod, W. D.; Brown, D. W.; Friedman, A. J.; Burrows, D. G.; Maynes, O.; Pearce, R. W.; Wigren, C. A. and Bogar, R. G. *Standard analytical procedures of*

the NOAA National Analytical Facility, 1985-1986. Extractable toxic organic compounds. 2nd. Edition, U. S. Department of Commerce, NOAA/NMFS. NOAA Tech. Memo. NMFS F/NWC-92, 1985, 121 pp.

(11) Ramos, L.; Prohaska, P. G. *J. Chromatogr.*, **1981**, *211*, 284-289.

(12) Krahn, M.M.; Wigren, C.A.; Pearce, R.W.; Moore, L.K.,; Bogar, R.G.; MacLeod, W.D.; Chan, S-L. and Brown, D.W. *Standard analytical procedures of the NOAA National Analytical Facility, 1988. New HPLC cleanup and revised extraction procedures for organic contaminants.* NOAA Tecn. Memo, 1988, 52,pp.

(13) Puri, R.K., Orazio, C.E., Kapila, S., Clevenger, T.E., Yanders, A.F., McGrath, K.E., Buchanan, A.C., Czarnezki, J. and Bush, J. *Studies on the Transport and Fate of Chlordane in the Environment.* In: D.A. Kurtz (ed.), Long Range Transport of Pesticides, Lewis Publishers, 1990, pp 271-289.

(14) Muir, D.C.G.; Norstrom, R.J.; Simon, M. *Environ. Sci. Technol.*, 1988, *22*, 1071-1079.

(15) Kawano, M.; Inoue, T.; Wada, T.,; Hidaka, H.; Tatsukawa, R. *Environ. Sci. Technol.*, **1988**, *22*, 792-797.

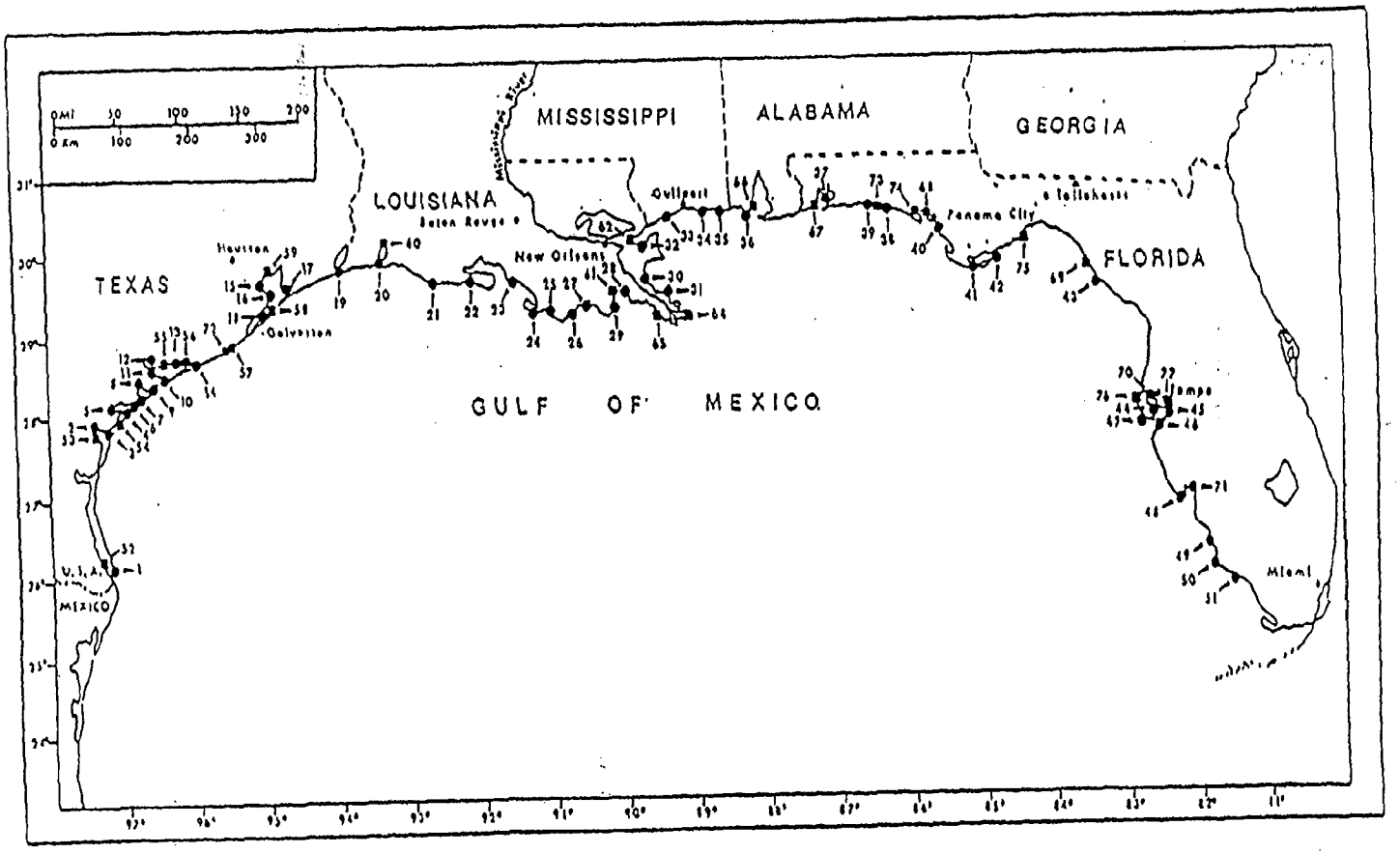


Fig. 1 Gulf of Mexico sampling site locations. Shown are the original sites (●) and the sites added to the sampling program (■)since 1988.

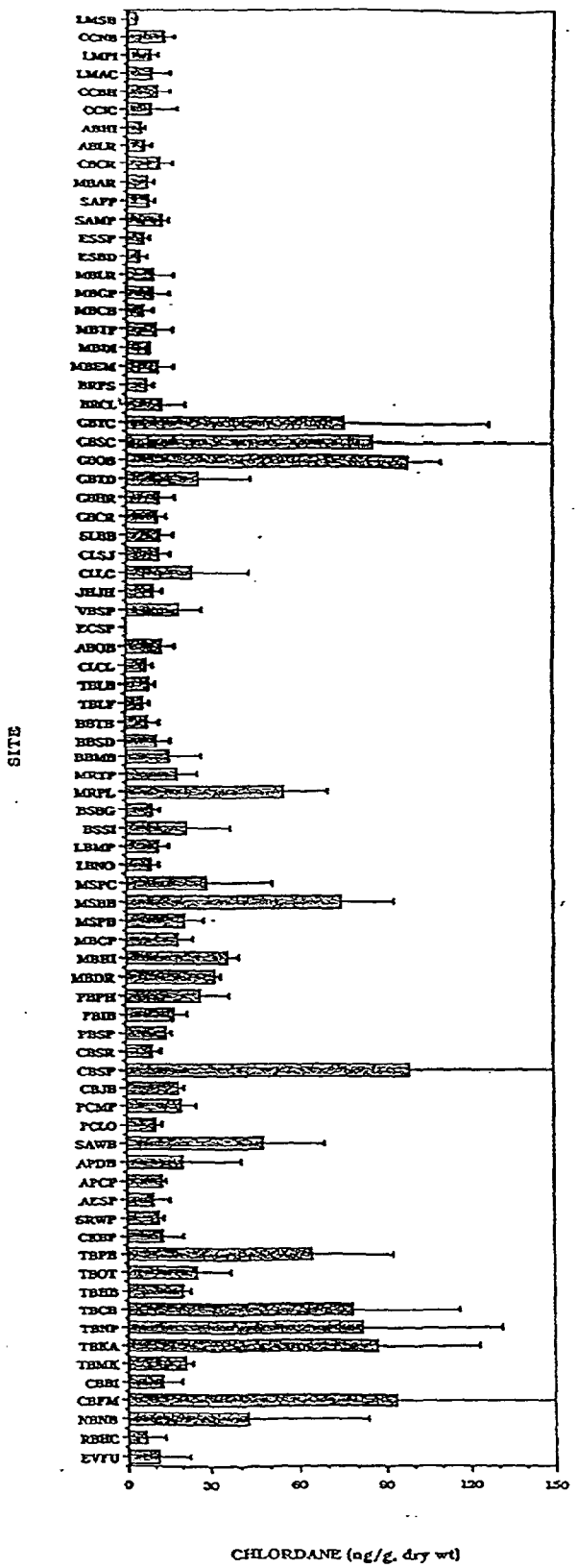


Fig. 2 Average total Chlordane concentrations in oyster samples from the Gulf of Mexico.

Table I: Chlordane-related Compound Concentrations[a] and Distribution Frequencies in Gulf of Mexico Oysters (Original Sites), 1986-1990

	concn, ng/g			% distribution				
	median	mean±1 STD	range	0.00-<0.25	0.25-<1.00	1.00-<10.0	10.0-<100	100[+], (ng/g)
1986 (n=147)								
Heptachlor	<0.25	0.51±0.69	<0.25-4.62	63	29	8		
Heptachlor Epoxide	1.87	2.71±3.31	<0.25-24.5	14	12	70	4	
Alpha-chlordane	5.23	10.9±14.4	0.91-96.3		1	72	27	
Trans-nonachlor	4.58	10.0±13.8	0.60-71.9		1	76	23	
ΣChlordane	13.1	24.1±30.3	2.00-175			36	61	3
1987 (n=143)								
Heptachlor	<0.25	0.54±0.99	<0.25-7.04	77	14	9		
Heptachlor Epoxide	2.45	3.30±3.93	<0.25-27.3	2	11	82	5	
Alpha-chlordane	6.42	14.1±29.0	0.65-292		1	71	27	1
Trans-nonachlor	4.78	11.6±27.7	<0.25-289	1	5	73	20	1
ΣChlordane	14.4	29.5±58.5	2.12-590			28	66	6

Table I (continuation)

	concn, ng/g			% distribution				
	median	mean±1 STD	range	0.00-<0.25	0.25-<1.00	1.00-<10.0	10.0-<100	100+, (ng/g)
1988 (n=132)								
Heptachlor	<0.25	0.49±0.66	<0.25-5.81	73	16	11		
Heptachlor Epoxide	1.69	2.44±2.33	<0.25-14.3	3	15	80	2	
Alpha-chlordane	5.80	9.76±10.7	0.40-60.2		2	72	26	
Trans-nonachlor	5.02	8.98±11.5	<0.25-81.6	1	4	70	25	
∑Chlordane	13.9	21.7±22.8	1.29-132			35	64	1
1989 (n=135)								
Heptachlor	0.33	0.78±1.03	<0.25-8.23	40	40	20		
Heptachlor Epoxide	0.60	1.25±1.41	<0.25-9.33	20	41	39		
Alpha-chlordane	3.14	7.00±9.84	<0.25-48.3	1	7	76	16	
Trans-nonachlor	2.33	7.29±14.5	<0.25-99.1	1	16	69	14	
∑Chlordane	7.68	16.3±25.0	1.37-159			61	37	2

Table I (continuation)

	concn, ng/g			% distribution				
	median	mean±1 STD	range	0.00-<0.25	0.25-<1.00	1.00-<10.0	10.0-<100	100+, (ng/g)
1990 (n=138)								
Heptachlor	0.72	1.34±1.81	<0.25-15.2	30	28	41	1	
Heptachlor Epoxide	1.20	2.63±4.53	<0.25-29.9	27	19	50	4	
Alpha-chlordane	4.16	5.81±5.66	<0.25-36.3	5	6	72	17	
Trans-nonachlor	3.13	5.55±6.49	<0.25-29.8	4	17	62	17	
ΣChlordane	10.7	15.3±14.0	<1.00-69.4		4	42	54	

[a] Concentrations on a dry weight basis

Table II: Chlordane-related Compound Concentrations[a] and Distribution Frequencies in Gulf of Mexico Oysters (Complete Data Set), 1988-1990

	conch, ng/g			% distribution				
	median	mean±1 STD	range	0.00-<0.25	0.25-<1.00	1.00-<10.0	10.0-<100	100+, (ng/g)
1988 (n=189)								
Heptachlor	<0.25	0.58±0.81	<0.25-5.81	68	18	14		
Heptachlor Epoxide	1.73	2.95±3.63	<0.25-21.5	5	15	75	5	
Alpha-chlordane	6.31	11.4±13.3	0.40-88.4		2	66	32	
Trans-nonachlor	5.46	10.5±12.8	<0.25-81.6	1	3	65	31	
ΣChlordane	14.3	25.4±27.7	1.29-182			34	64	2
1989 (n=186)								
Heptachlor	0.32	0.74±0.98	<0.25-8.23	43	38	18	1	
Heptachlor Epoxide	0.88	1.50±1.65	<0.25-10.7	16	37	46	1	
Alpha-chlordane	4.01	10.2±15.3	<0.25-116	1	5	69	24	1
Trans-nonachlor	3.02	11.8±22.7	<0.25-183	1	13	64	21	1
ΣChlordane	9.63	24.2±38.4	1.37-302			52	43	5

Table II (continuation)

	conc'n, ng/g			% distribution				
	median	mean±1 STD	range	0.00-<0.25	0.25-<1.00	1.00-<10.0	10.0-<100	100+, (ng/g)
1990 (n=203)								
Heptachlor	0.84	1.36±1.63	<0.25-15.2	24	30	45	1	
Heptachlor Epoxide	1.24	2.57±4.10	<0.25-29.9	26	19	51	4	
Alpha-chlordane	4.97	7.74±8.37	<0.25-59.0	4	6	65	25	
Trans-nonachlor	4.37	7.72±9.85	<0.25-73.8	3	12	59	26	
ΣChlordane	12.6	19.4±19.3	<1.00-139		1	36	62	1

[a] Concentrations on a dry weight basis

Table III. Total Chlordane Concentrations in Oyster Samples from NS&T Program Sites, 1986-1991[a]

Site	Location	State	mean±1 SD				
			1986	1987	1988	1989	1990
1 LMSB	Laguna Madre	TX	3.77±0.60	2.83±0.71	3.68±3.92	3.63±1.06	1.77±0.87
2 CCNB	Corpus Christi	TX	19.1±12.3	13.9±4.37	13.8±10.5	8.18±2.52	13.2±2.48
52 LMPI	Laguna Madre	TX	-	-	8.78±2.81	-	-
78 LMAC*	Laguna Madre	TX	-	-	-	-	8.80±7.43
53 CCBH	Corpus Christi	TX	-	-	14.9±7.50	-	7.76±1.35
3 CCIC*	Corpus Christi	TX	23.8±1.61	-	4.62±1.70	3.46±1.07	3.70±2.90
54 ABHI	Aransas Bay	TX	-	-	5.07±1.77	-	-
4 ABLR*	Aransas Bay	TX	8.82±3.72	9.76±2.22	4.86±1.15	3.57±1.05	4.49±2.48
5 CBCR^	Copano Bay	TX	8.84±2.41	12.1±0.21	9.70±5.48	-	19.4±11.8
6 MBAR*	Mesquite Bay	TX	8.56±2.95	11.0±3.13	8.22±2.33	5.09±2.01	6.17±5.40
7 SAPP	San Antonio Bay	TX	9.61±3.61	9.20±2.38	-	-	4.96±2.82
8 SAMP	San Antonio Bay	TX	11.9±2.42	14.8±3.52	-	-	-
9 ESSR	Espiritu Santo	TX	5.08±1.81	8.97±0.59	-	-	3.81±1.59
10 ESBD	Espiritu Santo	TX	-	-	7.97±1.65	3.19±0.82	2.76±3.12
11 MBLR*	Matagorda Bay	TX	16.5±6.08	16.4±2.31	-	4.71±1.40	1.76±1.26
12 MBGP	Matagorda Bay	TX	8.21±0.22	18.8±17.2	9.36±3.33	3.69±1.04	-
56 MBCB	Matagorda Bay	TX	-	-	9.04±4.58	-	3.65±1.98

Table III (continuation)

	Site		Location	State mean±1 SD				
				1986	1987	1988	1989	1990
13	MBTP*	Matagorda Bay	TX	14.8±10.3	19.7±4.40	8.79±2.95	4.50±1.23	6.66±5.26
55	MBDI	Matagorda Bay	TX	-	-	8.41±0.21	-	-
14	MBEM*	Matagorda Bay	TX	16.3±9.52	19.5±10.9	8.49±3.19	6.96±1.17	7.51±4.29
57	BRFS	Brazos River	TX	-	-	10.1±1.76	7.25±3.04	5.85±4.86
72	BRCL	Brazos River	TX	-	-	-	7.31±6.90	19.3±5.69
15	GBYC*	Galveston Bay	TX	124±24.9	136±30.3	60.8±8.95	20.4±3.57	42.1±9.63
59	GBSC	Galveston Bay	TX	-	-	139±38.5	35.2±16.6	51.7±13.6
58	GBOB	Galveston Bay	TX	-	-	88.2±15.2	98.2±11.7	111±35.9
16	GBTD	Galveston Bay	TX	25.5±4.68	55.2±8.13	14.2±2.93	11.4±1.25	27.7±6.03
17	GBHR	Galveston Bay	TX	10.7±2.14	15.6±7.89	8.06±2.42	7.36±0.48	21.0±13.4
18	GBCR	Galveston Bay	TX	9.92±1.78	14.8±2.78	14.2±7.33	7.93±2.37	11.0±1.20
19	SLBB	Sabine Lake	TX	11.9±4.31	9.23±1.36	20.4±11.4	6.99±1.18	12.4±6.83
20	CLSJ*	Calcasieu Lake	LA	14.0±1.35	16.2±3.71	14.5±3.19	7.94±7.19	6.65±4.28
60	CLLC	Calcasieu Lake	LA	-	-	45.1±3.88	6.63±1.56	20.2±4.51
21	JHJH	Joseph Harbor	LA	11.2±3.03	11.1±1.22	5.26±0.78	7.24±3.16	14.0±9.92
22	VBSP	Vermillon Bay	LA	15.2±2.36	21.4±6.25	15.2±1.19	12.0±1.60	32.7±14.9
23	ECSP	East Cote Blanche	LA	-	-	-	-	-
24	ABOB*	Atchafalaya Bay	LA	13.8±1.34	20.3±6.84	15.1±3.00	9.11±1.48	8.32±2.78

Table III (continuation)

	Site	Location	State	mean±1 SD			
			1986	1987	1988	1989	1990
25	CLCL Caillou Lake	LA	8.30±0.91	10.2±4.35	5.28±1.11	4.77±2.56	9.02±3.44
26	TBLB Terrebone Bay	LA	6.77±3.25	8.88±2.34	11.8±9.95	5.38±1.05	9.97±4.51
27	TBLF Terrebone Bay	LA	6.69±2.01	5.67±2.23	5.55±1.78	3.94±1.31	10.1±4.40
61	BBTB Barataria Bay	LA	-	-	7.86±4.62	-	-
28	BBSD Barataria Bay	LA	10.7±1.86	12.0±7.60	20.0±7.25	7.21±2.51	8.20±1.92
29	BBMB* Barataria Bay	LA	35.3±21.6	16.2±3.98	14.3±1.53	7.86±3.69	7.60±0.86
65	MRTP Mississippi River	LA	-	-	27.1±11.0	17.3±2.39	13.3±2.04
64	MRPL Mississippi River	LA	-	-	61.3±17.1	66.9±1.37	39.0±15.4
30	BSBG Breton Sound	LA	10.5±7.14	10.8±3.51	12.5±4.60	5.71±1.75	8.03±3.33
31	BSSI* Breton Sound	LA	45.4±18.0	12.8±1.39	28.8±10.2	13.1±6.14	12.6±3.35
32	LBMP Lake Borgne	LA	12.6±5.75	10.4±4.57	11.0±9.94	7.11±2.22	17.4±2.16
62	LBNO Lake Borgne	LA	-	-	8.82±3.25	-	-
33	MSPC Mississippi Sound	MS	21.5±3.13	69.0±41.2	15.9±4.63	22.7±7.70	14.8±2.03
34	MSBB Mississippi Sound	MS	98.0±67.8	86.0±55.6	71.4±22.3	71.2±18.7	49.8±16.2
35	MSPB Mississippi Sound	MS	12.0±4.25	18.9±1.34	28.0±7.81	18.5±7.60	28.6±32.7
36	MBCP Mobile Bay	AL	14.8±7.07	24.1±16.3	14.7±3.36	25.3±10.9	17.1±3.54
66	MBHI Mobile Bay	AL	-	-	34.3±7.12	40.3±3.22	34.3±2.98
79	MBDR Mobile Bay	AL	-	-	-	-	31.6±2.09

Table III (continuation)

	Site	Location	State	mean±1 SD			
			1986	1987	1988	1989	1990
67	PBPH Pensacola Bay	FL	-	-	35.6±1.67	17.0±0.80	28.6±8.36
37	PBIB Pensacola Bay	FL	15.0±0.88	24.5±4.55	17.4±2.31	13.7±1.13	-
80	PBSP Pensacola Bay	FL	-	-	-	-	14.9±1.84
38	CBSR Choctawhatchee Bay	FL	10.5±3.63	8.37±1.43	14.5±5.66	5.92±1.25	8.92±3.39
39	CBSP Choctawhatchee Bay	FL	57.5±20.5	288±256	45.4±20.8	65.8±36.8	39.3±26.6
73	CBJB Choctawhatchee Bay	FL	-	-	-	17.4±13.9	20.2±8.25
68	PCMP Panama City	FL	-	-	25.8±9.48	17.0±1.29	16.9±7.55
74	PCLO Panama City	FL	-	-	-	8.42±1.00	12.1±1.46
40	SAWB* St. Andrew Bay	FL	79.8±26.7	57.8±7.39	37.5±22.4	38.5±8.33	26.1±10.3
41	APDB Apalachicola Bay	FL	8.28±1.49	13.2±0.44	53.9±34.1	2.95±0.86	22.7±7.62
42	APCP Apalachicola Bay	FL	12.1±1.92	11.6±2.14	14.9±4.72	13.5±2.61	11.4±3.51
75	AESP Apalachee Bay	FL	-	-	-	5.11±2.31	13.7±12.7
69	SRWP Suwannee River	FL	-	-	11.2±1.91	-	-
43	CKBP Cedar Key	FL	8.90±3.15	14.4±3.16	25.6±31.2	8.18±5.33	6.71±2.33
44	TBPB Tampa Bay	FL	58.0±13.2	98.9±66.7	60.1±20.1	81.4±3.90	24.0±10.9
70	TBOT Tampa Bay	FL	-	-	34.8±5.85	28.1±11.8	12.2±6.96
45	TBHB Tampa Bay	FL	21.8±4.46	17.6±1.04	-	-	-
46	TBCB Tampa Bay	FL	73.8±16.6	45.1±8.58	107±47.2	127±27.3	41.3±3.42

Table III (continuation)

	Site	Location	State	mean±1 SD			
				1986	1987	1988	1989	1990
76	TBNP	Tampa Bay	FL	-	-	-	117±66.3	47.1±5.99
77	TBKA	Tampa Bay	FL	-	-	-	113±50.8	62.0±1.07
47	TBMK	Tampa Bay	FL	23.7±4.20	21.0±3.63	21.4±13.4	18.2±8.05	23.0±17.9
48	CBBI	Charlotte Harbor	FL	9.56±2.98	23.8±15.9	-	8.69±1.17	8.35±3.31
71	CBFM	Charlotte Harbor	FL	-	-	73.3±4.81	172±114	36.8±11.0
49	NBNB*	Naples Bay	FL	114±27.4	40.0±17.2	31.3±9.16	17.0±2.58	11.5±3.08
50	RBHC	Rookery Bay	FL	4.26±2.52	3.87±2.13	19.4±15.8	3.51±0.23	3.00±1.07
51	EVFU	Everglades	FL	2.51±0.46	5.70±2.31	22.7±12.0	1.91±0.72	25.3±20.8

[a] Concentration (ng g-1 on a dry weight basis); -, no sample; * or ^ indicate sites that have shown statistically concentration increases or decreases, respectively, with time (see text)

Preprint 4

Concurrent Chemical and Histological Analyses: Are They Compatible?

J.L. Sericano, T.L. Wade, E.N. Powell, and J.M. Brooks

CONCURRENT CHEMICAL AND HISTOLOGICAL ANALYSES: ARE THEY COMPATIBLE?

J. L. Sericano[1]; T. L. Wade[1]; E. N. Powell[2] and J. M. Brooks[1]

[1] Geochemical and Environmental Research Group, College of Geosciences, Texas A&M University, 833 Graham Rd, College Station, Texas 77845.

[1] Department of Oceanography, Texas A&M University, College Station, Texas 77840.

Bivalves are often used as sentinel organisms in monitoring programs for trace organic contaminants. The animal's physiological state may be important in interpreting trends in contaminant body burden. Simultaneous evaluation of physiological state and organic contaminant concentration in bivalves typically involves removal of a lipid-rich cross-section of the body mass for histopathological and/or gonadal analysis.

In this study, the bias introduced by this technique in the final trace organic, e.g. polynuclear aromatic hydrocarbons, chlorinated pesticides and polychlorinated biphenyls, concentrations are evaluated on five different size groups of oysters. As a test case, we evaluated the use of this method in the NOAA's Status & Trends Mussel Watch (NS&T) program. The average biases introduced by this technique in the final trace organic concentrations in Gulf of Mexico oysters have been increasing since 1986 as a consequence of a continuous decrease in the sizes of the individuals sampled.

INTRODUCTION

Seasonal variations in organic contaminant concentrations in bivalves have been attributed to a number of different factors including the stage of the reproductive cycle, nutritional status and ambient temperature (Wormell, 1979; Neff & Anderson, 1981; Jovanovich & Marion, 1987). Several studies have indicated the importance of considering the bivalve's physiological state when measuring contaminant loads (Fossato & Canzonier, 1976; Boehm & Quinn, 1977; Mix & Schaffer, 1979; Lunsford & Blem, 1982; Widdows *et al.*, 1982; Jovanovich & Marion, 1987). Monitoring programs that use bivalves as sentinel organisms typically try to assess some of these problems. In NOAA's National Status and Trends Mussel Watch (NS&T) Program, for example, reproductive state, condition index and disease incidence in oyster samples from the Gulf of Mexico have been monitored since 1986 (e.g. Craig *et al.*, 1989; Wilson *et al.*, in preparation).

One aspect of the problem involves the determination of the reproductive state, which typically requires a histological analysis in most bivalves (e.g. Morales-Alamo & Mann, 1989). In some cases, where the seasonal variability in contaminant concentrations in bivalves was followed in relation to their reproductive cycle, the chemical and biological analyses were performed on two different groups of individuals collected at the same site (e.g. Jovanovich & Marion, 1987). Since the reproductive state of bivalves may vary considerably among individuals at certain times of the year (e.g.

Wilson *et al.*, in preparation), adequate comparison requires a large sample size and the approach necessarily restricts statistical analysis.

An alternative approach is to take a cross-section of tissue from the same individual that is used for trace organic analyses. Bivalves where the gonadal material is in the mantle, such as mussels (Bullogh, 1970), present only a minor problem; but oysters, where the gonadal tissue surrounds the visceral mass (Morales-Alamo & Mann, 1989), require removal of a tissue cross-section that may be rich in trace organic contaminants. Any additional histopathological analysis would, of course, require removal of a larger tissue cross-section in either species.

The objective of this study was to evaluate the bias in the final organic contaminant concentrations introduced by the selective removal of a tissue cross section for histopathological or gonadal analysis. Five groups of oysters, *Crassostrea virginica*, of different average sizes were dissected and the portions normally used for histopathological and trace organic analysis were separately analyzed for selected polynuclear aromatic and chlorinated hydrocarbons to evaluate this bias.

MATERIALS AND METHODS

Oysters were collected from Galveston Bay, Texas, near the Houston Ship Channel in December 1988. This area is one of the 71 sites that

was sampled during the NS&T program in the Gulf of Mexico (Sericano *et al.*, 1990). The site, Galveston Bay Ship Channel (GBSC), is located at the mouth of Goose Creek in Tabbs Bay. Immediately after collection, the oysters were transported to the laboratory and sorted into five different size groups. A cross-section of the body of the oysters was separated by first making a transverse cut where the palps and gills meet. A second parallel cut was made about 5 mm from the first cut toward the center of the organism. This cross-section contained portions of gonad, stomach, intestine, digestive diverticula and connective tissue as well as mantle and gill. In standard practice, 3 to 5 mm sections are cut for histological analysis; consequently, the 5 mm cross-section would represent a maximum estimate of any bias incurred. The cross-section and remaining body tissues from oysters within each size group were pooled into two separated samples and analyzed for PAHs, chlorinated pesticides and PCBs. The methods used to measure the analyte concentrations were fully described elsewhere (e.g. Sericano *et al.*,1990).

RESULTS AND DISCUSSION

Average lengths of the five different groups of oysters used in this study ranged from 6.1 to 9.5 cm (Table 1). Also shown are the mean percent contribution on a dry weight basis of the cross-section and

remaining body tissues to the total body mass, and the percentage of extractable lipids corresponding to each of these fractions.

In general, the concentrations of PAHs, pesticides and PCBs measured in oysters are similar in each of the five size groups when the same subsamples, cross-section (A) or remaining body tissues (B), are compared (Table 2). In contrast, the trace organic concentrations of the two subsamples differ substantially in all five size classes. The cross-section (A) is the portion that normally would have been used for histological analysis. Since aromatic and chlorinated hydrocarbons are hydrophobic, they tend to be associated with lipid-rich tissues. This could in part explain the higher concentrations measured in the cross-section tissues which contain between 35 to 50% more extractable lipid than the remaining body tissues (Table 1).

The removal of the tissue cross-sections from the sample analyzed for trace organic compounds will introduce a bias towards lower total concentrations in the sample. The magnitude of this bias will largely depend on the sizes of the oysters sampled. In this study, the tissue cross-sections accounted for about 15% of the tissue dry weight in a 9 cm oyster, but for nearly 23% in a 6 cm oyster (Table 1). Accordingly, in large oysters, a proportionally smaller fraction is used for biological assays whereas, in smaller oysters, a 5 mm cross-section represents the removal of a comparatively large fraction of the total body mass. In the extreme, the cross-section of a very small specimen may include all of the tissues from where the palps and

gills meet to the adductor muscle which removes most of the lipid-rich internal organs.

The concentrations of PAHs, chlorinated pesticides and PCBs can be corrected for the contribution to the total body burden of the cross-section removed for histology. The differences between the uncorrected, which represent the values that would normally be reported, and corrected concentrations for each of the oyster groups are shown in Figure 1. As expected, the biases in the individual concentration of trace organic compounds increase as the oyster sizes decrease. Average biases are 6.1±2.0, 8.8±2.4, 10.5±2.7, 14.0±2.6, and 14.3±2.4%, for PAHs, 10.5±2.1, 11.7±0.7, 13.3±1.0, 13.9±0.8 and 16.8±1.5, for pesticides, and 6.3±0.9, 8.9±1.7, 10.9±1.2, 13.3±1.4 and 13.9±0.9, for PCBs, in oysters groups I to V, respectively.

As an example for this study, we consider the NS&T program in the Gulf of Mexico. In this program, oysters are used as sentinel organisms to monitor the current status and long-term trends of selected organic and inorganic environmental contaminants along the Atlantic, Pacific and Gulf coasts of the United States. In 1986, the overall average oyster size collected for the Gulf of Mexico portion of the NOAA's S&T Program was 8.5±1.4 cm (Brooks *et al.*, 1987) (Figure 2). During the following sampling years there was a continuous decrease in the sizes of the oysters that were sampled. In 1987, the average oyster size for the Gulf of Mexico was 7.6±1.8 cm (Brooks *et al*, 1988); in 1988, the average oyster size was 7.2±1.4 cm (Brooks *et al.*, 1989); and in 1989, the average oyster size was 7.0±1.3 cm (Brooks *et al.*, 1990).

Wilson *et al.* (in preparation) discuss the possible reasons for this decline in the sizes of the sampled oysters and concluded that the trend toward smaller sizes was probably a manifestation of decreased population health. For our purposes, this downward trend could introduce a bias in the trace organic values.

The bias imposed by the continuous decrease in oyster sizes with the successive sampling years in the observed PAH concentrations in the Gulf of Mexico can be estimated from the regression lines in Figure 1. Assuming that the cross-section was 5 mm in each case, the average percent biases in PAH concentrations that were reported for 1986, 1987, 1988 and 1989 can be estimated as 9.7, 11.7, 12.5 and 13.0%, respectively. Similarly, average percent biases for chlorinated pesticides and PCBs can be estimated as 12.6, 13.8, 14.4 and 14.7% and 9.7, 11.4, 12.2 and 12.6%, respectively. However, under the protocol used for the NS&T program, a cross-section of tissue is removed from only 10 of the 20 oysters collected per sampling station. Thus, the estimated bias for each group of analytes would be about half of these values.

In order to avoid misleading interpretations of comparative spatial and temporal data, it is imperative to understand how the methodology affects the trace organic concentration measurements in bivalves. This understanding is of particular importance if tissue cross sections are removed for histological analysis and it is especially important in sites where considerable variability exists in the sizes of the individuals sampled over the years and in cases where smaller organisms must be used. The development of non-

histologically based gonadal indices (e.g. Choi *et al.*, 1989; 1990) offers one way to avoid this problem.

ACKNOWLEDGEMENTS

This research was supported by the National Oceanic and Atmospheric Administration, contract No. 50-DGNC-5-00262, through the Texas A&M Research Foundation, Texas A&M University.

References

Boehm, P.D. and Quinn, J.G. (1977). The persistence of chronically accumulated hydrocarbons in the hard shell clam, *Rangia cuneata*. *Marine Biology*, 44, 227-233.

Brooks, J.M., Wade, T.L., Atlas, E.L., Kennicutt II, M.C., Presley, B.J., Fay, R.R., Powell, E.N. and Wolff, G. (1987). *Analyses of bivalves and sediments for organic chemical and trace elements from Gulf of Mexico estuaries.* Annual Report, Geochemical and Environmental Research Group, College of Geosciences, Texas A&M University, TX, 618 pp.

Brooks, J.M., Wade, T.L., Atlas, E.L., Kennicutt II, M.C., Presley, B.J., Fay, R.R., Powell, E.N. and Wolff, G. (1988). *Analyses of bivalves and sediments for organic chemical and trace elements from Gulf of Mexico estuaries.* Annual Report, Geochemical

and Environmental Research Group, College of Geosciences, Texas A&M University, TX, 644 pp.

Brooks, J.M., Wade, T.L., Atlas, E.L., Kennicutt II, M.C., Presley, B.J., Fay, R.R., Powell, E.N. and Wolff, G. (1989). *Analyses of bivalves and sediments for organic chemical and trace elements from Gulf of Mexico estuaries.* Annual Report, Geochemical and Environmental Research Group, College of Geosciences, Texas A&M University, TX, 678 pp.

Brooks, J.M., Wade, T.L., Atlas, E.L., Kennicutt II, M.C., Presley, B.J., Fay, R.R., Powell, E.N. and Wolff, G. (1990). *Analyses of bivalves and sediments for organic chemical and trace elements from Gulf of Mexico estuaries.* Annual Report, Geochemical and Environmental Research Group, College of Geosciences, Texas A&M University, TX.

Bullogh, W.S. (1970). *Practical invertebrate anatomy.* Macmillian and Co. Ltd., London, 483 pp.

Choi, K-S, Wilson, E.A., Lewis, D.H, Powell, E.N. and Ray, S.M. (1989). The energetic cost of Perkinsus marinus parasitism in oysters: quantification of the thioglycollate method. *Journal of Shellfisheries Research*, 8, 125-131

Choi, K-S, Lewis, D.H. and Powell, E.N. (1990). Quantitative evaluation of gonadal proteins in male and female oysters (Crassostrea virginica) using an immunological technique. *Journal of Shellfisheries Research*, 8, 431 (abstract).

Craig, A., Powell, E.N., Fay, R.R. and Brooks, J.M. (1989). Distribution of Perkinsus marinus in Gulf coast oyster populations. *Estuaries*, 12, 82-91.

Fossato, V.U. and Canzonier, W.J. (1976). Hydrocarbon uptake and loss by the mussel *Mytilus edulis*. *Marine Biology*, 36, 243-250.

Jovanovich, M.C. and Marion, K.R. (1987). Seasonal variation in uptake and depuration of anthracene by the brackish water clam *Rangia cuneata*. *Marine Biology*, 95, 395-403.

Lunsford, C.A. and Blem, C.R. (1982). Annual cycle of Kepone residue and lipid content of the estuarine clam, Rangia cuneata. *Estuaries*, 5, 121-130.

Mix, M.C. and Schaffer, R.L. (1979). Benzo(a)pyrene concentrations in mussels (*Mytilus edulis*) from Yaquina Bay, Oregon, during June 1976-May 1978. *Bulletin of Environmental Contamination and Toxicology*, 23, 667-684.

Morales-Alamo, R. and Mann, R. (1989). Anatomical features in histological sections of Crassostrea virginica (Gmelin, 1971) as an aid in measurement of gonad area for reproductive assessment. *Journal of Shellfisheries Research*, 8, 71-82.

Neff, J.M. and Anderson, J.W. (1981). *Response of marine animals to petroleum and specific petroleum hydrocarbons*, Applied Science Publishers Ltd, London, 177 pp.

Sericano, J.L., Atlas, E.L., Wade, T.L. and Brooks, J.M. (1990). NOAA's Status and Trends Mussel Watch Program: Chlorinated pesticides and PCBs in oysters (*Crassostrea*

virginica) and sediments from the Gulf of Mexico, 1986-1987. *Marine Environmental Research*, 29, 161-203.

Widdows, J., Bakke, T., Bayne, B.L., Donkin, P., Livingstone, D.R, Lowe, D.M., Moore, M.N., Evans, S.V. and Moore, S.L. (1982). Responses of *Mytilus edulis* on the exposure to the water-accomodate fraction of north sea oil. *Marine Biology*, 67, 15-31.

Wilson, E.A., Powell, E.N., Wade, T.L., Taylor, R.J., Presley, B.J. and Brooks, J.M. Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: The role of local and large-scale climatic controls (in preparation).

Wormell, R.L. (1979). *Petroleum hydrocarbon accumulation patterns in Crassostrea virginica: analyses and interpretations.* Ph.D. Dissertation, Rutgers University, The State University of New Jersey (new Brunswick), 189 pp.

TABLE 1. Average shell length and percent contribution of the cross-section and remaining-body tissues to the total body weight corresponding to the five group of oysters analyzed. Lipid percentages for each fraction are also indicated.

Oyster Size	n	Shell Length (cm)	Cross-Section Tissues		Remaining Body Tissues	
			Dry Weight. (%)	Lipids (%)	Dry Weight (%)	Lipids (%)
I	8	9.5±0.8	15.6±2.7	14.2	84.4±2.7	9.6
II	8	9.3±0.9	17.0±1.9	13.3	83.0±1.9	9.0
III	8	8.5±1.4	18.5±3.0	12.7	81.5±3.0	8.9
IV	14	6.7±0.9	22.2±3.4	14.9	77.8±3.4	10.2
V	14	6.1±0.6	22.5±2.7	14.5	77.5±2.7	10.9

TABLE 2. (cont.)

Analyte	Oyster size										Average		
	I		II		III		IV		V				
	A	B	A	B	A	B	A	B	A	B	A	B	Δ%
Clorinated Pesticides													
Gamma-chlordane	20.0	11.2	21.1	12.1	21.3	12.2	23.1	13.8	23.7	13.2	21.8±1.52	12.5±1.01	74
Alpha-chlordane	18.9	11.8	21.4	13.0	21.9	12.9	23.8	14.7	23.4	13.9	21.9±1.94	13.3±1.10	65
Trans-nonachlor	17.2	10.0	18.7	10.9	19.2	10.8	20.0	12.1	20.8	11.6	19.2±1.36	11.1±0.80	73
p-p'DDE	42.2	28.5	48.1	28.6	43.5	26.5	50.2	31.7	47.8	28.6	46.4±3.37	28.8±1.86	61
p-p'DDD	45.2	25.2	48.0	28.8	49.1	28.3	51.6	31.9	53.8	30.2	49.5±3.31	28.9±2.49	71
PCBs													
52	71.3	48.1	82.5	49.7	75.8	46.6	81.9	52.3	79.0	47.6	78.1±4.64	48.9±2.23	60
101	102	75.3	109	77.1	127	76.3	132	78.1	122	78.1	118±12.5	77.0±1.20	53
105	26.6	18.4	32.6	20.7	32.2	20.4	34.1	22.2	33.6	20.9	31.8±3.02	20.5±1.37	55
118	74.0	54.2	82.6	56.3	82.9	55.6	93.3	57.7	92.2	55.6	85.0±7.94	55.9±1.27	52
138	52.5	38.5	64.6	42.8	67.5	42.8	66.0	42.1	68.0	42.1	63.7±6.41	41.7±1.80	53

A= Cross-section Tissues

B= Remaining-body Tissues

TABLE 2. Cross-section and remaining-body PAH, pesticide and PCB concentrations, ng g^{-1}, measured in the five different groups of oysters. Average concentrations for each analyte in the subsamples and percent differences are also listed.

Analyte	Oyster size										Average		
	I		II		III		IV		V				
	A	B	A	B	A	B	A	B	A	B	A	B	Δ%
PAHs													
2,3,4 Trimethyl Naphthalene	95.2	64.6	106	64.5	98.4	57.2	101	59.6	124	68.2	105±11.3	62.8±4.38	67
1 Methyl Phenanthrene	111	86.3	112	91.8	123	80.7	104	63.3	158	93.0	121±21.5	83.0±12.1	46
Fluoranthene	615	462	676	446	626	392	686	402	766	474	674±60.0	435±36.0	55
Pyrene	1300	1030	1430	1070	1470	970	1440	976	1750	1130	1480±165	1040±67.1	42
Benz(a)anthracene	210	132	229	147	204	132	214	131	219	142	215±9.47	137±7.26	57
Chrysene	392	281	439	277	426	264	443	273	487	321	437±34.2	283±22.1	54
Benzo(b+k)fluoranthene	220	170	221	147	232	169	254	172	299	186	245±33.0	169±14.0	45
Benzo(e)pyrene	253	201	282	172	267	200	298	204	352	226	290±38.3	201±19.2	44
Benzo(a)pyrene	86.4	58.6	84.6	55.8	100	58.3	98.3	59.8	107	62.1	95.3±9.51	58.9±2.30	62
Perylene	140	85.5	155	94.8	160	89.6	173	96.0	182	101	162±16.3	93.4±5.99	73

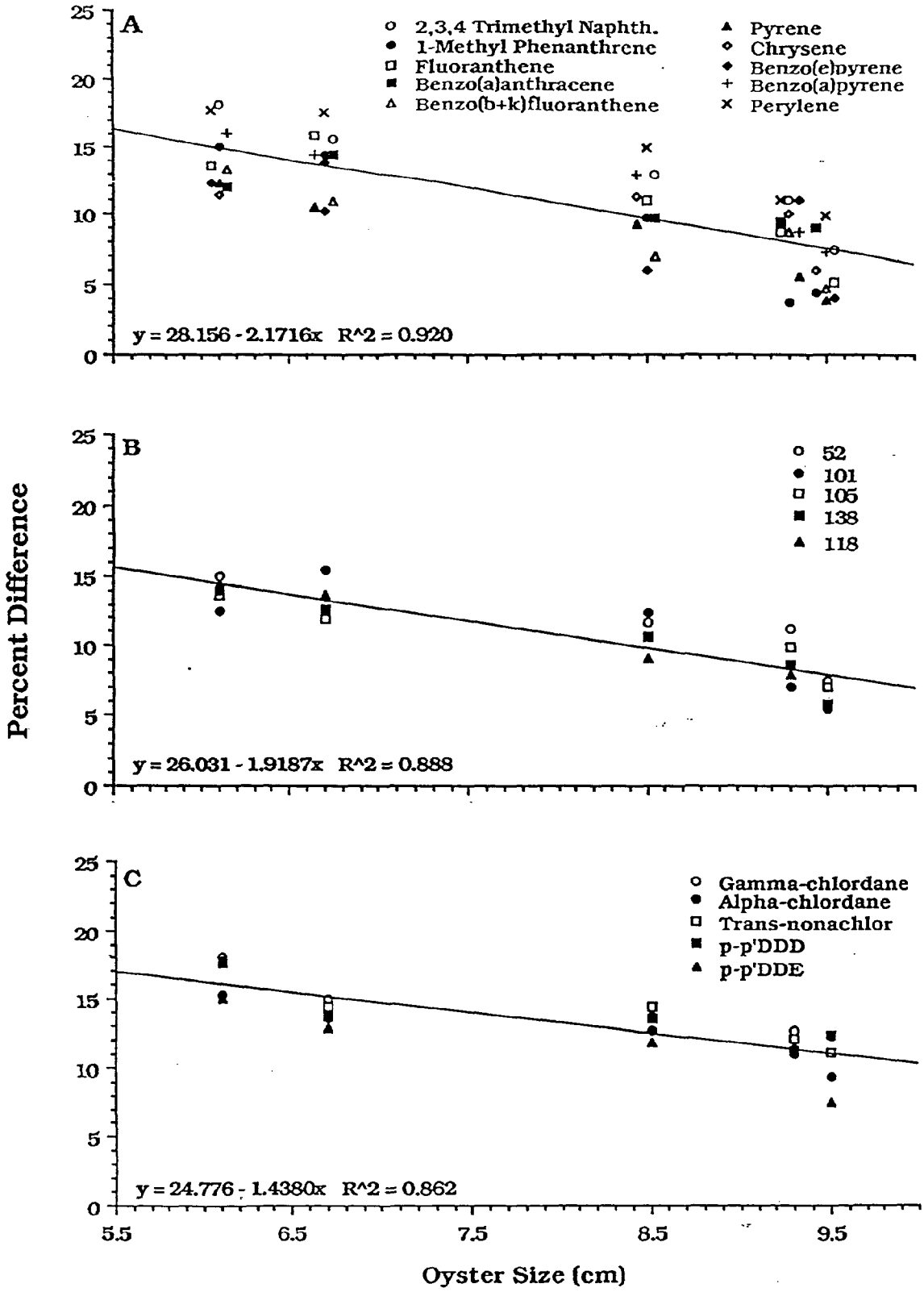
FIGURE CAPTIONS

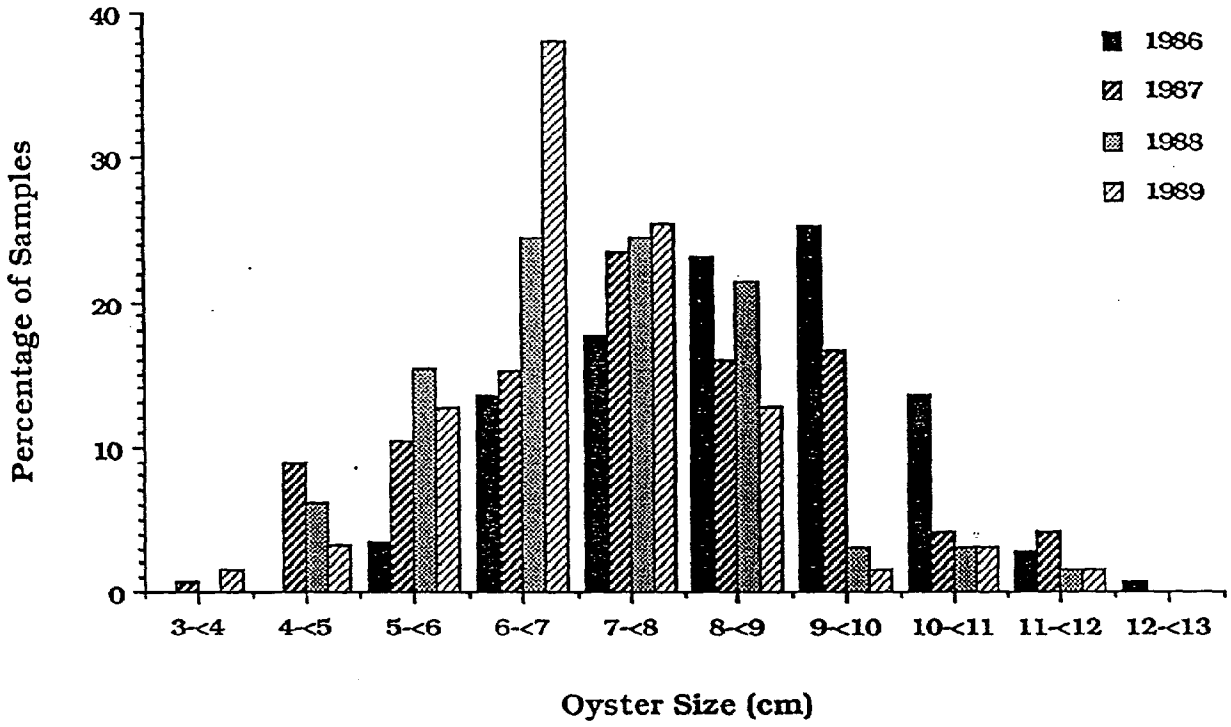
Figure 1.

Percent differences between corrected and remaining-body PAH, chlorinated pesticide and PCB concentrations versus oyster size.

Figure 2.

Size distributions of oysters sampled in the Gulf of Mexico during the NOAA's S&T Program between 1986 and 1989.


A
2,3,4 Trimethyl Naphth.
1-Methyl Phenanthrene
Fluoranthene
Benzo(a)anthracene
Benzo(b+k)fluoranthene
Pyrene
Chrysene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
y = 28.156 - 2.1716x R^2 = 0.920
B
52
101
105
138
118
y = 26.031 - 1.9187x R^2 = 0.888
C
Gamma-chlordane
Alpha-chlordane
Trans-nonachlor
p-p'DDD
p-p'DDE
y = 24.776 - 1.4380x R^2 = 0.862
Percent Difference
Oyster Size (cm)
0
5
10
15
20
25
5.5
6.5
7.5
8.5
9.5


Percentage of Samples
40
30
20
10
0
1986
1987
1988
1989
3-<4
4-<5
5-<6
6-<7
7-<8
8-<9
9-<10
10-<11
11-<12
12-<13
Oyster Size (cm)

Preprint 5

Environmental Significance of the Uptake and Depuration of Planar PCB Congeners by the American Oyster (*Crassostrea virginica*)

José L. Sericano, Terry L. Wade, Amani M. El-Husseini, and James M. Brooks

Environmental Significance of the Uptake and Depuration of Planar PCB congeners by the American Oyster (*Crassostrea virginica*)

José L. Sericano, Terry L Wade, Amani M. El-Husseini and James M. Brooks

Geochemical and Environmental Research Group
College of Geosciences, Texas A&M University
College Station, Texas 77845, U.S.A.

Uptake and depuration of three highly toxic PCB congeners, i.e. PCBs 77, 126 and 169, by the American oysters (*Crassostrea virginica*) were study under environmental conditions. Compared with other PCB congeners, these compounds can be considerably bioconcentrated, and retained, by bivalves and constitute a potential health hazard for higher consumers. To evaluate the health risks that these PCB congeners pose for human beings, concentrations in oyster samples from two of the largest bays on the northern Gulf of Mexico coast, Galveston and Tampa Bays, sampled as part of the NOAA's National Status and Trends "Mussel Watch" Program, are discussed.

Of the 209 possible PCB congeners that can be produced by the extensive chlorination of biphenyl, only 20 have non-*ortho* chlorine substitutions in the biphenyl rings. These congeners can attain planarity which makes their structure similar to the highly toxic dibenzo-p-dioxins and dibenzofurans (McKinney *et al.*, 1976, 1985; Hansen, 1987). Particularly important within this group are the PCBs having four, five or six chlorines in non-*ortho* positions, for example, congeners 3,3',4,4' tetrachlorobiphenyl (IUPAC No 77), 3,3',4,4',5 pentachlorobiphenyl (IUPAC No 126), and 3,3',4,4',5,5' hexachlorobiphenyl (IUPAC No 169) which are very potent mimics of the 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8 tetrachlorodibenzofuran (TCDF) both in P-450 induction and toxic effects, e.g. body weight loss, dermal disorders, liver damage, thymic atrophy, reproductive toxicity and immunotoxicity (Poland & Knutson, 1982; Safe, 1984, 1986, 1990; Goldstein & Safe, 1989).

Although these planar PCB congeners represent a small portion of the total technical PCB mixtures (Duinker & Hillebrand, 1983; Kannan *et al.*, 1987; Schulz *et al.*, 1989), a worldwide environmental occurrence should be expected and monitoring of these compounds is needed. Until recently, however, quantitation of individual non-*ortho* substituted PCB congeners was very difficult because of their extremely low concentrations and routine high-resolution capillary gas chromatography analyses failed to separate some of these planar PCBs from other *ortho*-PCB congeners. Although, this separation can now be achieved with, more expensive, techniques such as multidimensional gas chromatography (Duinker *et al.*, 1988), simpler and less expensive methods, carbon chromatography for

example, are available for routine analysis of planar PCBs (Hong & Bush, 1990; Kuehl *et al.*, 1991; Sericano *et al.*, 1991)

This paper, which is part of a more comprehensive study, reports the uptake and depuration of three highly toxic PCB congeners, i.e. PCBs 77, 126 and 169, by the American oysters (*Crassostrea virginica*) under environmental conditions using a newly developed carbon chromatographic method (Sericano *et al.*, 1991) and evaluate the health risks that these congeners pose in two of the largest bays on the northern Gulf of Mexico coast, Galveston and Tampa Bays. As part of the NOAA's National Status and Trends "Mussel Watch" Program, oyster samples from these, and other areas, have been analyzed for selected organic pollutants since 1986. Although PCBs are one of the most commonly found contaminants in Gulf of Mexico oysters (e.g., Sericano *et al.*, 1990a), the occurrence of planar PCB congeners have not been previously reported.

Materials and Methods

Uptake and depuration experiments

Approximately 250 oysters were collected by dredge at Hanna Reef, a relatively pristine area in Galveston Bay (Fig. 1). Collected oysters were immediately transplanted live in nets to a site near the Houston Ship Channel, an area where oysters have shown high PCB concentrations. Thereafter, oysters were sampled in groups of 20 individuals during the 3rd, 7th, 17th, 30th and 48th days after transplantation, respectively. During the uptake period, native oysters were collected from the Ship Channel area to compare their

concentrations of these trace organic contaminants with those encountered in transplanted Hanna Reef oysters. The remaining transplanted oysters, i.e. approximately 150 individuals, were re-located to the Hanna Reef area and sampled in groups of 20 individuals during the 3rd, 6th, 18th, 30th, and 50th days after transplantation.

Extraction and initial sample fractionation

The analytical procedure used for the extraction, initial fractionation and cleanup of oyster tissue samples for aliphatic and aromatic (PAHs) hydrocarbons, polychlorinated biphenyls (PCBs), including planar congeners, and chlorinated pesticides analyses is based on a method developed by MacLeod *et al.* (1985) with a few modifications that proved to be equivalent or superior to the original technique. This method and its modifications have been fully described elsewhere (Sericano *et al.*, 1990a) and is not repeated here.

Isolation of planar PCB congeners

For the isolation and analysis of planar PCB congeners, 250 ul fractions were withdrawn from the final 1 ml extract reserved for PCB analyses (Sericano *et al*, 1990a). Before proceeding with the isolation of planar congeners, PCB #81 was added to the extracts as internal standard.

The methodology to analyze planar PCBs in tissue samples has being published elsewhere (Sericano *et al.*, 1991). Briefly, glass chromatographic columns (10 mm i.d.) were packed in methylene chloride. Two grams of the adsorbent, a 1:20 mixture of activated

AX-21 charcoal (Super-A activated carbon) and LPS-2 silica gel (Low-pressure silica gel, particle size 37-53 μm, 450 m^2g^{-1}), were packed between two layers of anhydrous sodium sulfate. The adsorbent mixture was carefully checked for interfering compounds by running blanks with the solvent mixtures used to elute the columns and concentrating them to a final volume of approximately 0.1 times the working volume. Oyster tissue extracts were sequentially eluted from the column with 50 ml of 1:4 methylene chloride and cyclohexane, 30 ml of 9:1 methylene chloride and toluene, and 40 ml of toluene. The flow rate through the column was 1.5 to 2.0 ml min^{-1}. The first two solvent mixtures were collected as one fraction (f1) and contained the bulk of PCB congeners. The second fraction (f2), containing the planar PCB congeners with four, five and six chlorines in *meta* and *para* positions, was concentrated to a final volume of 0.1 ml, in hexane, for GC-ECD analysis.

Instrumental analysis

Planar PCB congeners were analyzed by fused-silica capillary column GC-ECD (Ni^{63}) using a Hewlett Packard 5880A GC in splitless mode. Capillary columns, 30 meters long x 0.25 mm i.d. with 0.25 mm DB-5 film thickness, were temperature-programmed from 100 to 150°C at 10°C min^{-1} and from 150 to 270°C at 6°C min^{-1} with 1 min hold time at the beginning of the program and the program rate change. A hold time of 3 min was used at the final temperature. Total run time was 30 min. Injector and detector temperatures were set at 275 and 325°C, respectively. Helium was used as carrier gas at a flow velocity of 30.0 cm sec^{-1} at 100°C.

Argon:methane (95:5) was used as make-up gas at a flow rate of 20 ml min^{-1}. The volume injected was 2 ul. Planar PCBs were quantitated against a set of authentic standards which were injected at four different known concentrations, i.e., 1, 5, 20 and 50 pg ul^{-1}, to calibrate the instrument and to compensate for a non-linear response of the electron capture detector. PCB congeners 103 and 198 were used as the GC internal standard to estimate the recovery of the internal standard. The detection limits for individual planar PCB congeners, calculated on the basis of 2 grams (dry weight) sample size with 2% by volume of the extract injected into the GC-ECD, was 50 pg g^{-1} dry weight.

Results and Discussion

Uptake and depuration of planar PCB congeners by transplanted oysters

The concentrations of the three highly toxic planar congeners, i.e., 3,3',4,4' (77), 3,3',4,4',5 (126) and 3,3',4,4',5,5' (169), in transplanted and indigenous oysters are summarized in Table 1. Planar PCBs were found at low concentrations, e.g. part per trillion (pg g^{-1}) to part per billion (ng g^{-1}). Congener 169 was present at concentrations near or below the detection limits.

Congeners 77 and 126 have well defined uptake and depuration curves as seen when the concentrations of these congeners versus time are plotted during both stages of this study (Fig. 2). The concentrations of these two planar PCB congeners in transplanted Hanna Reef oysters increased over the seven week exposure period.

PCB congener 77 reached a concentration similar to that encountered in indigenous Ship Channel oysters within 30 days. The uptake of congener 126 was slower and only approximated the concentration of Ship Channel oysters by the end of the exposure period. Contrasting with congeners 77 and 126, and because the extremely low concentration, it was not possible to observe a clear trend for congener 169.

The decreasing concentrations of accumulated planar PCBs with the increasing number of chlorines substituted in the biphenyl rings observed during this study in transplanted oysters were also reported to occur in transplanted green-lipped mussels (*Perna viridis*) during a 32 day exposure experiment in Hong Kong waters (Kannan *et al.*, 1989). Kannan *et al.* (1987) reported the concentrations of these planar congener in different commercial PCB mixtures. In general, congener 77 is 1 to 2 and 3 to 5 orders of magnitude higher than congeners 126 and 169, respectively. Comparing this relative concentrations with those observed in transplanted oyster samples, it appears that the high molecular weight congeners in oyster tissues are enriched with respect to congener 77. The same observation was made by Kannan *et al.* (1989). This is not surprising since the K_{ow} (octanol-to-water partition coefficient) increases with the IUPAC number of the PCB congener, e.g. 6.36, 6.89 and 7.42 for congeners 77, 126 and 169, respectively (Hawker & Connell, 1988).

When transplanted to the Hanna Reef area, exposed oysters slowly depurated the concentrated planar congeners. These PCBs were still present at relatively high concentrations by the end of the 50-days depuration period. Kannan *et al.* (1989) also observed that the

concentrations of these planar PCB congeners in transplanted green-lipped mussels (*Perna viridis*), at the end of the exposure period (32 days), were substantially higher than those found in native individuals.

Kinetics parameters describing the uptake and depuration of planar PCB congeners by the oyster *Crassostrea virginica* can be calculated according to the first-order equation:

$$dC_t/d_t = k_u C_w - k_d C_t \qquad (1)$$

where C_t is the concentration of the analyte in the tissue at time = t and C_w is the concentration in water. If the concentration in the depuration site is regarded as zero, i.e., $C_w = 0$, equation (1) can be reduced to:

$$dC_t/dt = - k_d C_t \qquad (2)$$

From this equation, the relationships to calculate Kd, biological half-life and time to reach a concentration equal to 90% the equilibrium concentration, i.e. concentration at time = infinity, can be deduced. Respectively, these equations are:

$$\text{Log } C_t = \log C_o - k_d\, t / 2.303 \qquad (3)$$

$$t_{1/2} = 0.693 / k_d \qquad (4)$$

$$t_{90\%} = 2.303 / k_d \qquad (5)$$

where Co is the initial concentration, i.e. time = zero, during depuration.

Depuration rate of congener 77 was higher than the rate observed for congener 126. The estimated depuration constants for congeners 77 and 126 were 0.0079 and 0.0064 $days^{-1}$, respectively. These values were lower than the range of values observed for other PCB congeners within the same homolog group (Sericano, unpublished data). This would indicate longer biological half-lives for congeners 77 and 126 (88 and 107 days, respectively) and longer time to reach a concentration within 10% the concentration at equilibrium (291 and 360 days, respectively; Fig. 3).

The estimated biological half-lives for these toxic planar PCB congeners during this study were significantly higher than those reported by Kannan *et al.* (1989) for mussels (9 and 13 days, respectively). However, it must be noted that all the reported biological half-lives for different PCB congeners corresponding to that transplantation study (i.e. Tanabe *et al.*, 1987; Kannan *et al.*, 1989) were significantly lower than the estimated half-lives during this study and previous reports involving different organisms (Table 2). Despite this disagreement, both studies indicate that, compared to other *ortho*-substituted congeners within the corresponding homolog groups, planar PCBs take longer to equilibrate into and out of the lipid pools of these organisms.

NOAA's National Status and Trends "Mussel Watch" Program

Details regarding site locations and oyster collection during this program are given elsewhere (Sericano *et al.*, 1990a, 1990b). The concentrations of PCB congeners 77, 126 and 169, as well as the concentrations of selected predominant mono- and di-*ortho*

substituted congeners and total PCBs in oyster samples from sites in Galveston and Tampa Bays (Fig. 4) are summarized in Table 3.

In Galveston Bay, the highest concentration of these planar PCBs was found in samples collected near the area where the Houston Ship Channel enters the upper Galveston Bay (GBSC) and decreases seaward. The second highest total concentration was encountered in samples from a site near the city of Galveston (GBOB). The general distribution of planar congener concentrations in Galveston Bay clearly indicates high values near population centers. The same correlation between urban centers and concentrations of planar PCBs can be observed in Tampa Bay. The highest concentrations were measured in samples collected near Tampa (TBKA).

As expected from the small contributions of these planar congeners to the total commercial PCB mixtures (Kannan *et al.*, 1987), these congeners were detected at much lower concentrations than other mono- and di-*ortho* substituted PCB congeners. However, as discussed previously, it appears that congeners 126 and 169 are enriched with respect to congener 77. On average, the sum of these three highly toxic congeners ranged from 0.26 to 0.62% and from 0.31 to 1.40% of the total PCB load in Galveston and Tampa Bays, respectively.

In a recent review, Safe (1990) discussed the environmental and mechanistic considerations behind the development of the Toxic Equivalent Factor (TEF) concept. He proposed provisional TEF values of 0.01, 0.1 and 0.05 for planar congeners 77, 126 and 169, respectively. Calculated TEF in oysters tissues collected from Galveston and Tampa Bay, as well as their averages, are listed in Table 4. In

Tampa Bay, the total TEF values ranged from 14 to 52 whereas in Galveston Bay the TEF values were between 13 and 280. The data show that, except for the sample collected near the Houston Ship Channel, oysters from Tampa and Galveston Bays are similar in terms of total toxicity. Oysters collected near the Houston Ship Channel (GBSC), in Galveston Bay, were clearly the most toxic. This area is closed to commercial or sport oystering.

In conclusion, two of the most toxic planar PCB congeners, i.e. congeners 77 and 126, were bioconcentrated by transplanted oysters during a seven-week exposure period. Congener 77 attained an equilibrium concentration in a shorter period of time than congener 126. When contaminated oysters were back transplanted to the Hanna Reef area, they significantly depurated both planar PCB congeners; however, the estimated depuration half-lives were significantly longer than those corresponding to different PCBs within the same homolog groups. Because of their potential toxicity, this persistency of highly toxic planar congeners is of significant importance in environmental studies. These congeners can be considerably bioconcentrated, and retained, by bivalves and constitute a potential health hazard for higher consumers, including human beings.

This research was supported by the National Oceanic and Atmospheric Administration (NOAA), contract No 50-DGNC-5-00262, through the Texas A&M Research Foundation, Texas A&M University.

Duinker, J.C. & Hillebrand, M.T.J. (1983). Characterization of PCB components in Clophen formulations by capillary GC-MS and GC-ECD techniques. *Environ. Sci Technol.* **17**, 449-456.

Duinker, J.C., Schulz, D.E. & Petrick, G. (1988). Multidimentional gas chromatography with electron capture detection for the determination of toxic congeners in polychlorinated biphenyl mixtures. *Anal. Chem.* **60**, 478-482.

Goldstein, J. A. & Safe, S. (1989). Mechanism of action and structure-activity relationships for the chlorinated dibenzo-p-dioxins and related compounds. In: *Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products*, (Kimbrough & Jensen (Eds.)). Elsevier Science Publishers, pp. 239-293.

Hansen, L.G. (1987). Environmental Toxicology of polychlorinated biphenyls. In: *Polychlorinated biphenyls (PCBs): Mammalian and environmental toxicology*, (Safe, S. and Hutzinger, O., (Eds)). Springer-Verlag Berlin Heidelberg, pp. 15-48.

Hawker, D.W. & Connell, D.W. (1988). Octanol-water partition coefficients of polychlorinated biphenyl congeners. *Environ. Sci. Technol.* **22**, 382-387.

Hong, C-S & Bush, B. (1990). Determination of mono- and non-ortho coplanar PCBs in fish. *Chemosphere* **21**, 173-181.

Kannan, N., Tanabe, S., Wakimoto, T. & Tatsukawa, R. (1987). Coplanar PCBs in Aroclor and Kanechlor mixtures. *J. Assoc. Off. Anal. Chem.* **70**, 451-454.

Kannan, N., Tanabe, S., Tatsukawa, R. & Phillips, D.J.H. (1989). Persistency of highly toxic coplanar PCBs in aquatic ecosystems:

uptake and release kinetics of coplanar PCBs in gree-lipped mussels (Perna viridis Linnaeus). *Environ. Pollut.* 55, 65-76.

Kuehl, D.W., Butterworth, B.C., Libal, J. & Marquis, P. (1991). An isotope dilution high resolution gas chromatographic - high resolution mass spectrometric method for the determination of coplanar polychlorinated biphenyls: application to fish and marine mammals. *Chemosphere* 22, 849-858.

MacLeod, W.D., Brown, D.W., Friedman, A.J., Burrows, D.G., Maynes, O., Pearce, R.W., Wigren, C.A. & Bogar, R.G. (1985). Standard analytical procedures of the NOAA National Analytical Facility, 1985-1986. Extractable toxic organic compounds. (2nd Ed.), U.S. Department of Commerce, NOOAA/NMFS. NOAA Tech. Memo. NMFS F/NWC-92.

McKinney, J., Chae, K., Gupta, B.N., Moore, J.A. & Goldstein, J.A. (1976). Toxicological assessment of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran in chicks. I. Relationship of chemical parameters. *Toxicol. Appl. Pharmacol.* 36, 65-80.

McKinney, J.D., Chae, K., McConnel, E.E. & Birnbaum, L.S. (1985). Structure-induction versus structure-toxicity relationships for polychlorinated biphenyls and related aromatic hydrocarbons. *Environ. Health Perspect.* 60, 57-68.

Oliver, B.G. (1987). Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by Oligochaete worms. *Environ. Sci Technol.* 21, 785-790.

Poland, A & Knutson, J.C. (1982). 2,3,7,8-tetrachlorodibenzo-p-dioxin and related halogenated aromatic hydrocarbons: examination of the toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22, 517-524.

Pruell, R.J., Lake, J.L., Davis, W.R. & Quinn, J. G. (1986). Uptake and depuration of organic contaminants by the blue mussels (Mytilus edulis) exposed to environmentally contaminated sediments. *Mar. Biol.* **91**, 497-507.

Safe, S. (1984). Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanisms of action. *CRC Crit. Rev. Toxicol.* **13**, 319-393.

Safe, S. (1986). Comparative toxicology and mechanisms of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Ann. Rev. Pharmacol. Toxicol.* **26**, 371-399.

Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and related compounds, environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol.* **21**, 51-88.

Schulz, D.E., Petrick, G. & Duinker, J.C. (1989). Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clorphen mixtures by multidimentional gas chromatography-electron capture detection. *Environ. Sci. Technol.* **23**, 852-859.

Sericano, J.L., Atlas, E.L., Wade, T.L & Brooks, J.M. (1990a). NOAA's Status and Trends Mussel Watch Program: Chlorinated pesticides and PCBs in oysters (*Crassostrea virginica*) and sediments from the Gulf of Mexico, 1986-1987. *Mar. Environ. Res.* **29**, 161-203.

Sericano, J.L., Wade, T.L. Atlas, E.L. & Brooks, J.M. (1990b). Historical perspective on the environmental bioavailability of DDT

and its derivatives to Gulf of Mexico oysters. *Environ. Sci Technol.* 24, 1541-1548.

Sericano, J.L., El-Husseini, A.M. & Wade, T.L. (1991). Isolation of planar polychlorinated biphenyls by carbon column chromatography. *Chemosphere* 23, 915-924.

Tanabe, S., Tatsukawa, R. & Phillips, D.J.H. (1987). Mussels as bioindicators of PCB pollution: A case study on uptake and release of PCB isomers and congeners in green-lipped mussels (Perna viridis) in Hong Kong waters. *Environ. Pollut.* 47, 147-163.

TABLE 1

Planar PCB concentrations in oysters (Crassostrea virginica) during the uptake and depuration phases in Galveston Bay

Sample	Sampling	Concentration of Planar PCBs			Total PCBs
		77	126	169	
		pg g^{-1}	pg g^{-1}	pg g^{-1}	ng g-1
HRSC[1]	3	330	110	ND	220
HRSC	7	560	140	ND	380
HRSC	17	630	140	ND	500
HRSC	30	920	160	ND	650
HRSC	48	1000	220	77	830
HRHR[2]	3	900	170	106	850
HRHR	6	800	250	ND	670
HRHR	18	750	210	76	470
HRHR	30	740	190	ND	400
HRHR	50	630	150	ND	380
SC[3]	3	1070	370	340	1500
SC	17	1040	250	120	1200
SC	30	1000	230	320	960
SC	48	980	220	96	1100

(1) Hanna Reef-to-Ship Channel oysters

(2) Hanna Reef-back-to-Hanna Reef transplanted oysters

(3) Ship Channel oysters

ND = not detected

TABLE 2

Biological half-lives of selected PCBs in different organisms

Congener	Oysters[a]	Mussels[b]	Mussels[c]	Worms[d]
Planar PCBs				
3,3',4,4' (77)	88	9	-	-
3,3',4,4',5 (126)	107	13	-	-
3,3',4,4',5,5' (169)	-	26	-	-
Selected *non*-planar PCBs				
2,4,4' (28)	17	7	16	-
2,2',5,5' (52)	55	6	28	-
2,2',4,5,5' (101)	76	7	37	50
2,2',3,3',4,4' (128)	51	9	46	92
2,2',4,4',5,5' (153)	27	6	-	36

[a] This study; [b] Tanabe *et al.* (1987) and Kannan *et al.* (1989); [c] Pruell *et al.* (1986); [d] Oliver (1987)

TABLE 3

Planar PCB concentrations in oysters (Crassostrea virginica) from Galveston and Tampa Bays

Sample	Concentration of Planar PCBs			Total PCBs
	77	126	169	
	pg g^{-1}	pg g^{-1}	pg g^{-1}	ng g-1
Galveston Bay				
GBSC	2000	2200	790	1100
GBYC	330	210	190	210
GBTD	140	120	54	110
GBHR	89	110	89	50
GBCR	100	94	51	77
GBOB	500	400	93	160
Tampa Bay				
TBOT	170	320	280	55
TBKA	1500	330	84	580
TBPB	85	100	51	75
TBNP	260	140	150	120
TBCB	200	290	100	49
TBMK	ND	ND	ND	38

ND = not detected

TABLE 4

Toxic Equivalent Factors (TEF) in *Crassostrea virginica* Oysters from Galveston and Tampa Bays.

Sample	Toxic Equivalent Factors			Total TEF
	77	126	169	
Galveston Bay				
GBSC	20	220	40	280
GBYC	3.3	21	9.5	34
GBTD	1.4	12	2.7	16
GBHR	0.9	11	4.5	16
GBCR	1.0	9.4	2.6	13
GBOB	5.0	40	4.7	50
Tampa Bay				
TBOT	1.7	32	14	48
TBKA	15	33	4.2	52
TBPB	0.9	10	2.6	14
TBNP	2.6	14	7.5	24
TBCB	2	29	5.0	36
TBMK	-	-	-	-

Figure captions

Fig. 1 Galveston Bay transplantation sites

Fig. 2 Planar PCB concentrations in Hanna Reef oysters during the uptake and depuration phases of the transplantation experiments at Galveston Bay. Planar concentrations in Ship Channel oysters during the uptake phase are also indicated

Fig. 3 Depuration constants (k_d) and biological half-lives (BHL) of planar PCB congeners compared to the ranges of values calculated for *non*-planar PCBs (Sericano, unpublished data).

Fig. 4 Location of Galveston and Tampa Bays sampling sites (NOAA's National Status and Trends Mussel Watch Program)

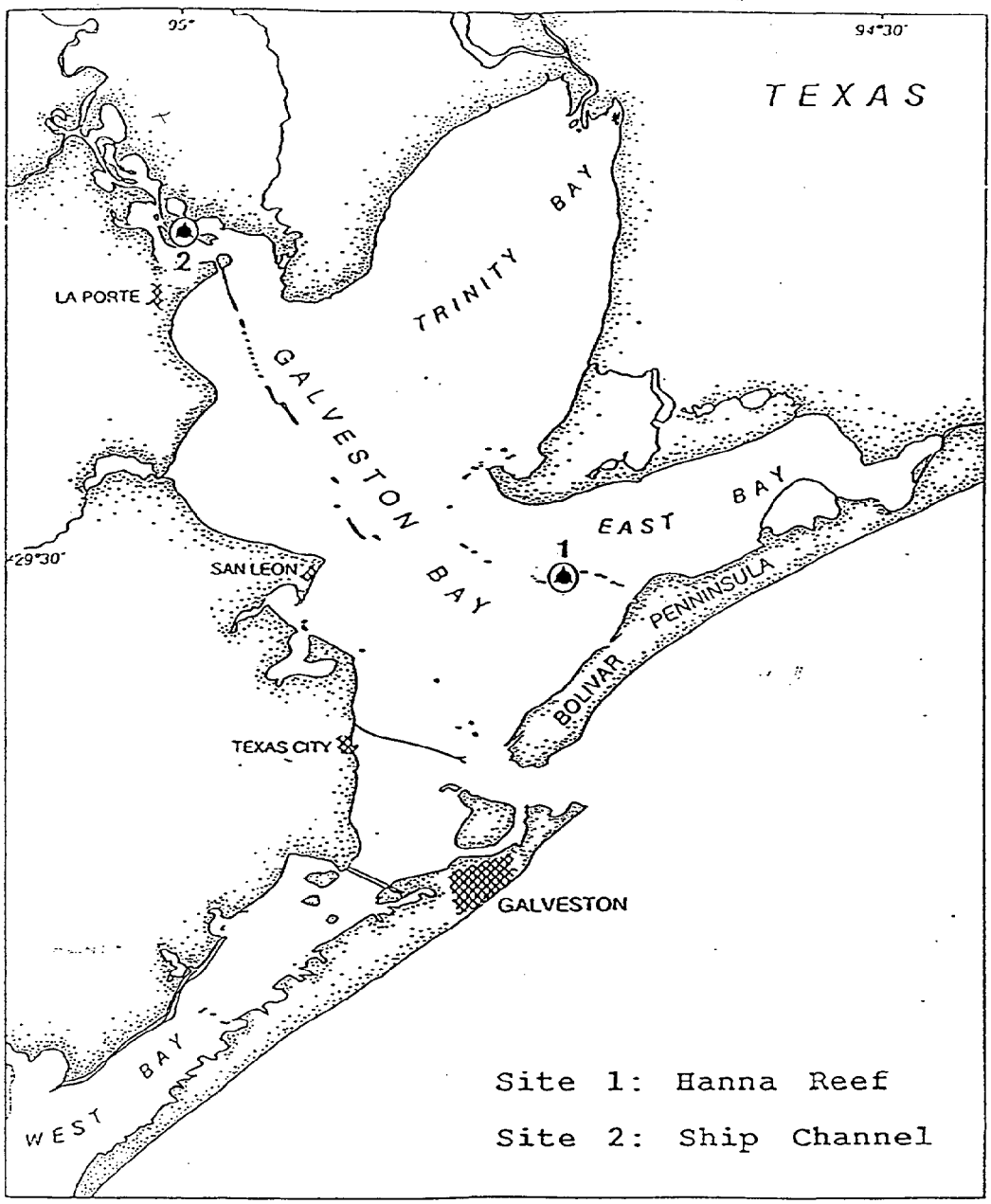

95°
94°30'
TEXAS
TRINITY BAY
2
LA PORTE
GALVESTON BAY
EAST BAY
29°30'
SAN LEON
1
BOLIVAR PENNINSULA
TEXAS CITY
GALVESTON
WEST BAY
Site 1: Hanna Reef
Site 2: Ship Channel

Fig. 1

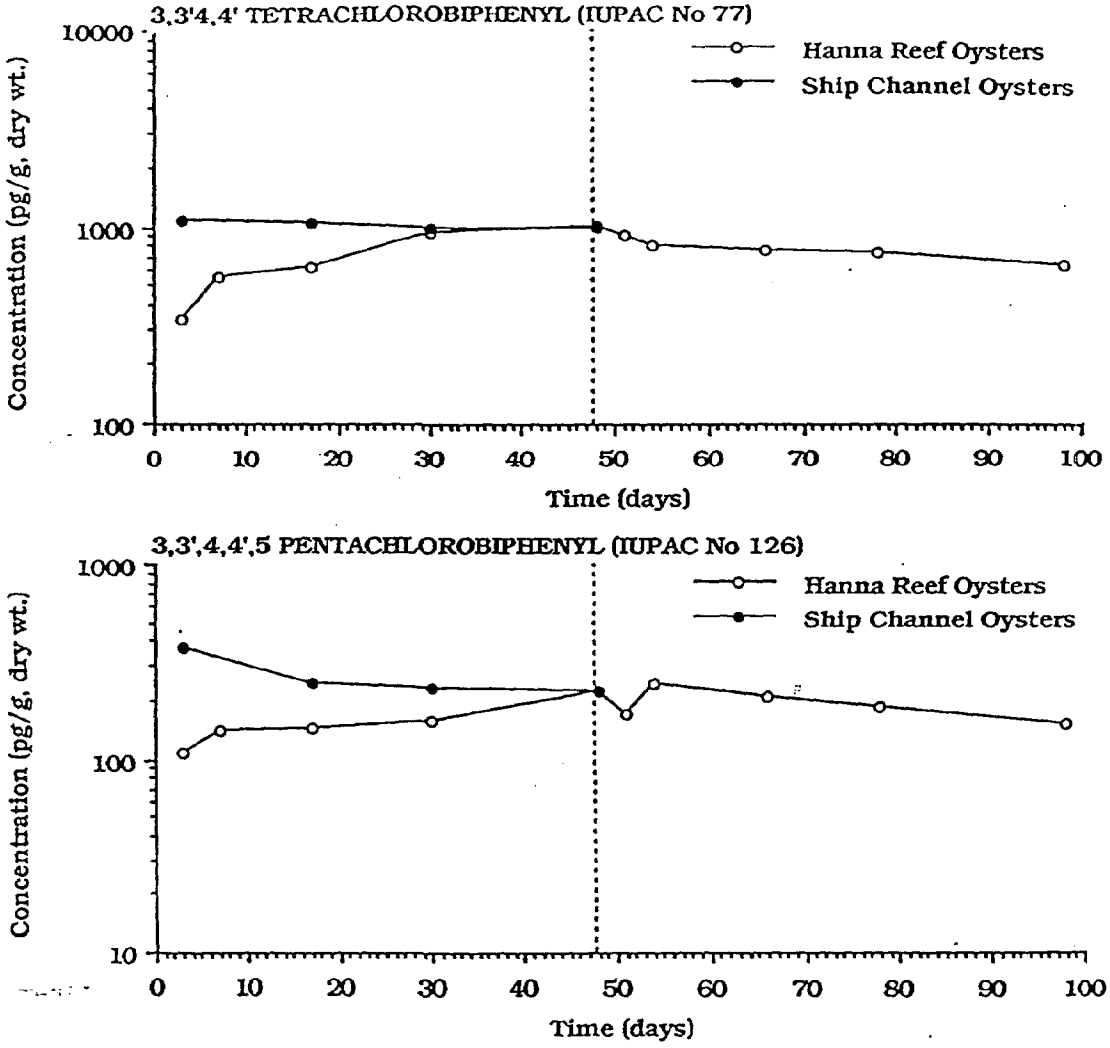
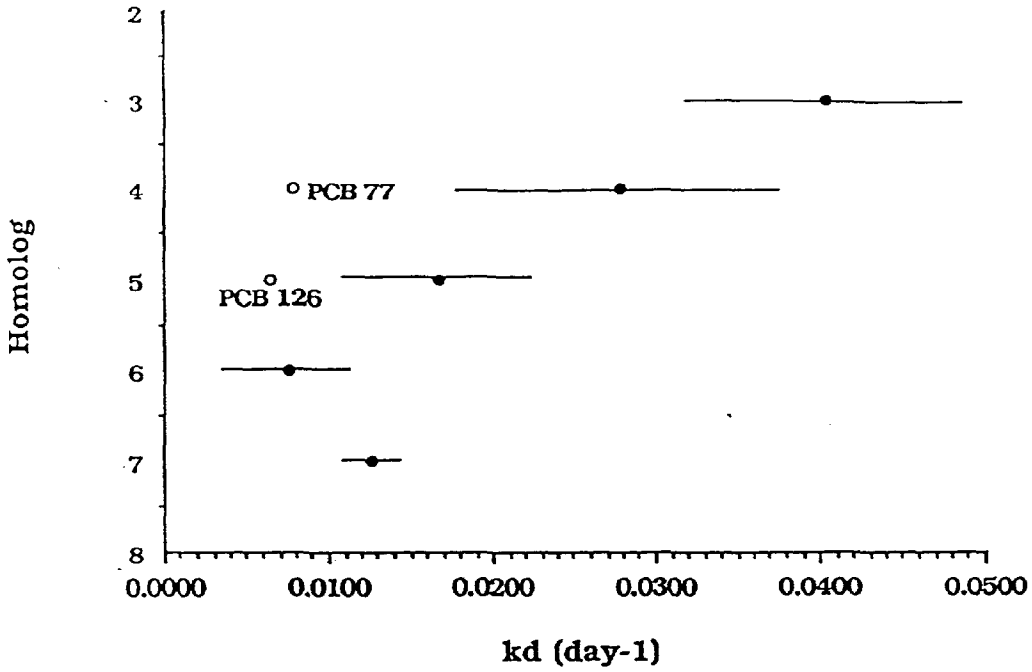

3,3'4,4' TETRACHLOROBIPHENYL (IUPAC No 77)
Hanna Reef Oysters
Ship Channel Oysters
Concentration (pg/g, dry wt.)
10000
1000
100
0
10
20
30
40
50
60
70
80
90
100
Time (days)
3,3',4,4',5 PENTACHLOROBIPHENYL (IUPAC No 126)
Hanna Reef Oysters
Ship Channel Oysters
Concentration (pg/g, dry wt.)
1000
100
10
0
10
20
30
40
50
60
70
80
90
100
Time (days)

Fig. 2


Homolog
2
3
4
5
6
7
8
PCB 77
PCB 126
0.0000
0.0100
0.0200
0.0300
0.0400
0.0500
kd (day-1)

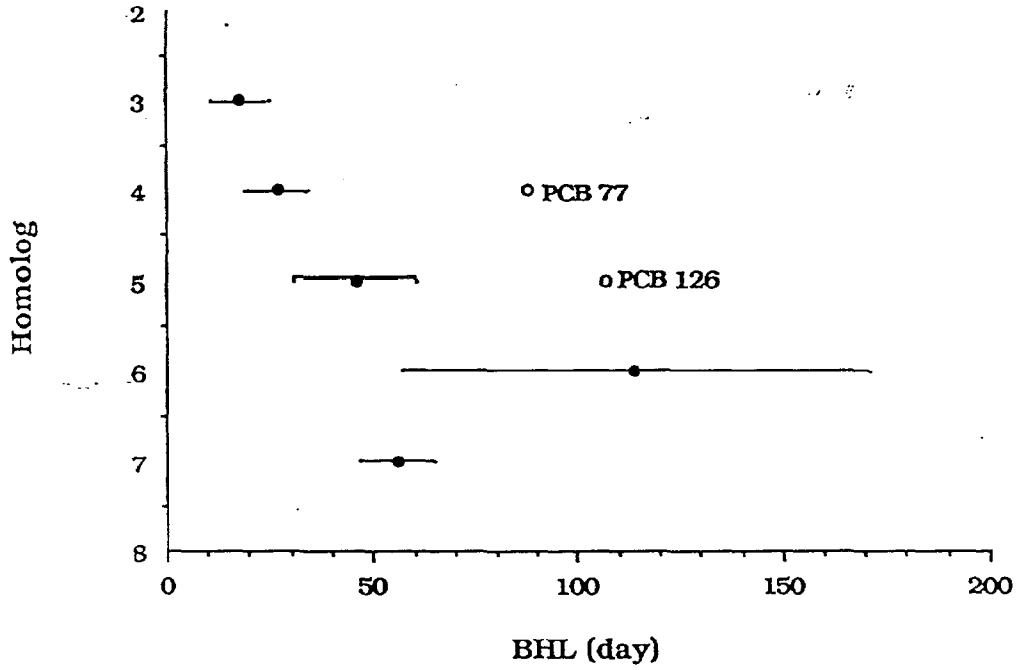
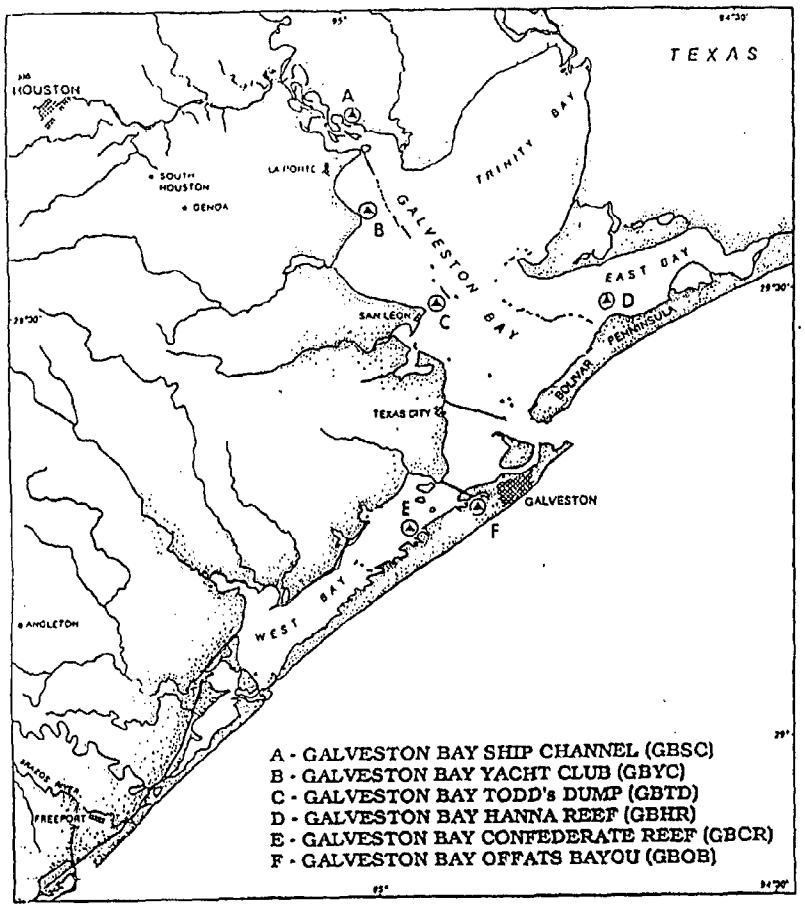

Homolog
2
3
4
5
6
7
8
PCB 77
PCB 126
0
50
100
150
200
BHL (day)

Fig. 3


TEXAS
HOUSTON
SOUTH HOUSTON
GENOA
LA PORTE
TRINITY BAY
GALVESTON BAY
EAST BAY
SAN LEON
BOLIVAR PENINSULA
TEXAS CITY
GALVESTON
ANGLETON
WEST BAY
FREEPORT
A - GALVESTON BAY SHIP CHANNEL (GBSC)
B - GALVESTON BAY YACHT CLUB (GBYC)
C - GALVESTON BAY TODD's DUMP (GBTD)
D - GALVESTON BAY HANNA REEF (GBHR)
E - GALVESTON BAY CONFEDERATE REEF (GBCR)
F - GALVESTON BAY OFFATS BAYOU (GBOB)

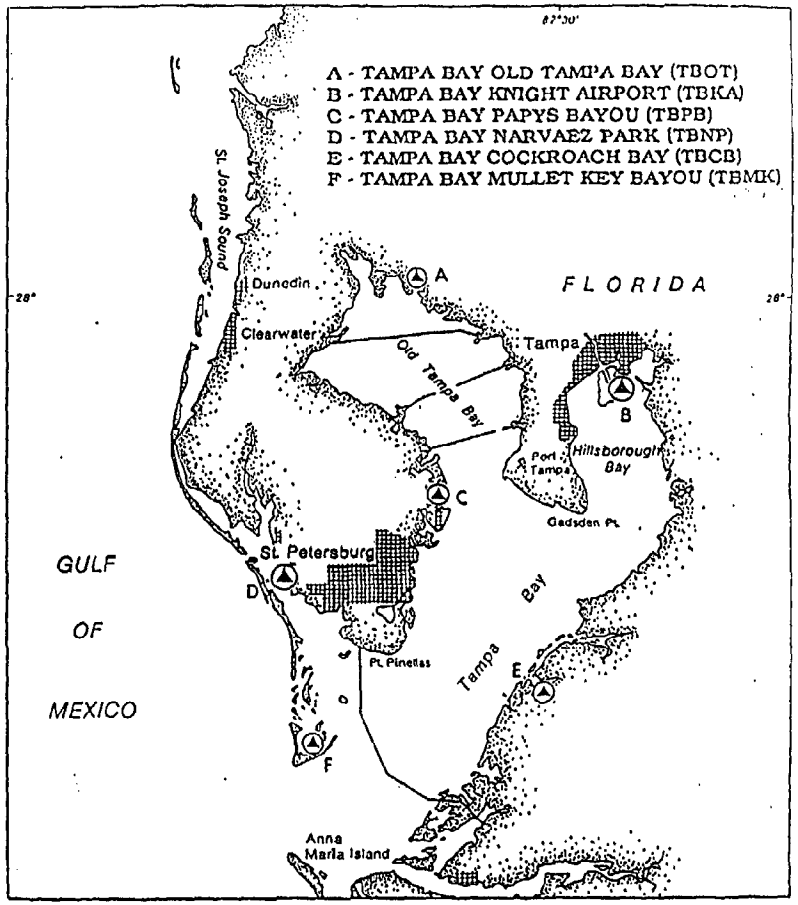

A - TAMPA BAY OLD TAMPA BAY (TBOT)
B - TAMPA BAY KNIGHT AIRPORT (TBKA)
C - TAMPA BAY PAPYS BAYOU (TBPB)
D - TAMPA BAY NARVAEZ PARK (TBNP)
E - TAMPA BAY COCKROACH BAY (TBCB)
F - TAMPA BAY MULLET KEY BAYOU (TBMK)
St. Joseph Sound
Dunedin
Clearwater
FLORIDA
Tampa
Old Tampa Bay
Port Tampa
Hillsborough Bay
Gadsden Pt.
St. Petersburg
Pt. Pinellas
Tampa Bay
GULF
OF
MEXICO
Anna Maria Island

Fig. 4

5.0 Trace Metals Results

5.1 Laboratory Intercalibration Results

As was discussed in the Laboratory Procedures section, standard reference materials, U.S.G.S. standard rocks and other materials of known trace metal concentrations were analyzed with almost every batch of samples. In the case of INAA, these materials were used to quantify the amount of trace element in the sample, whereas in the AAS analysis, working curves made from commercial standards were used for quantification and the reference materials were used to verify results and identify recovery problems.

In addition to the reference materials we had obtained from NIST, USGS and other sources, we received intercalibration materials from the National Research Council of Canada (Dr. Shier Berman) again this year as we have each year since the NS&T program began.

5.2. Trace Metal Concentrations in Year 6 Oysters

In an attempt to bring out geographic and temporal trends, trace metal concentrations in oysters (the average of the three stations for each site), were plotted as a function of site location from Lower Laguna Madre, Texas, through the Everglades, Florida, in the annual reports for the first five years of this project. Each plot showed the geographic distribution of one of the trace metals determined. The plots of averaged data for the first five years are shown updated here by addition of the Year 6 data (Figures 5.1 to 5.13). Some observations based on these plots are given below, with emphasis on higher than average values for a given element that persist for more than one year and values that changed significantly in year 6 when compared to average values for the first 5 years. The plots will continue to be updated annually as the project proceeds, and both geographic and temporal trends will be sought.

Silver concentration in oysters was very high at Copano Bay, Texas, and at the nearby San Antonio and Matagorda bays during Year 1 of the project. During Year 2, the Copano Bay site, which at 7ppm was highest in Ag not only for the Gulf but for the entire U.S. in Year 1, was about 50% lower in Ag. Four other Gulf sites had higher Ag concentrations than it did. It was, however, still enriched relative to most other sites along the Gulf Coast and remained enriched in Year 3 with a concentration very similar to the Year 2 value. Year 4 saw a further decrease at this site to a near average Ag value. However, in Year 5, two of the three stations at this site gave oysters which were very enriched in Ag, even more so than in Year 1 of the project. In year 6 Ag was again down at this site.

The East Matagorda site was similar in Ag concentration in Years 1 and 2 and was greatly enriched compared to most other sites. It decreased to average values in Years 3 and 4 but was again enriched in Year 5. The other three sites from Matagorda Bay were not enriched in Ag in Years 1 and 2, but one site showed enrichment in Year 3. In general during Years 4 and 5, slight enrichments were seen throughout this part of Texas, but in year 6 Ag decreased to average values at all but one of these sites. East Matagorda oysters increased in year 6.

The south central Texas (Matagorda) areas where Ag is so variable are generally areas of low population density and relatively little commercial activity. There are, however, several large isolated petrochemical plants in the area as well as a large aluminum refining plant (ALCOA). It seems unlikely that human activity is involved in Ag variability in this area, but it's not impossible.

The Galveston Bay area is much more industrialized than is the Matagorda Bay area, but it produced oysters lower in Ag for the first 4 years. In Year 5, for some unknown reason, all stations at one site (GBCR) in Galveston were highly enriched in Ag and this same site was again greatly enriched in year 6. It seems that Ag input to this area of Texas varies from year to year, but we can not explain why or why the enrichments are so geographically localized.

Sabine Lake, Texas, just east of Galveston, was average in Ag in Year 1, very high in Year 2, and moderately high in Years 5 and 6. This area is heavily industrialized and was enriched in several metals (Ag, Cd, Cu) in Year 2 compared to Year 1, but slightly depleted in others (Cr, Fe, Zn). These large changes in Sabine Lake oysters might be due to inputs of specific pollutants at specific times. (Cu, for example, changed from a less than average value in Year 1 to a value more than three times greater than average in Year 2 to an average value in Year 3 and 4, whereas Zn was very much above average in Year 1, decreased in Year 2, and decreased further in Years 3, 4, 5 and 6.) However, we have no data on pollutants inputs that would confirm this hypothesis.

In Louisiana, a high Ag value of about twice the Gulf average was found at Vermilion Bay in central Louisiana in both Years 1 and 2. There was a slight decrease at this site in Year 3 but in Year 4 it had the highest Ag in the Gulf and it remained high in Years 5 and 6. As with the Texas situation, a variable input of Ag from some unknown source is suggested. Further east, the Ag concentration dropped drastically (Figure 5.1) through the next five sites and reached a distinct minimum at Barataria Bay, just west of the Mississippi River delta in Years 1 and 2. The pattern was similar in Years 3, 4, 5 and 6, but the minimum at Barataria Bay was not as distinct because the

nearby Terrebonne Bay samples were also very low in Ag. Moving eastward from Barataria Bay, scattered high values were found east of the Southwest Pass of the Mississippi River delta, especially at the Pass A Loutre site on the Mississippi River Delta, during Year 5, but this site was not sampled for Year 6. Farther east, site MBHI first sampled in Year 3 almost doubled in Ag in Year 6 to become one of the highest sites in the Gulf. Nearby site MBDR was high last year but was not sampled this year.

Both high and low Ag values are found in Florida. Like the Louisiana sites, the Florida sites were generally, but not always, very similar for all 6 years, whether they were high, low, or average in silver concentration. For example, Choctawhatchee Bay was much above average all 6 years and Tampa Bay Mullet Key was much below average. The CBSP site in Choctawhatchee Bay gave oysters averaging 6.4ppm in year 6, the highest in the Gulf. Oysters from this site were also enriched in Pb and Se.

Barataria Bay, and the surrounding bays with low concentrations of Ag in oysters, have probably been as physically disturbed by man as any bays on the Gulf Coast based on the information we have at this time. These are areas of extensive petroleum development and widespread dredging and channel cutting. Almost every square foot has been disturbed by man. Furthermore, these bays are directly downstream of the Mississippi River outflow, which is usually considered to be a major source of pollutants to the Gulf of Mexico. Why, then, is Ag so low and does only Ag show this apparently anomalous behavior? The second part of the question is easy. Several other metals show distribution patterns almost identical to that of Ag (e.g. Cd and Cu; Figures 5.3 and 5.5); other metals (e.g. Fe, Cr, Se, and Hg) show similar but not identical patterns. Something about the muddy, frequently stirred Louisiana bays may be keeping the concentration of some trace metals in oysters low. Perhaps the large amount of fine-grained clay from the Mississippi River effectively competes with the oysters by adsorbing dissolved metals. The clay itself would not become greatly enriched in trace metals due to dilution by its large mass (~ 3×10^{14}g of sediment are transported by the Mississippi River each year) and would not be a clear indicator of pollution.

The idea that the amount and/or kind of suspended material in the water might control the amounts of trace metals in oysters by controlling the concentration of dissolved trace metal is one of several possible explanations for the patterns seen. It is also possible that local anthropogenic inputs influence trace metal concentration patterns, even though such inputs have not yet been identified. As noted above, it is interesting that oysters from Sabine Lake, Texas, were greatly enriched in Cu in Year 2 compared to Year 1, and also in Ag and Cd, but not in Fe, Hg, Pb or other metals. The extreme

enrichment in Ag of oysters taken at Confederate Reef in Galveston Bay during Years 6 should also be noted, as well as the big increase in Cu at a Lake Borgne site (LBMP) in Year 5 and the big increases in Cu, Pb and Zn at Knight's Airport in Tampa Bay in Year 6. This shows that oysters can change drastically in trace metal content in a one year period under certain circumstances, even though the general pattern in the Gulf of Mexico is to have similar concentrations of a given metal at a site year after year. The implication is that the oysters do respond to added pollutants.

Other anthropogenic-looking trace metal values include high Hg in Lavaca Bay, Texas, and at some Florida sites, especially Old Tampa Bay; very high Pb, increasing in concentration each year at one of the two Choctawhatchee Bay sites; a two fold increase in Pb at the Houston ship channel site in Year 5; very high Zn at the old Tampa Bay site; and higher than average Ag, Cd and Cu at the Vermilion Bay site. These abnormally high values may well be a result of anthropogenic inputs of metals. It is also possible that some of the tissue metal concentration variability is determined by the oysters themselves through "natural" processes. As mentioned earlier, such parameters as size and sexual stage of the oysters are being examined in this study because trace metal levels in oysters are reported to vary with these and other physiological parameters. In other work we have sampled oysters in Mobile Bay four times over a year period and Galveston Bay oysters in June and September (vs NS&T sampling in December). In repeated sampling from the same reefs, we have found concentrations of some metals as much as a factor of two lower in September than in March-June. Thus, physiology may play a role in some metal variation.

We have not yet completed attempts to correlate oyster metal data with the other information we have about the oysters, but this is being done and preliminary work shows no simple relationships applicable to the whole data set. One observation made in our earlier reports is that the oysters around Barataria Bay, which were much lower than average in trace metal content, were among the last oysters collected in Year 1 of the project, and apparently were among the few that had either just spawned, or were about to spawn, although spawning state is not easy to determine for Gulf oysters. This relationship obviously cannot explain all variability in the data, however. Otherwise, all metals would correlate perfectly with each other, which they do not. In fact, in some cases some metals are in high concentration precisely where others are low. Likewise, the sampling that occurred after Year 1 in Louisiana apparently did not collect oysters at the same spawning state as the first year sampling, yet trace metal levels were similar to those found in Year 1.

The Louisiana oysters for most years have been larger than average, and trace metal content of all Gulf of Mexico oysters showed a weak negative correlation with size, although there were exceptions to

this tendency. We have not seen any correlation between metals in oysters and salinity, water depth, or such variables. In short, there is no strong indication that physiological parameters have obscured metal concentration variations which are due to environmental factors such as variable input of pollutant metals. The strategy of sampling during the winter each year does seem to reduce "natural" variability in oyster metals.

Some of the high metal levels in oysters from the clear waters of western Florida are surprising. Arsenic especially is much higher in some of the Florida oysters than it is elsewhere on the Gulf Coast, yet some Florida oysters, for example those from most sites in Tampa Bay, were very low in As all 6 years. Only the Tampa Bay site at Navarez Park near the city of St. Petersburg was significantly enriched in As. It was first sampled in Year 4, at which time it had the highest As concentration in the Gulf. In Year 5 the As level was even higher and in Year 6 the concentration more than doubled from the high Year 5 value. Arsenic at this site is now four times higher than that at any other site. The new site at Knight Airport on the edge of the city of Tampa was low in As in Years 4, 5, and 6. It is possible that the extensive phosphate rock deposits in Florida are a source of arsenic, but based on the limited data we have, there is no correlation between phosphate rock occurrence, shipping, or mining, and As concentration in oysters. For example, a phosphate plant is reportedly adjacent to the TBHB site, yet oysters from it were low in As.

The As distribution in Florida does seem to call for some kind of local environmental control, as do certain other metal distributions. There seems to be no other explanation for high and low values of trace metals to occur at adjacent sites, often in a given bay, and to have these values repeat year after year. This suggestion is further strengthened by the Year 5 and 6 results which show the As level at CBBI, NBNB and RBHC dramatically lowered than was found through the first four years. Some unknown environmental change, perhaps rainfall and runoff or a change in pest control practices, must be responsible for the As decrease.

The local patterns discussed above are imposed on regional trends; for example, Hg is enriched in Florida sites where twelve of the 25 sites are well above average. The oysters from Old Tampa Bay are especially high in Hg, rivaling even those from Lavaca Bay, Texas, which are known to be contaminated with Hg and to be a human health threat. Se, Cd, and Ag, on the other hand, are generally lower in Florida oysters than those collected elsewhere. Other metals show no obvious regional trends, but subtle trends may exist.

Zn shows especially great variability from place to place, not only in Florida but throughout the Gulf Coast, and can even vary widely within a given bay. For example, Old Tampa Bay oysters averaged 8300

ppm Zn in Year 3 and 6700 ppm Zn in Years 4, 5, and 6, whereas oysters from Mullet Key in Tampa Bay averaged only 240 ppm in Year 3 and only about 325 ppm in Years 4, 5, and 6. Apalachicola Bay oysters were even lower in Zn than those from Mullet Key during the six years of the project. Should the APDB low Zn values be considered background for all Florida oysters, or does the natural background concentration vary by more than the observed factor of 20 from site to site? It seems very unlikely that the background would vary so drastically within a given bay. Human activity must somehow be involved in these drastic differences in Zn content. This suggestion is supported by the observation that Zn concentrations in oysters does seem in a qualitative way to correlate with proximity to population and industry.

St. Andrews Bay in north Florida provided oysters greatly enriched in Zn and Cu in Year 1 but somewhat less enriched in later years. These same oysters were depleted in Cd by almost a factor of four during all six years and had less than average concentrations of several other metals. This may be a case in which large amounts of one or two metals inhibit the uptake of other metals, but again the situation is ambiguous because high Cu and Zn in Sabine Lake and Vermillion Bay oysters are accompanied by high Ag, Cd, etc. It is likely that the form (species) of metal in the environment is as important as the amount where uptake by oysters is concerned.

5.3 Summary and Conclusions from Six Years of Trace Metals in Oysters Data

The trace metal concentrations found in the Gulf of Mexico oysters were generally less than or equal to literature values from other parts of the world that are thought to be uncontaminated by anthropogenic activity. A few sites, however, did show apparent trace metal pollution, and other sites gave anomalous values that cannot be readily explained by either known anthropogenic or natural causes.

The range of values for the overall data set (maximum/minimum) varied from 15-fold for Mn to more than 600-fold for Pb, whereas the coefficient of variation (standard deviation/mean) was generally in the 50-60% range for most metals. Variations were much greater between stations than between years at a given station. Enrichments usually occurred in suites of 3-4 elements with Ag, Cd, Cu and Zn being the most common suite; thus, several strong inter-element correlations were found. There was, however, little correlation between metal levels in oysters and in sediments from the collection sites even when sediment data were ratioed to Al. There was likewise little correlation between oyster metal levels and size, sex, or reproductive stage of the oysters.

Geographically, appreciably elevated (>3 x average) metal levels were generally restricted to single sites within bays or estuaries which implies local control (Reprint 8). On the other hand, Ag, Cd and Se levels were somewhat higher in Texas oysters than in those from Florida, whereas the reverse was true for As and Hg. Concentrations were lower than average for several metals in oysters from central Louisiana, especially Ag, Cd, and Cu. Thus, the Mississippi River outflow and extensive offshore oil development do not seem to enrich oysters in trace metals.

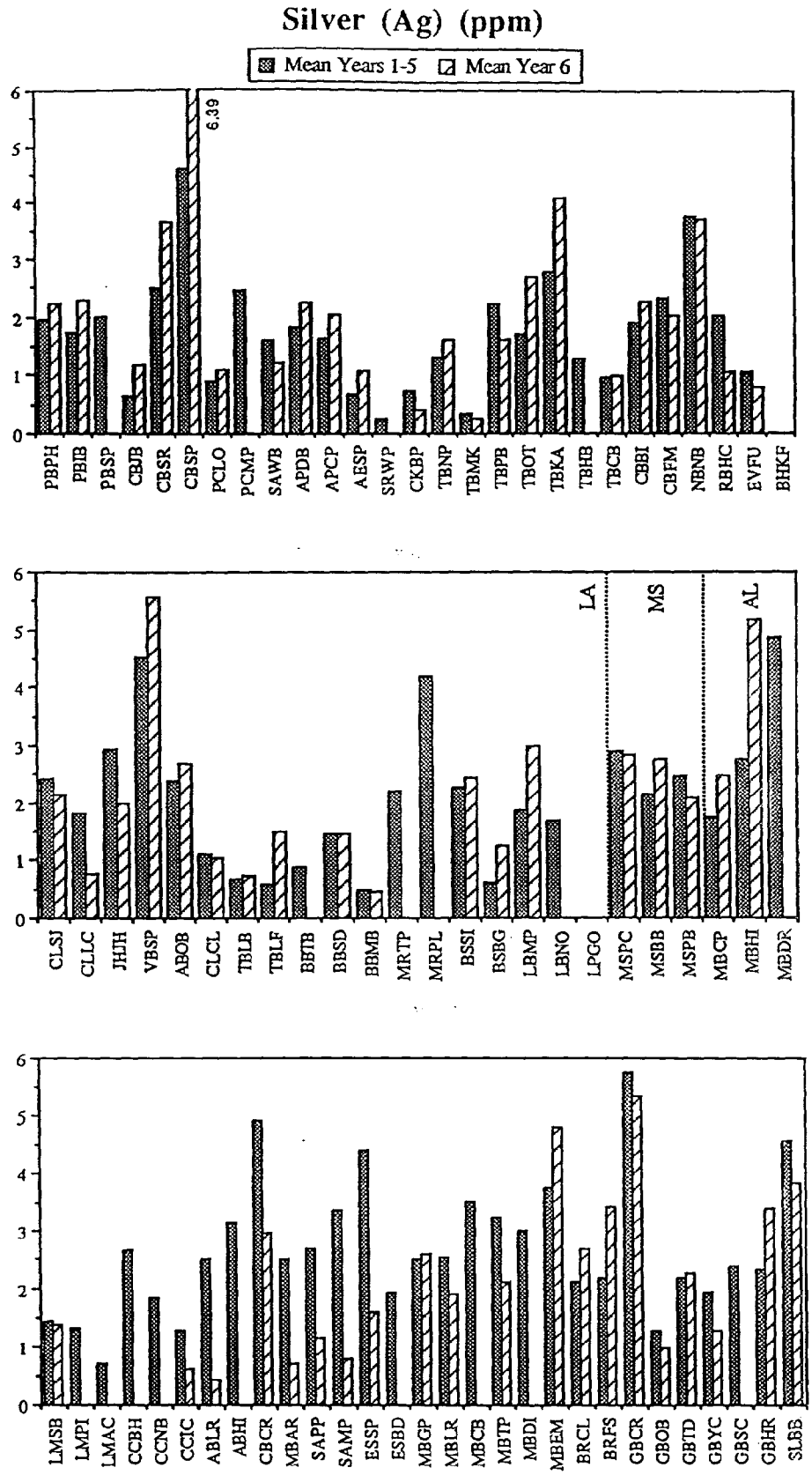


Figure 5.1 Average silver concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

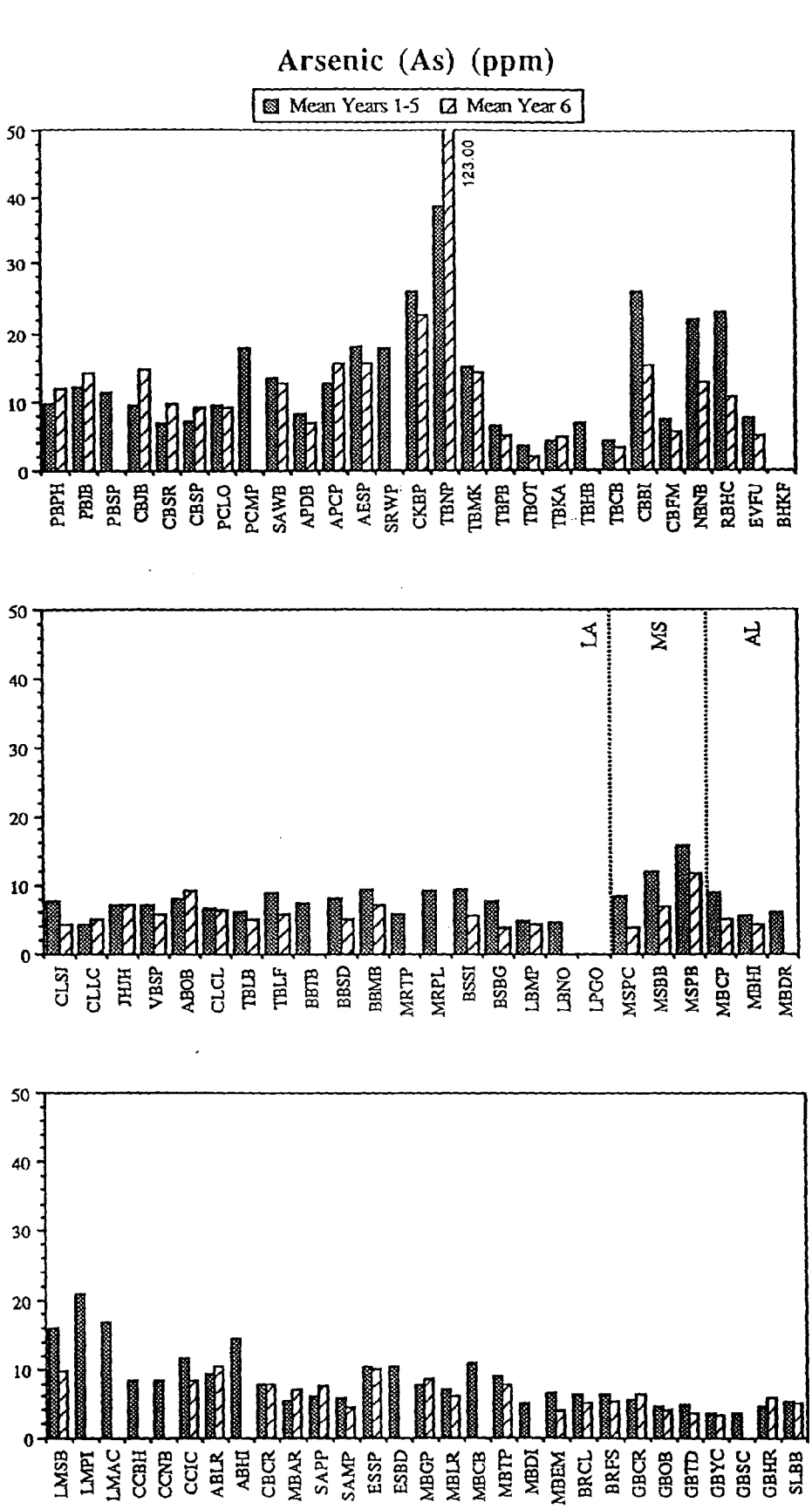


Figure 5.2 Average arsenic concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

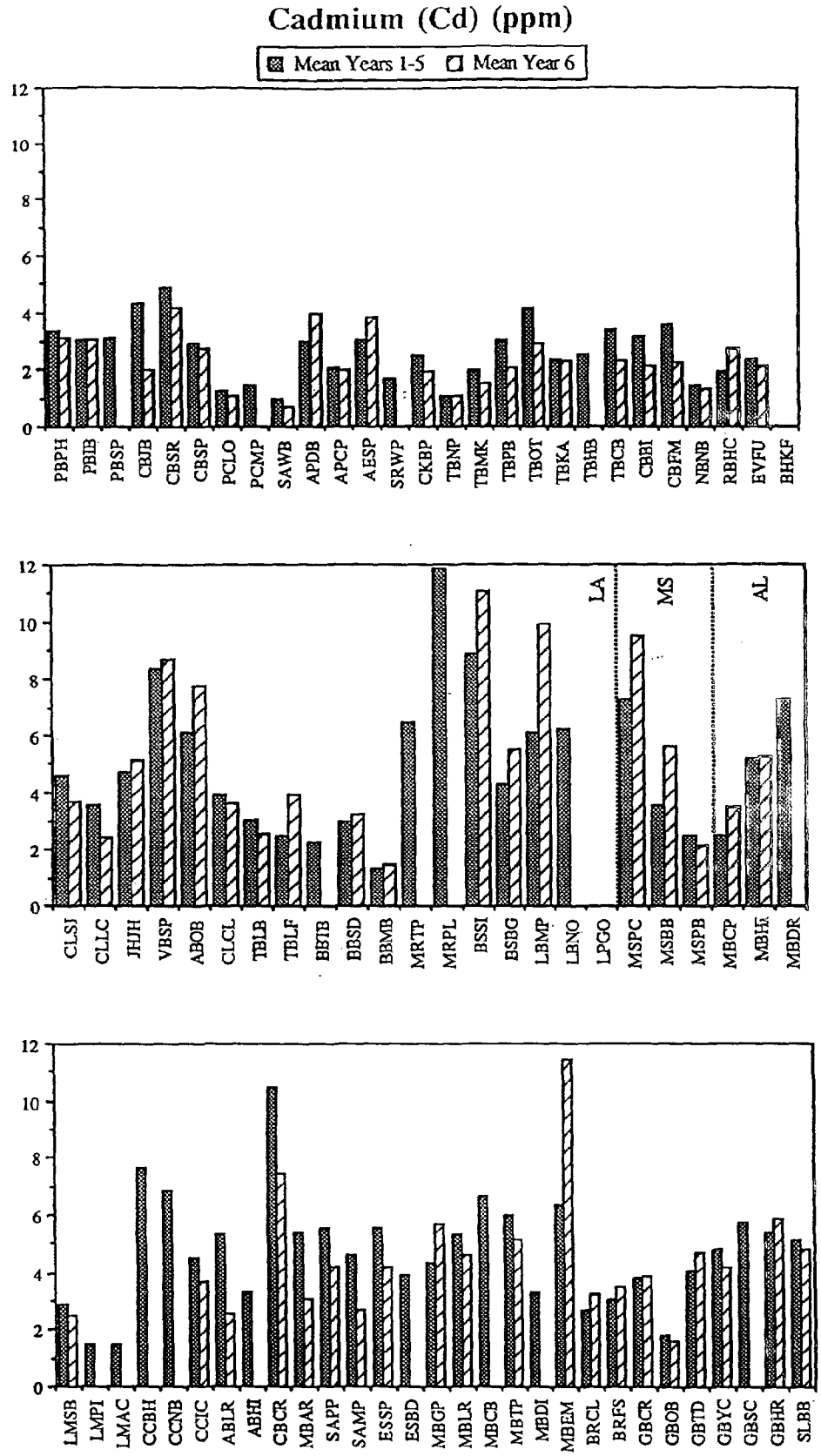


Figure 5.3 Average cadmium concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

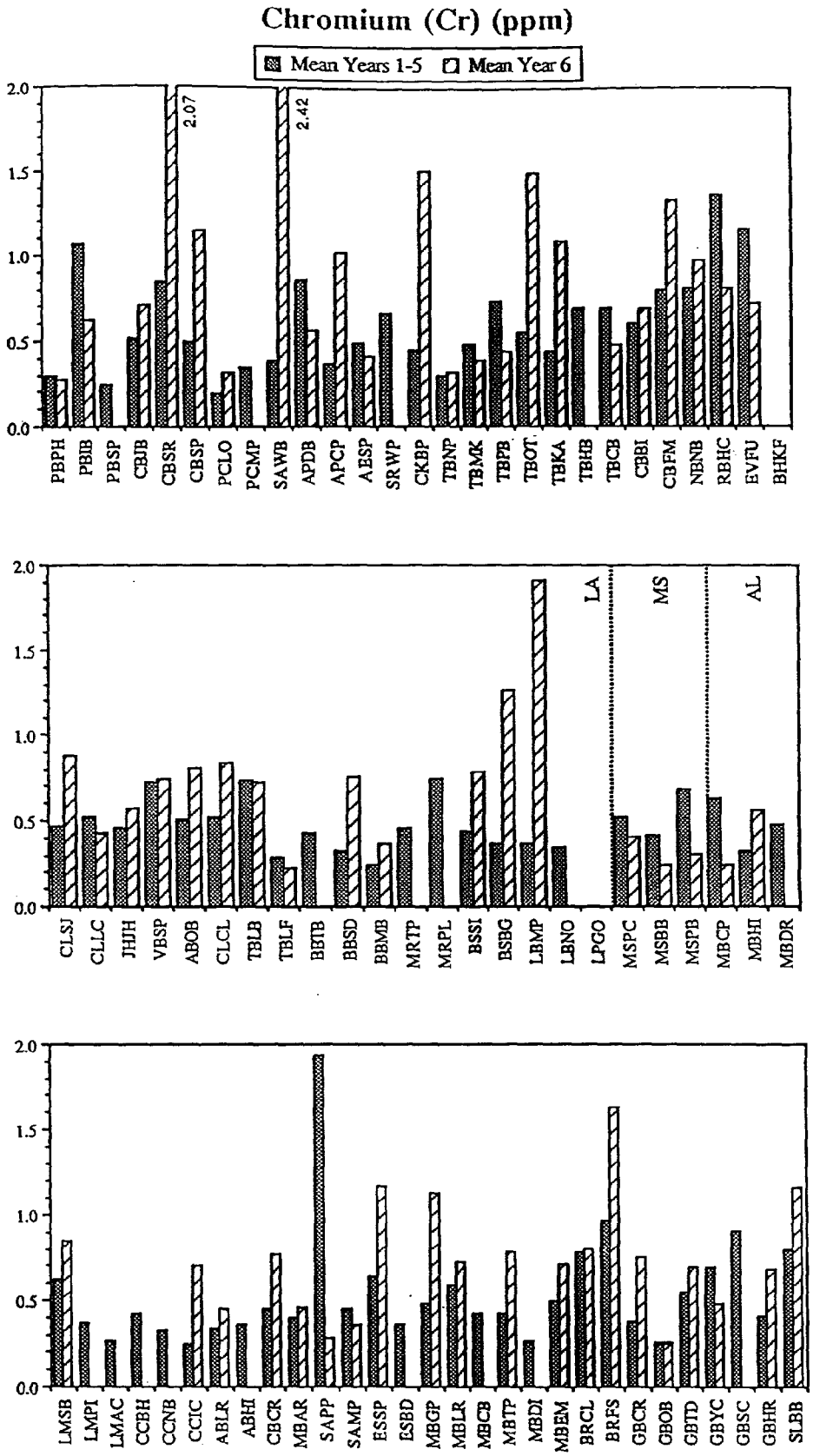


Figure 5.4 Average chromium concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

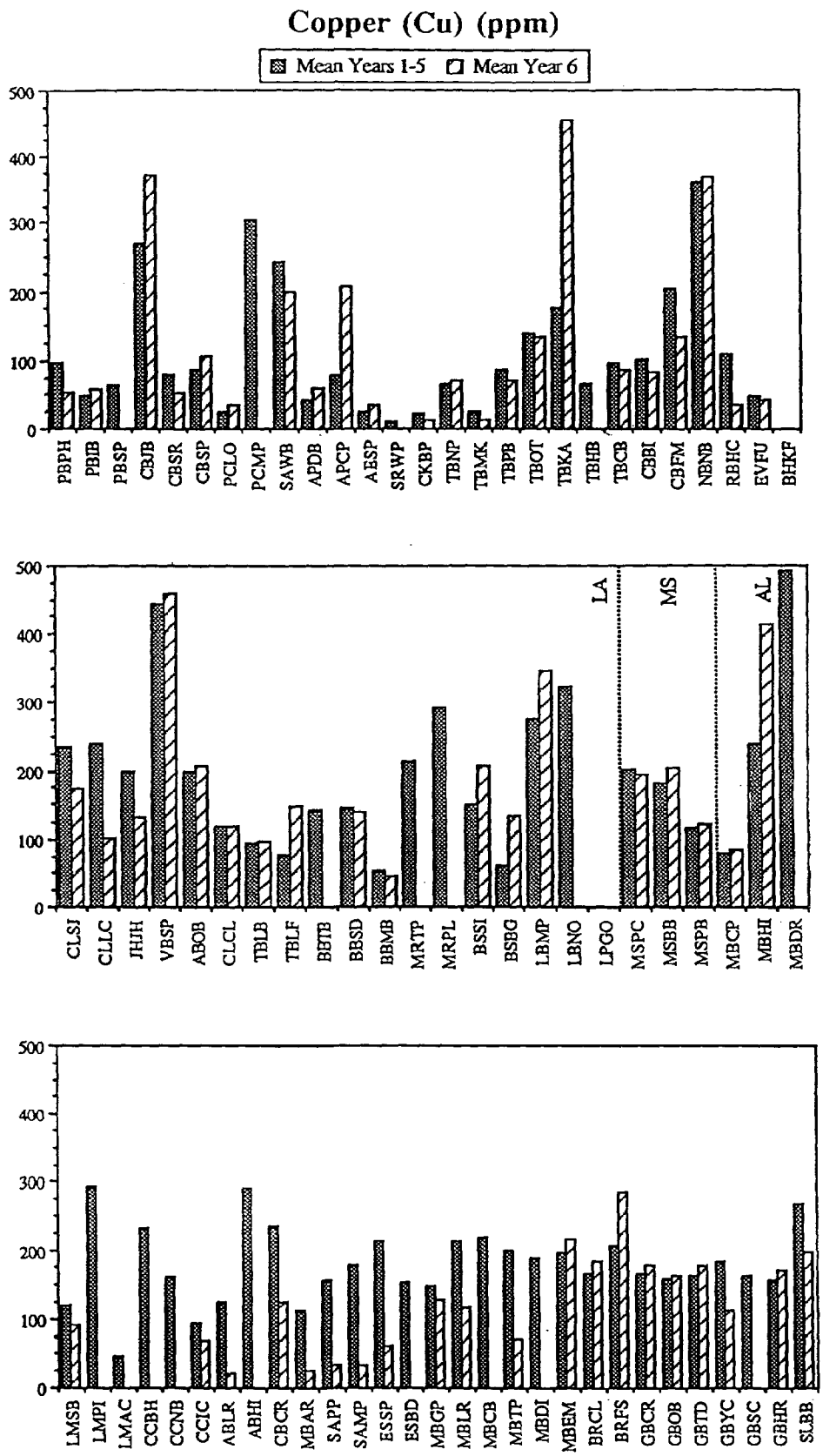


Figure 5.5 Average copper concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

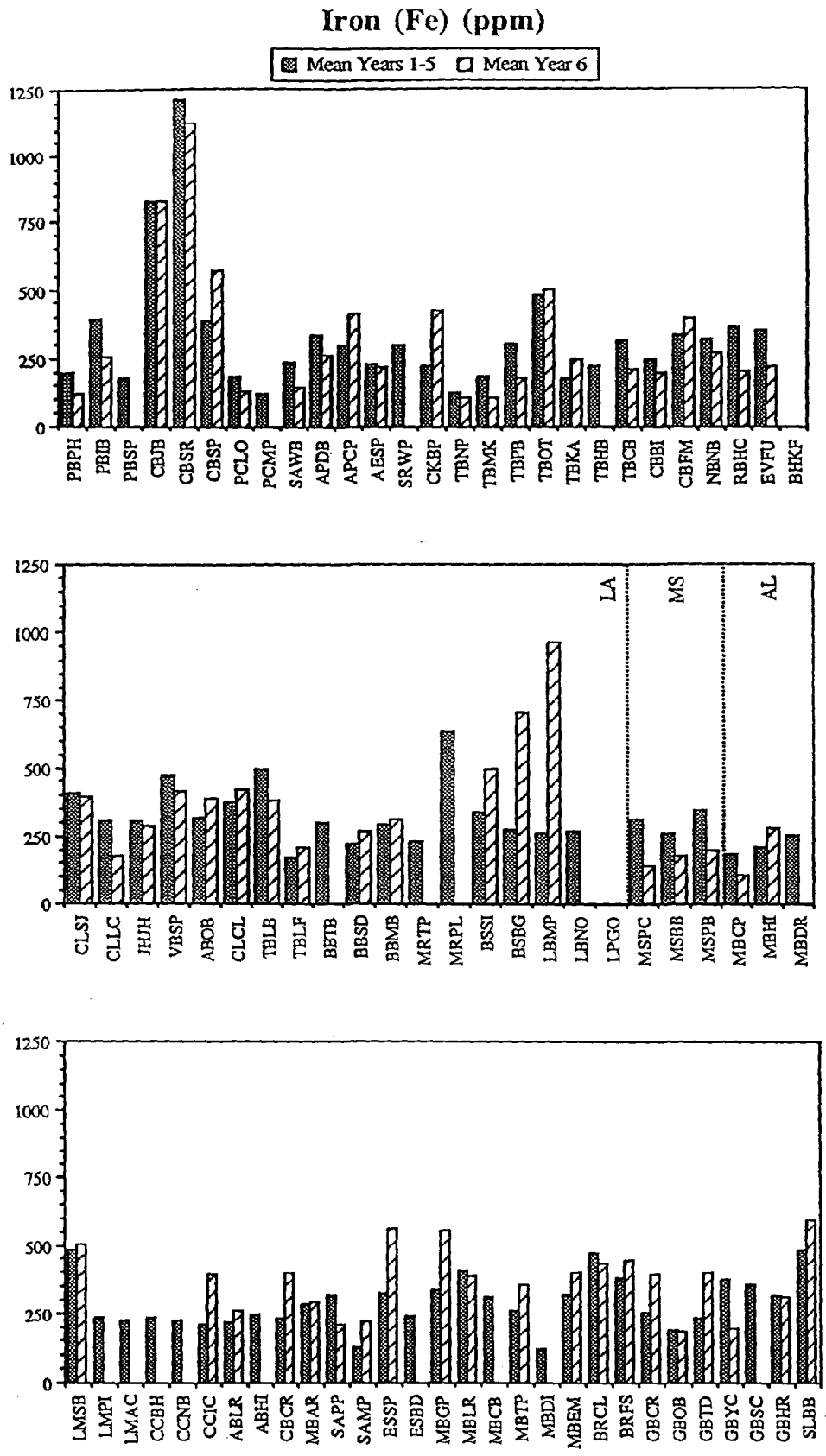


Figure 5.6 Average iron concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

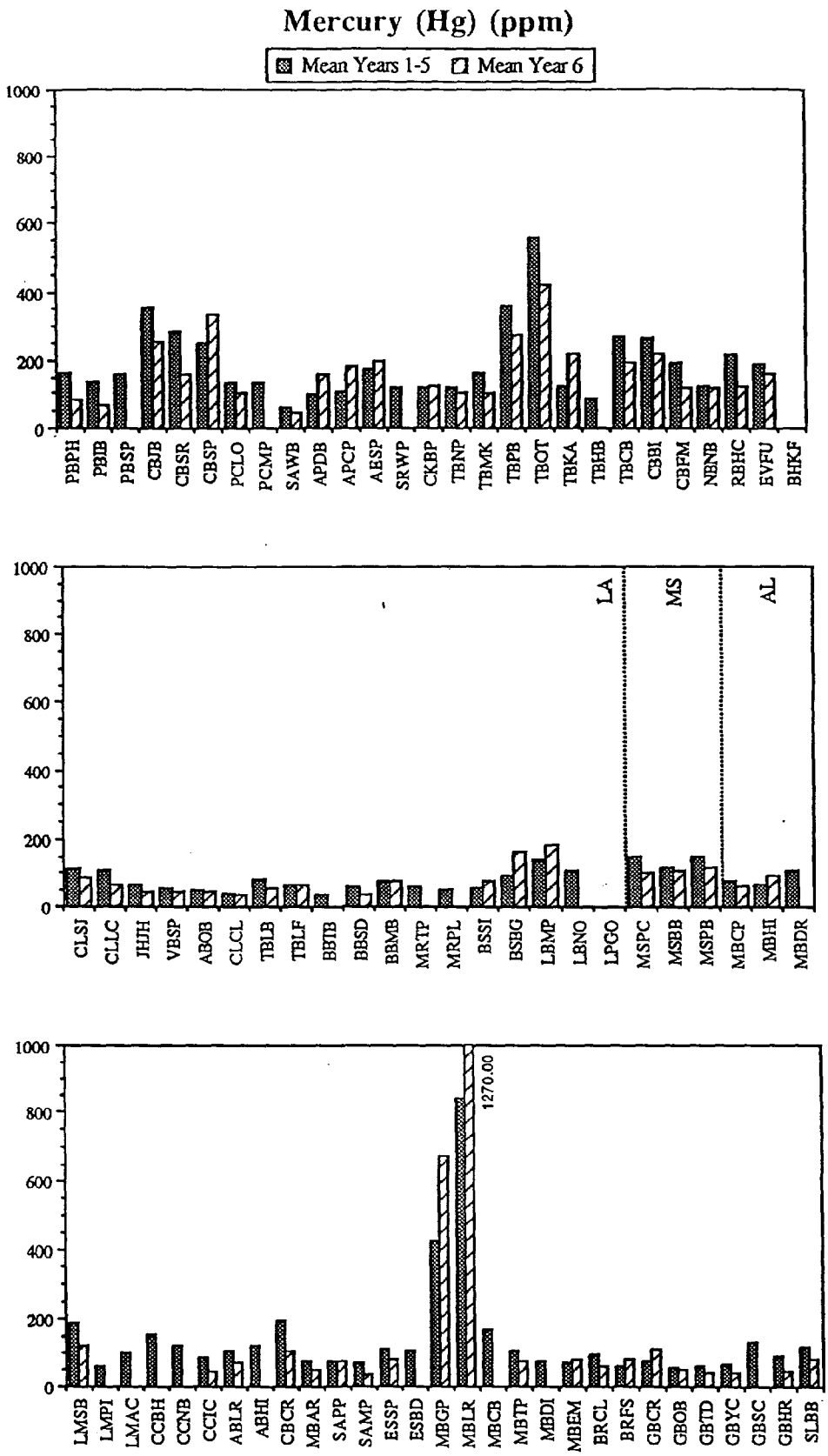


Figure 5.7 Average mercury concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

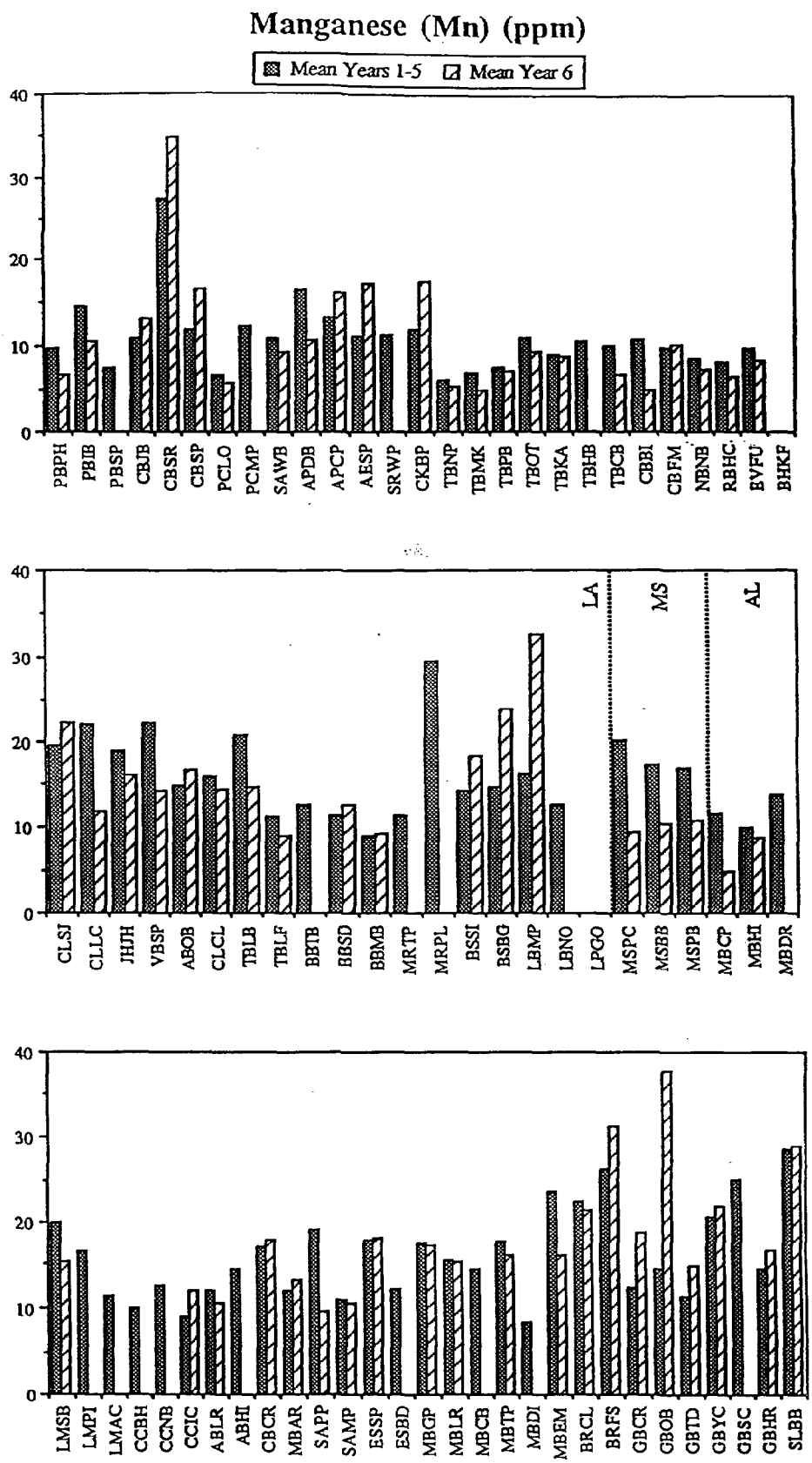


Figure 5.8 Average manganese concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

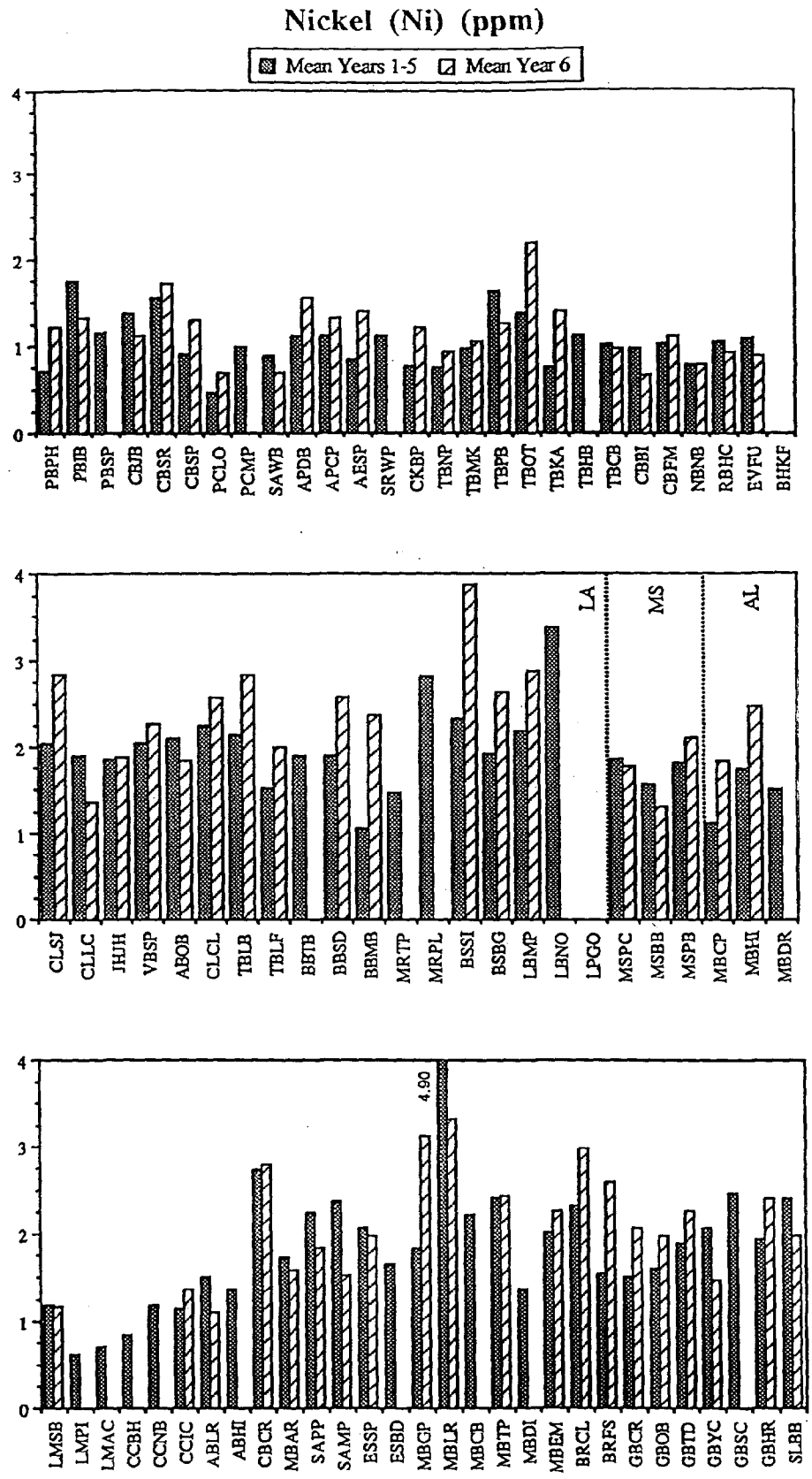


Figure 5.9 Average nickel concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

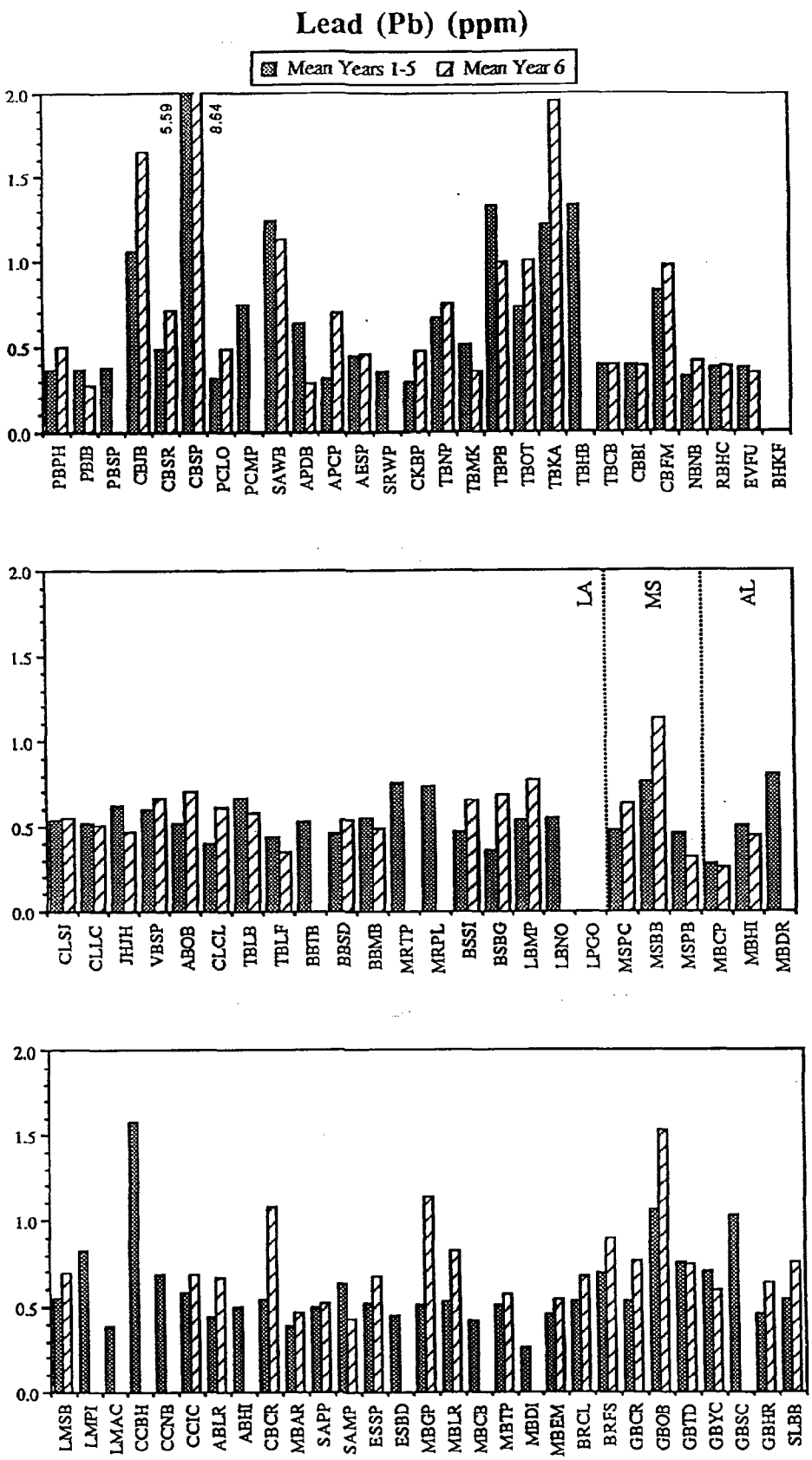


Figure 5.10 Average lead concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

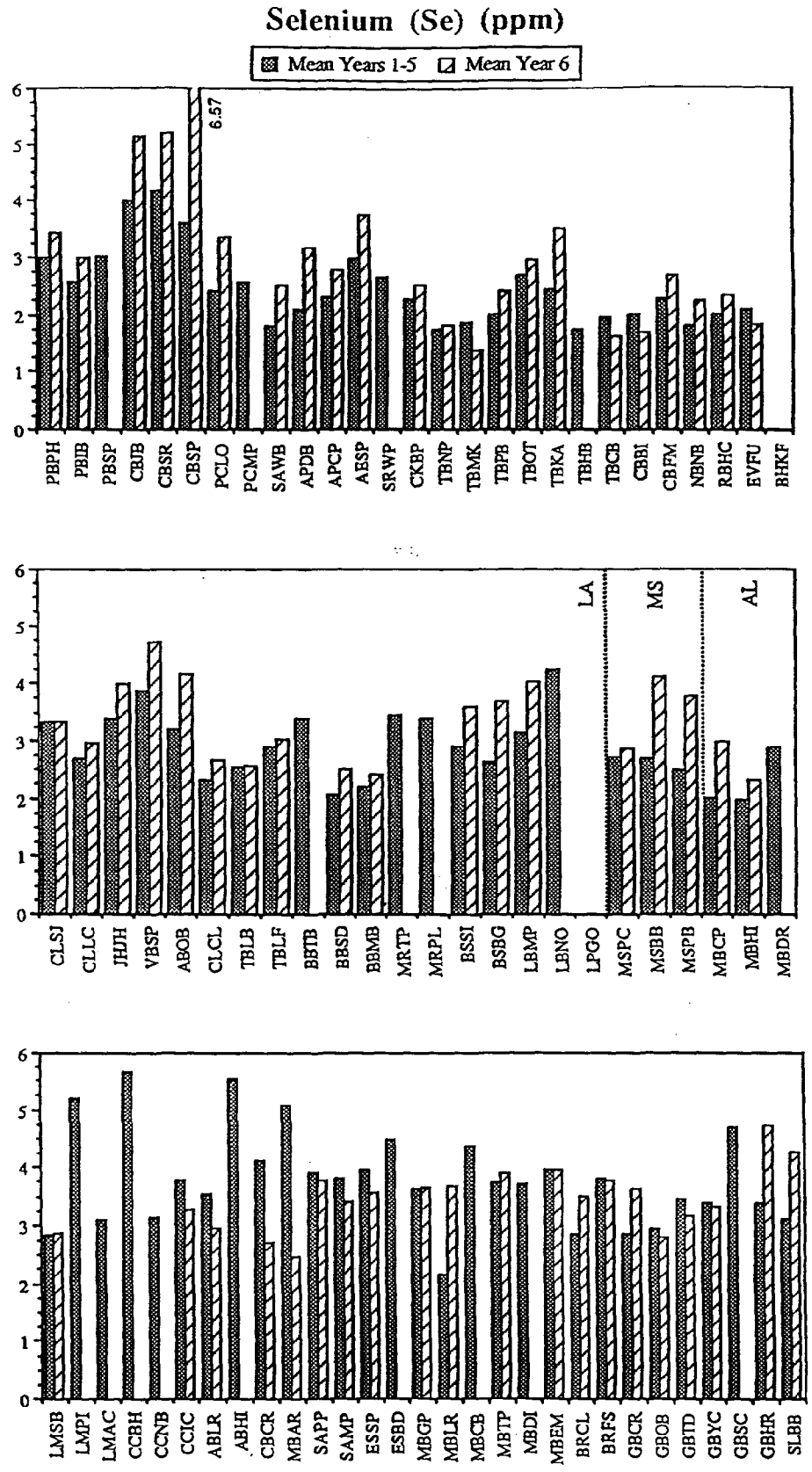


Figure 5.11 Average selenium concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

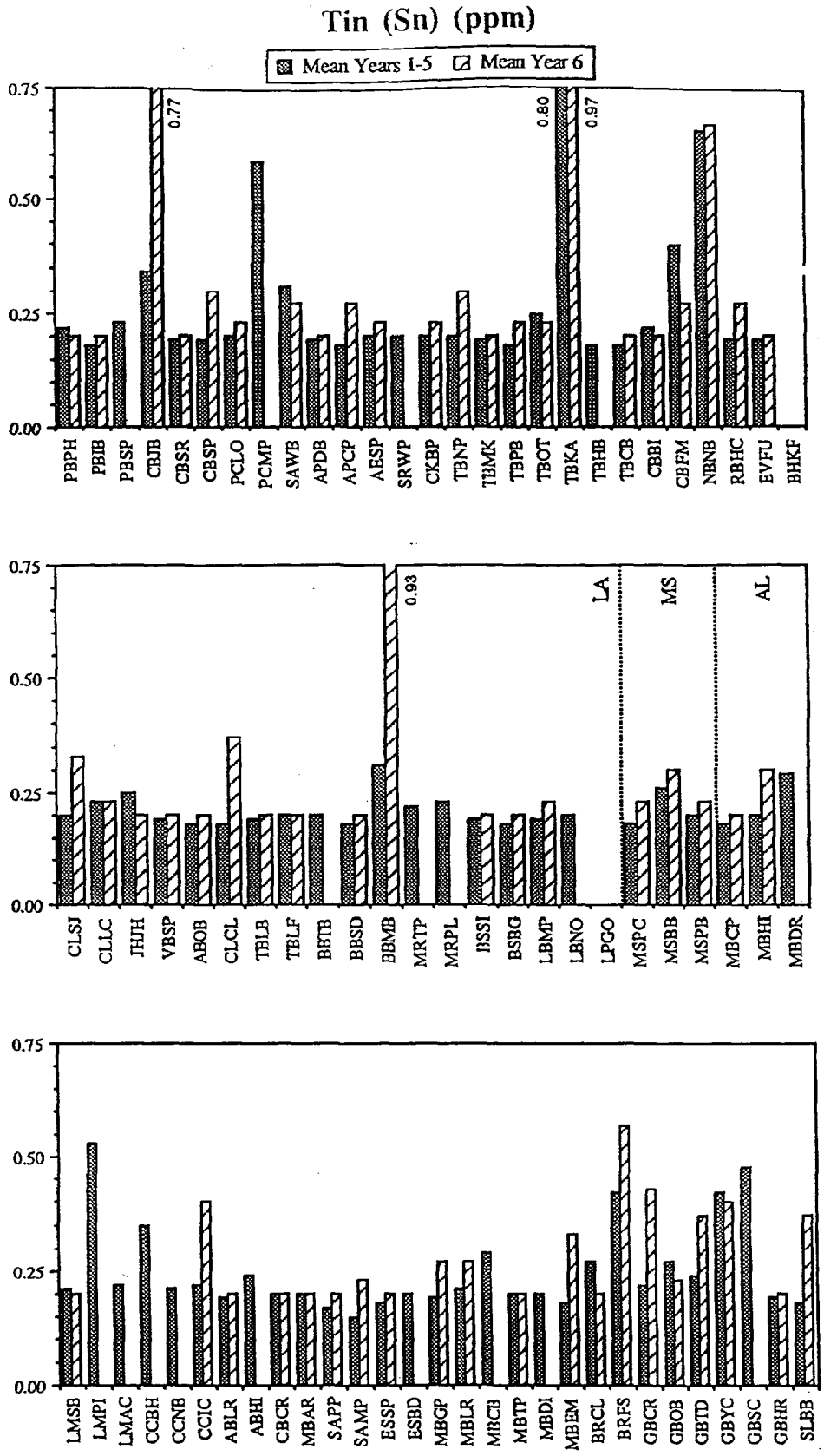


Figure 5.12 Average tin concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

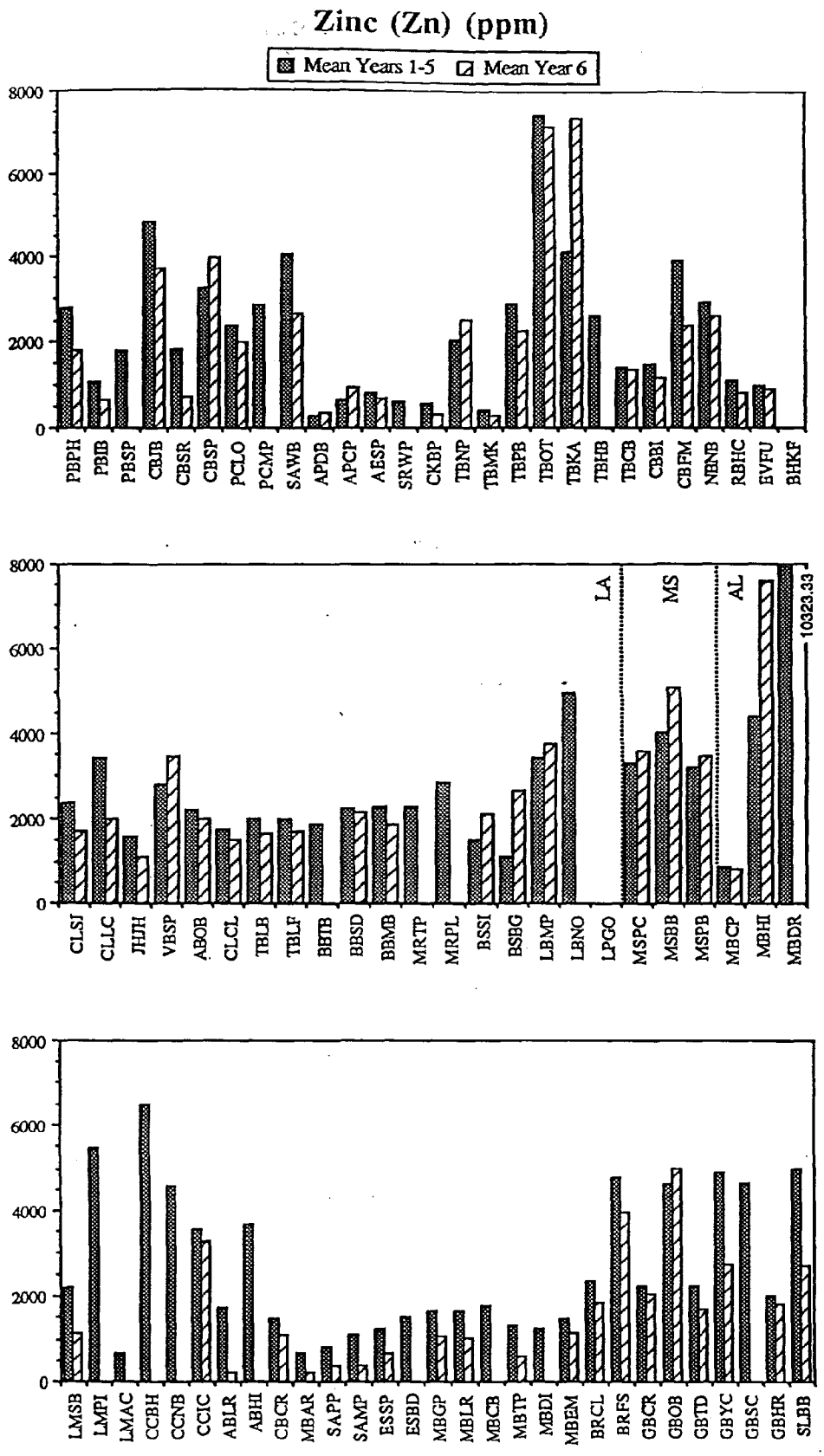


Figure 5.13 Average zinc concentrations in oysters from each NS&T Mussel Watch Gulf of Mexico sampling site for Years 1-5 and Year 6.

Reprint 8

Trace Metals in Galveston Bay Oysters

B.J. Presley, R.J. Taylor, and P.N. Boothe

Trace Metals in Galveston Bay Oysters

B. J. Presley, R. J. Taylor and P. N. Boothe
Oceanography Department, Texas A&M University

Oysters and other bivalves have been used as "sentinel" organisms for assessing the pollution status of marine water bodies for almost twenty years. For example, Goldberg, *et al.*, (1983) report data for a U.S. EPA funded "Mussel Watch" program conducted in 1976-78 and the current NOAA funded "National Status and Trends Program" (NS&T) is an outgrowth and extension of the "Mussel Watch" concept. Bivalves are widely recognized as being responsive to changes in pollutant levels in the environment, good accumulators of pollutants, widely distributed along coasts, and easy to collect and analyze. They integrate pollutant levels in the environment over weeks to months and therefore allow areas to be compared even when sampling is done only once or twice per year.

Oysters (*Crassostrea virginica*) were collected at six different sites in Galveston Bay during 1986-1989 as part of NS&T (see Fig. 1, page 69). Each site was on an identifiable oyster reef and at each, twenty oysters were taken from each of three stations, the stations being 100 to 500 m apart. Each site was sampled once each year, except two of the sites (stations 58, 59) were not sampled the first two years. The twenty oysters from each station were combined and analyzed as a single sample each year. In most cases stations were located hundreds of meters to many kilometers away from any obvious point sources of pollutant inputs in an attempt to characterize large areas of Galveston Bay, rather than to identify specific point sources of pollutant input.

Frozen oysters were returned to the lab where they were opened under clean room conditions. The oyster tissue was put into teflon jars which were loaded into an industrial paint shaker and shaken vigorously for 15-20 minutes to completely homogenize the samples. An aliquot of the combined and homogenized sample was freeze-dried, re-homogenized by ball milling in plastic, and weighed into a digestion vessel. Digestion of the approximately 200 mg dry weight samples of oyster tissue used three ml of a four to one mixture of ultra-pure nitric and perchloric acids.

Two blanks and two reference materials were digested with every set of 20-40 samples. Repeated analysis of these reference materials and participation in several intercalibration exercises give an estimate of ten percent or better for both the precision and accuracy of the data reported here.

All data reported here were obtained by atomic absorption spectrophotometry (AAS). The samples were analyzed for Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Si, Sn, and Zn. Flame AAS was used for Cu, Fe, and Zn which exhibit high concentrations in oysters, cold vapor AAS for mercury, and graphite furnace AAS for the remaining elements.

Trace metal concentrations found in oysters collected along the entire Gulf of

Mexico coastline during the first four years of NS&T were generally similar to those reported in oysters taken from non-contaminated water in other parts of the world (Texas A&M Geochemical and Environmental Research Group, 1990). Only a few sites showed obvious trace metal pollution and these were restricted geographically such that nearby sites were usually unaffected. Abnormally high or low values at a site did, however, usually repeat year after year suggesting local control. Abnormal sites for most metals were just as likely to be visibly pristine as to be highly industrialized.

The oysters collected in Galveston Bay for NS&T were similar in trace metal content to those collected elsewhere along the Gulf coastline, i.e., there is no indication of generalized trace metal pollution in Galveston Bay (Table 1). The average Ag, Cd, Cr, Fe, Mn and Pb in Galveston Bay oysters differs by 10% or less from the Gulf-wide average. Copper is 13% higher in Galveston Bay, while Ni is 15% higher and Se is 16% higher. A "t-test" of the significance of those differences shows that only the Se averages are significantly different at the 95% confidence level. Arsenic in Galveston Bay oysters is less than one-half the Gulf-wide average, but the Gulf average is greatly influenced by several sites in southern Florida that produce oysters greatly enriched in As. Oysters from other Texas and Louisiana bays are similar in As content to those in Galveston Bay. Tin seems to be about 20% lower than Gulf averages in Galveston Bay, but all Sn values are near the detection limit of the method used and a 20% difference is not significant. Finally, Zn is 43% higher in Galveston Bay oysters than in Gulf-wide average oysters.

Table 1. Average trace metal concentrations in 1200 oysters from Galveston Bay and 14,000 oysters from the entire U.S. Gulf coastline. All elements in ppm, μg/g dry wt.

	Ag	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sn	Zn
GB avg.	2.35	4.74	4.29	0.53	166	279	.0815	16.2	2.01	0.66	3.46	0.26	3220
GOM avg.	2.13	9.94	4.06	0.57	148	309	0.135	15.1	1.75	0.68	2.96	0.33	2250
GB/GOM	1.10	0.48	1.06	0.93	1.12	0.90	0.60	1.07	1.15	0.97	1.17	0.79	1.43

Discussion of metals in Galveston Bay oysters averaged over all sites and all years obviously cannot show possible geographic and temporal trends within the bay. In the case of Zn, for example, three of the six Galveston Bay sites had oysters with near Gulf average Zn, with relatively little year to year variation. The other three sites had much higher Zn. Two of the high Zn sites, Ship Channel and Yacht Club, are in extreme northwestern Galveston Bay near industrial waste water inputs and boat basins where Zn contamination might be expected. The other high Zn site was in Offatt's Bayou on Galveston Island and is surrounded by residential development and private boat moorings. This apparent local control on Zn, and in some cases on other metals, is seen not only in Galveston Bay but also throughout the Gulf of Mexico. Large site to site and time to time changes in trace metal concentration might be due to man, but the exact activity responsible has not been identified.

Cadmium, Pb, Ag and Hg are often added to the environment by man in amounts rivaling those added by nature but there is no evidence of anthropogenic inputs of these metals in the Galveston Bay oyster data. Rather, except for Zn, trace metal concentrations in oysters from Galveston Bay are similar to those in oysters from pristine areas elsewhere and do not reflect the big differences in proximity to population and industrialization of the different sites in the bay.

Literature Cited

Texas A&M Geochemical and Environmental Research Group. 1990. NOAA status and trends mussel watch program for the Gulf of Mexico. Technical Report submitted to National Oceanic and Atmospheric Administration, Rockville, MD.

Goldberg, E. D., M. Koide, V. Hodge, A. R. Flegal and J. Martin. 1983. U.S. mussel watch: 1977-1978 results on trace metals and radionuclides. Estuarine, Coastal and Shelf Science, 16: 69-93.

6.0 Butyltin Results

The analyses of butyltins as part of the National Status and Trends (NS&T) Project for Marine Environmental Quality, Mussel Watch Project was initiated in 1987 by GERG. Butyltin analyses was added to the NS&T project because of the growing concern about the effects of tributyltin from antifouling paints on non-target organisms. This section serves as an overview of several papers that have resulted from the butyltin studies performed by GERG (Table 1.1).

Organotin compounds have a range of toxicity and as such have found a broad spectrum of applications including use as fungicides, bactericides, pesticides, anti-cancer agents, and biochemicals. Tributyltins (TBTs) were a major component of many antifouling paints because they are 10-100 times more effective than copper-containing paints. It is estimated that 140,000 kg of TBT-containing antifouling paint was used each year prior to 1988 in the United States on commercial and recreational boats and ships to retard fouling. The U.S. Navy estimates that it could save $155 million annually by repainting its fleet of 550 ships with TBT-containing antifouling paint, but has not begun this process because of the mounting evidence that TBT compounds may have acute and chronic effects on non-target organisms. However, the recreational and smaller commercial fleets are potentially the most deleterious to estuarine resources because these vessels spend most of their time in port and their antifouling paints are formulated to give high static release rates.

Studies conducted in the United Kingdom and France have determined that the short-term acute toxicity of TBT in water is at the nanogram per liter level for oysters and other non-target molluscan species. The results of these studies and the finding that TBT water column concentrations appear to be increasing in selected harbors and marinas in the United States have prompted the U.S. Environmental Protection Agency (EPA) to initiate a special review study.

After review of the available data, the U.S. EPA concluded that low concentrations (20 ppt) of TBT in the water can cause irreversible chronic effects to a broad spectrum of aquatic organisms. This led to the President signing the Organotin Paint Control Act of 1988 (OPACA). The act contained interim and permanent TBT use restrictions as well as research and monitoring requirements. The application of TBT antifoulant was prohibited from vessels under 25 meters (82 ft.) and the maximum average daily release rate was set at 4 $mg/cm^2/day$. These requirements of OPACA should reduce the total amount of TBT entering the marine environment to about 10% of its pre-OPACA levels. Furthermore, most of the reduction should come in

estuarine and fresh water areas where small vessels are used and moored and where the risk from TBT input is greatest.

Bivalves (oysters and mussels) have been widely used as sentinel organisms for monitoring the contamination burden of estuarine ecosystem because they filter feed and bioaccumulate contaminants. Oysters have been found to have bioconcentration factors for TBT that range from 2300 to 11,400 times the water concentration, rapidly reaching an equilibrium plateau and slowly depurating. Thus, analysis of TBT concentrations in bivalves from coastal waters should provide information by which to assess the extent of butyltin contamination.

In 1987, GERG analyzed bivalves (mussels and oysters) and sediments from 36 coastal sites distributed on the Atlantic, Gulf, and Pacific Coasts, including one site in Hawaii. These selected S&T sites were chosen as the NS&T sites closest to suspected sources of input (for example, near marinas, dry docks, etc.). It was anticipated that no butyltins would be detected because the half-life of tributyltin in the water column measured by C^{14} label techniques was estimated to be 2-14 days. However, all but one of the 36 bivalve samples analyzed contained TBT and its less toxic breakdown products (dibutyltin and monobutyltin). The concentrations of TBTs ranged from <5 top 1560 (366 av) ng of Sn/g dry weight as tin and accounted on average for 74% of the tin present as butyltins. Replicate oyster samples from a specific site concentrate TBT to the same level. Concentrations of TBT found in oysters varied both spatially and temporally. Both oysters and mussels concentrate TBT from their environment and are therefore excellent sentinel organisms to monitor the environmental levels of TBT available to marine organisms.

Butyltin concentrations in sediment samples from U.S. coastal areas ranged from <5 to 282 ng Sn/g. Butyltins were detected in 75% of the sediment samples analyzed. The predominant butyltin was TBT, which is also the most toxic. DBT and MBT were detected in 30% of the sediment samples analyzed: the TBT degradation products were only found when TBT was present, usually at high concentrations. Mean bivalve butyltin concentrations were 18 times higher than mean sediment concentrations. Based on bivalve analyses, bioavailable butyltins were present at all the sites where butyltins were detected in the sediment. The sediments are one possible source of these bioavailable butyltins. However, the lack of correlation between sediments and bivalve butyltin concentration indicates that other sources may be predominant.

The purposes of the NS&T project is to determine the current status and the long term trends of contamination in U.S. Coastal areas. With the limitations imposed on the usage of TBT antifouling paint by OPACA, a decrease in the concentration of TBT in the environment would be expected. Studies done at GERG were designed to examine

the uptake and depuration rates of TBT compounds in oysters (*Crassostrea virginica*) through transplantation experiments at two locations in Galveston Bay, Texas. Oysters from a relatively uncontaminated area (Hanna Reef) were transplanted to a new site known to have indigenous oysters with higher TBT concentrations (Houston Ship Channel). Total butyltin concentrations increased rapidly from 62 to 380 ng Sn/g during the exposure period (48 days) with TBT accounting for most of the increase. After the uptake period, transplanted and indigenous oysters were relocated to the relatively pristine location. During the depuration period (50 days), oysters originally from the clean location depurated at a faster rate than oysters from the chronically exposed population. This is reflected in half-lives for TBT of 15 and 26 days for these oysters, respectively.

These experiments indicate that we should see a decrease in environmental levels of TBT in NS&T oyster samples from the Gulf of Mexico. This was indeed found to be the case. The concentration of total butyltins and tributyltin in oysters are decreasing at many Gulf of Mexico sites (Reprint 6 and Preprint 6). The decrease is more than a factor of two for 75% of these sites. The environmental half-life for butyltins for some sites is less than 3 years. At several sites there was no measurable decrease in butyltin concentration, but no site had an increase in concentration between 1987 and 1990. The indications of a decrease in TBT environmental levels appear to be reflected in the 1990 data. However, it is only one data point for these sites. The data from the 1991 collection will determine if the trend of decreasing concentrations continues.

Preprint 6

Butyltin Concentrations on Oysters from the Gulf of Mexico during 1989-1991

Bernardo Garcia-Romero, Terry L. Wade,
Gregory G. Salata, and James M. Brooks

Butyltin Concentrations in Oysters from the Gulf of Mexico during 1989-1991

Bernardo Garcia-Romero, Terry L. Wade, Gregory G. Salata & James M. Brooks

Department of Oceanography, Texas A&M University,
Geochemical and Environmental Research Group,
833 Graham Road
College Station, Texas 77845, USA

ABSTRACT

Oyster samples from 53 Gulf of Mexico coastal sites were collected and analyzed for butyltins during 1989, 1990, and 1991. The geometric mean tributyltin concentrations were 85, 30, and 43 ng Sn/g for 1989,1990, and 1991, respectively. The tributyltin concentrations are best represented by a log normal distribution. A decline of the butyltin concentrations at sites with relatively low butyltin concentrations for 1989 compared to 1990 and 1991 was observed, while at relatively high butyltin concentrations (>400 ng Sn/g), there was almost no difference between 1989 and 1991 but lower concentrations were present in 1990. Continued monitoring is needed in order to determine if butyltin contamination of the coastal marine environment is decreasing in response to use limitations.

INTRODUCTION

The presence of tributyltin and its degradation products in the environment continues to be of environmental concern. Tributyltin (TBT) anti-fouling paints are a solution to the costly problem of fouling organisms

which attach to the bottom of the hulls of boats and ships (Huggett *et al.*, 1992). Although an effective antifouling agent, tributyltin was found to adversely affect non-target organisms (Bushong *et al.*, 1987; Hall & Pinkney, 1985; Minchin *et al.*, 1987; Short & Thrower, 1986; Thain, 1986; Thompson *et al.*, 1985; Alzieu, 1991). For example, commercially valuable species were adversely affected in France (Alzieu, 1991). The presence of TBT and its degradation products, dibutyltin (DBT) and monobutyltin (MBT), in samples removed from input sources (Wade *et al.*, 1988; 1991b) suggest that environmental half-lives in the marine environment may be longer than reported values (Lee *et al.*, 1987; Olson & Brinckman, 1986; Seligman *et al.*,1986a,b & 1988a). After the use of TBT-based paints was limited in countries such as France, England, and the United States, the concentration of organotins in water and oysters was shown to decline (Short & Sharp, 1989; Wade *et al.*, 1991b; Alzieu, 1991; Page & Widdows, 1991; Valkirs *et al.*, 1991; Waite *et al.*, 1991). In the United States, however, continuous monitoring is needed in order to provide information on the long term response of butyltin concentrations in the marine environment to these regulations.

Oysters are excellent sentinels of TBT contamination. Bivalves have been used in uptake and depuration studies (Laughlin, *et al.*, 1986; Langston & Burt, 1991; Sericano *et al., in press;* Alzieu *et al., 1991;* Ritsema *et al., 1991;* Salazar & Salazar, *1991*) and to determine temporal and spatial variations of butyltin concentrations (Short & Sharp, 1989; Wade *et al.*, 1988; Page & Widdows, 1991). These studies indicated that oysters integrate bioavailable TBT with equilibration rates on the order of weeks. This indicates that continuous and carefully planned sampling should be

carried out in order to determine trends in the variation of TBT concentrations in the environment.

Tributyltin and its degradation products have been determined in oysters from 53 sites in the Gulf of Mexico from 1989 to 1991. The overall butyltin concentrations showed a decline from 1989 to 1990 (Wade *et al.*, 1991a,b). If this decline resulted from the implementation of the limitations on the use of TBT in the United States by the Organotin Anti-Fouling Paint Control Act of 1988 (OAPCA), a continuous decline would be expected. The results are now available for 1991. This report compares three years of data for the Gulf of Mexico to determine if there is a trend in butyltin concentrations.

METHODS

Oyster (*Crassostrea virginica*) samples were collected at 73 different sites along the Gulf of Mexico coast in the winter of 1989, 1990, and 1991. Table I shows the geographic location of the sites sampled and the symbols used to identify each site. Although known point sources of TBT such as marines or dry docks were avoided, some locations are closer to such TBT sources. A complete description of field sampling and logistics has been reported (GERG, 1991).

The same sampling and analytical procedures were used for all oyster samples reported. A detailed description of these procedures has been previously reported (Wade *et al.*, 1988; Wade & Garcia-Romero, 1989). Briefly, oyster tissues were homogenized, weighed, spiked with a surrogate standard, extracted with 0.2% tropolone in methylene chloride, hexylated, purified using Si/Al columns, and analyzed by gas chromatography with a tin

selective flame photometric detector. Quality control consisted of duplicate samples, procedural blanks, and spike blanks. Quadruplicate analysis of one sample yielded the following means and standard deviations 395 ± 14.5 ng Sn/g for TBT; 74.5 ± 5.80 for DBT; and 32.5 ± 6.5 for MBT. Method detection limit (MDL) on average for TBT and DBT was 5 ng Sn/g and for MBT was 10 ng Sn/g.

RESULTS and DISCUSSION

Annual Variation of Butyltins at Individual sites

Oyster butyltin concentrations determined in 1989, 1990, and 1991 were compared. In order to simplify the presentation of data, the sites sampled have been divided into three geographical zones: Florida, Louisiana-Mississippi-Alabama (LA MS AL), and Texas. Only 73% of the sites reported were sampled during all three years. In some instances, some sites were not sampled because no oysters were available. Butyltin concentrations in oysters are reported in ng Sn/g dry weight (Maguire, 1991). Sites with an incomplete set of data are indicated with a star in Table I and Figures 1, 2, and 3.

The concentration of total butyltins in 1989, 1990, and 1991 ranged from below the limit of detection (<5 ng Sn/g) to 1880 (TBKA), 850 (TBKA), and 1300 ng Sn/g (BBMB), for 1989, 1990, and 1991, respectively. In general the butyltin concentrations decreased from 1989 to 1990 and then increased slightly between 1990 and 1991.

Tributyltin, the most toxic butyltin, was the predominant butyltin found in oysters during the three-year sampling. Percentages of TBT

determined were 85±15% for all years. Near the limit of detection the percentage of TBT is more variable. The high percentage of TBT for *C. virginica* agrees with other reports (Wade *et al.*, 1988; Uhler *et al.*, 1989). Uhler *et al.* (1989) reported that bivalves have approximately constant ratios of TBT/DBT. The TBT percentages observed are the result of the uptake of TBT and DBT from the water column (Lee *et al.*, 1987; Olson & Brinckman, 1986; Seligman *et al.*, 1986a; 1988); TBT degradation to DBT by oysters (Lee, 1985); and different rates of depuration for TBT, DBT, and MBT (Lee, 1991). There is no evident relationship between the TBT concentration and the percentage of TBT present in the oysters for this study. Therefore, the fluctuation of percentage TBT around 85% is probably the result of a dynamic equilibrium between uptake, metabolism, and depuration.

The TBT concentrations determined for each site during 1989, 1990, and 1991 are shown in Figure 1. Sites are shown in geographical order from Texas to Florida. Tributyltin concentrations ranged from <5 ng Sn/g to 1450 (TBKA), 770 (BBMB), and 1160 ng Sn/g (BBMB) in 1989, 1990, and 1991, respectively. TBT concentrations increased monotonically at some sites from 1989 to 1991, while at others sites concentrations decreased monotonically. For example, oyster TBT concentrations increased from 1989 to 1991 at CLLC, BBMB and GBTD (Figure 1). Decreasing TBT concentrations from 1989 to 1991 were observed for oysters from PBPH, SAWB, TBCB, MBLR, and MBEM (Figure 1). Concentrations of TBT were the same at TBOT and GBCR during all three years. In general, higher concentrations of TBT were determined in Florida sites than in Texas, Louisiana, Mississippi, or Alabama sites. TBT was below the detection limit at 1 of 53 sites in 1989 and at 10 and 11 sites during 1990 and 1991,

respectively. Although the concentrations were low, butyltins were detected in oysters from every site sampled in at least one sampling year.

Dibutyltin concentrations determined in oysters during 1989, 1990, and 1991 are shown in Figure 2. Dibutyltin concentrations ranged from <5 ng Sn/g to 380 (TBKA), 160 (TBKA), and 200 ng Sn/g (TBKA), in 1989, 1990, and 1991, respectively. Sites sampled in Florida had the highest DBT concentrations. With the exception of five sites (CBJB, TBKA, CBFM, BBMB, and BRFS), annual variation of DBT concentrations did not mimic the annual variation of TBT concentrations. Ship and boating activity have been cited as potential factors that may affect DBT fluctuations (Short & Sharp, 1989; Uhler *et al.*, 1989). Also, the commercial usage of DBT as a stabilizer for plastics including PVC pipes may be another important source of input to the marine environment and may result in DBT fluctuations that do not mimic TBT fluctuations (Fent *et al.*, 1991; Maguire, 1991). At this point it is not possible to estimate the influence of the factors discussed above on the DBT concentrations present in the oysters. Monotonic increases or decreases of DBT were observed at specific sites during the three year period. For example, Middle Bank (BBMB, Figure 1 and 2) showed not only increasing concentrations of TBT during the three year sampling period but also showed a steady increase of DBT in the same period. DBT was detected in 39, 38, and 33 out of the 53 sites sampled each of the three years. In many instances DBT was not detected in any of the sampling years.

Regional MBT concentrations are shown in Figure 3. Since the MBT concentrations are low, annual variations in MBT concentrations for each site are large. The precision of MBT determination is also not as good as that of TBT and DBT (Wade *et al.*,1988). Monobutyltin concentrations ranged from <5 ng Sn/g to 145 (NBNB), 25 (CCIC), and 42 ng Sn/g (TBKA),

in 1989, 1990, and 1991, respectively. Generally, sites with high TBT concentrations had high MBT concentrations. MBT was detected in 21, 4, and 19 of the 53 sites during 1989, 1990, and 1991, respectively. During all three years, MBT was only detected at three sites in Florida (CBJB, TBKA, and CBFM) and at one site in Texas (CCIC). The fact that MBT was found in lower concentrations than DBT, and DBT was found in lower concentrations than TBT is consistent with the fact that TBT is the major constituent of antifouling paints while DBT and MBT are environmental breakdown products of TBT. This may indicate that only a limited degradation of TBT has occurred or that the more water soluble DBT and MBT are assimilated by the oysters at a slower rate than TBT.

Annual Variation of Butyltins in the Gulf of Mexico

A graphic representation of the TBT data for the 53 sites sampled in 1989, 1990, and 1991 is shown in Figure 4. The graph is a plot of 1989 concentrations vs 1990 and 1991. The "x" and "y" scales are identical. If no change occurs in the TBT concentration at a site, that data will be plotted on the center line. Sites that fall below the line show a decrease while points that rise above the line show an increase compared to 1989. Two other lines also appear in Figure 4. These are the lines that form the boundary of sites with a factor of 2 increase (top line) or decrease (bottom line). Only 6 sites for 1990 and 8 for 1991 of the 53 sites plotted for each year are above the center line. Therefore, over 85% of the TBT concentrations in 1990 and 1991 were less than the concentration measured in oysters at that site in 1989. There were 30 sites (57%) in 1990 and 20 sites (38%) in 1991 that had decreases of more than a factor of

two. There was only one site that had an increase of TBT concentration of more than a factor of 2.

In order to detect temporal trends, the butyltin oyster concentrations for the entire Gulf of Mexico from 1989 to 1991 are compared. Annual variation of butyltins for the entire Gulf of Mexico are not readily apparent in Figures 1, 2, or 3 where only annual concentrations at individual sites are compared. Comparisons of arithmetic mean, geometric mean, and medians (Table II) for butyltin concentrations determined during 1989, 1990, and 1991 are based only on the 53 sites that were sampled all three years. All of these parameters were calculated by assigning 5 ng Sn/g to all of those samples with concentrations below the limit of detection. The percentage of samples below the detection limit is listed in Table II. The median and geometric means are similar in all cases, while the arithmetic mean is always higher. The median or the geometric means appear to be the better estimators of the central tendency of the data. Based on the median or the geometric means, there was a decrease in TBT oyster concentrations when 1989 is compared to 1990 or 1991.

A complete view of butyltin concentrations for the whole Gulf of Mexico for a given year can be achieved using either cumulative percentage distribution or probability distribution curves (Mackay & Paterson, 1984; O'Connor & Ehler, 1990; Jackson *et al.*, in press). Although both types of curves may describe a distribution of butyltin concentration for each year, probability distribution curves were chosen because they are more easily compared. Use of this type of curve assumes that the log of the concentration produces a normal distribution. Log normal distributions have already been reported for environmental data obtained in the NOAA National

Status and Trends Mussel Watch Program (O'Connor & Ehler, 1990; Jackson *et al.*, in press).

TBT log distribution curves are shown in Figure 5 for 1989, 1990, and 1991. These curves were obtained by using the following equation (Milton & Arnold, 1986):

$$f(x) = \{s\,[SQR(2\pi)]\}^{-1}\ EXP\ -\ \{1/2\ [(x - X)/s\ ]^2\ \} \qquad (1)$$

where f(x) is the distribution probability of the log butyltin concentration, s is the standard deviation, SQR is the square root, x is the log of the butyltin concentration, and X is the geometrical mean. Then each f(x) was divided by the sum of the f(x) as shown by equation (2)

$$f'(x)_i = f(x)_i\ /\ \Sigma\ f(x)_i \qquad (2)$$

in such a way that

$$\Sigma\ f'(x)_i = 1 \qquad (3)$$

TBT concentrations curves from 1989, 1990, and 1991 (Figure 5) are Gaussian with some degree of skewness. DBT had a log normal distribution only in 1989 and 1990, while MBT does not follow a log normal distribution for any of the years. The geometric mean concentrations are indicated by solid lines for 1989, dotted for 1990, and dashed for 1991 (Figure 5) and are also reported in Table IIa. The TBT, DBT and MBT concentrations for plus and minus one standard deviation from the geometric mean are listed in Table IIb. Probability distribution curves of TBT in oysters from the Gulf of Mexico provide information about annual variations at low, medium, and high ranges of concentration. Although the standard deviations quantify the spread of a data set, they provide no information about how low or high concentrations changed with time. TBT concentrations decreased from 1989 to 1990 at all concentrations; while TBT concentration decreased from 1989 to 1991 at low and medium concentrations, but were similar at

high concentrations (Figure 5). This decrease may be the result of the TBT regulation of 1988 and/or development and use of lower release rate TBT paint formulations. Initial TBT regulations probably resulted in a marked reduction in private boat owners painting their own vessels. The fact that newer TBT containing paints are rated to be good for up to 5 years and TBT was not banned but its use limited probably leads to decreased TBT inputs. This may have resulted in the observed decreases in TBT concentrations in 1990 and 1991. The decrease observed at high concentrations from 1989 to 1990 but not in 1991 may be due to the naturally higher variation of TBT concentrations near input areas (Seligman *et al.*, 1988). Therefore, TBT lower concentrations ranges may have decreased as a consequence of TBT regulations or changes in TBT-based paint formulations, but the effect are not as apparent at sites with high TBT concentrations. Distribution curves for DBT and MBT concentrations did not follow a log normal distribution but also show annual variations. This may be due to the high percentage of values below the MDL (Table II).

CONCLUSION

Oysters are valuable biomonitors for butyltins. The percentage of TBT present with respect to the total butyltins oscillated around 85% during the three years sampled. There was a decrease of the butyltin concentration from 1989 to 1990 or 1991. However at high concentrations there was little difference between 1989 and 1991. Environmental response to the TBT regulation in 1988 is not yet apparent. The decline between 1989 and 1990, 1991 may have resulted from previous changes in antifouling paint formulation with lower TBT release rates or suspension of painting activities

by individual boat owners after 1988. Because the newer TBT paints were formulated to last 5 years or more, there are many boats still in use that were painted with TBT containing paints before the ban. Consequently, continuous monitoring is necessary to determine trends in butyltin contamination of the marine environment.

ACKNOWLEDGMENTS

This Research was supported by the National Oceanic and Atmospheric Administration Grant Number 50-DGNC-5-00262 (National Status and Trends Mussel Watch Program).

REFERENCES

Alzieu C. (1991) Environmental problems caused by TBT in France: Assessment, regulation, prospects. *Mar. Environ. Res.* **32**,7-17.

Alzieu C., Michel P., Tolosa I., Bacci E., Mee L.D. & Readman J.W. (1991) Organotin compounds in the Mediterranean: A continuing cause for concern. *Mar. Environ. Res.* **32**,261-270.

Bushong S.J., Hall W.S., Johnson W.E. & Hall L.W.Jr. (1987) Toxicity of tributyltin to selected Chesapeake Bay biota. *Proceedings of the Oceans '87 International Organotin Symposium*, vol. 4. pp. 1499-1503.

Cleary J.J. (1991) Organotin in the marine surface microlayer and subsurface waters of Southwest England: Relation to toxicity thresholds and the UK environmental quality standard. *Mar. Environ. Res.* **32**, 213-222.

Fent K., Hunn J., Renggli D. & Siegrist H. (1991) Fate of tributyltin in sewage sludge treatment. *Mar. Environ. Res.* **32**, 223-231.

GERG (1991) NOAA Status and Trends, Mussel Watch Program. Field Sampling and logistics. Year VI. The Geochemical and Environmental Research Group, Texas A&M Research Foundation. Technical Report 91-046, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Ocean Assessment Division. 212 pp.

Hall L.W. Jr. & Pinkney A.E. (1985) Acute and sublethal effects of organotin compounds on aquatic biota: An interpretative literature evaluation. *CRC Critical Reviews in Toxicol.* **14**, 159-209.

Huggett R.J., Unger M.A., Seligman P.F. & Valkirs A.O. (1992) The marine biocide tributyltin. Assessing and managing the environmental risks. *Environ. Sci. Technol.* **26**, 232-237.

Jackson T.J., Wade T.L., McDonald T.J, Wilkinson D.L. & Brooks J.M. (Submitted) Polyaromatic hydrocarbon contaminants in National Status and Trends oysters from the Gulf of Mexico (1986-1990). *Oil. Chem. Poll.*

Langston W.J. & Burt G.R. (1991) Bioavailability and effects of sediment-bound TBT in deposit-feeding clams, *Scrobicularia plana*. *Mar. Environ. Res.* **32**, 61-77.

Laughlin R.B., French W. & Guard H.E. (1986) Accumulation of bis(tributyltin) oxide by the marine mussel *Mytilus edulis*. *Environ. Sci. Technol.* **20**, 884-890.

Lee R.F. (1985) Metabolism of Tributyltin oxide by crabs, oysters and fish. *Mar. Environ. Res.* **17**, 145-148.

Lee R.F., Valkirs A.O., & Seligman P.F. (1987) Fate of tributyltin in estuarine waters. *Proceedings of the Oceans '87 International Organotin Symposium*, vol. 4. pp. 1411-1415.

Lee R.F. (1991) Metabolism of tributyltin by marine animals and possible linkages to effects. ***Mar. Environ. Res.* 32**, 29-35.

Mackay D. & Paterson S. (1984) Spatial concentration distributions. *Environ. Sci. Technol.* **18**, 207A-214A.

Maguire R. J. (1991) Aquatic environmental aspects of non-pesticidal organotin compounds. *Water Poll. Res. J. Canada.* 26, 243-360.

Milton J.S. & Arnold J.C. (1986) *Probability and statistics in the engineering and computing sciences.* McGraw-Hill Book Company, New York-Toronto. 643 pp.

Minchin D., Duggan C.B. & King W. (1987) Possible effects of organotins on scallop recruitment. *Mar. Poll. Bull.* **18**, 604-608.

O'Connor T.P. & Ehler C.N. (1990) Results from the NOAA National Status and Trends Program on distribution and effects of chemical contamination in the coastal and estuarine United States. *Environ. Monitor. Assessment* **16**, 1-17.

Olson G.J. & Brinckman F.E. (1986) Biodegradation of tributyltin by Chesapeake Bay microorganisms. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1196-1201.

Page D.S. & Widdows J. (1991) Temporal and spatial variation in levels of alkyltins in mussel tissues: A toxicological interpretation of field data. *Mar. Environ. Res.* **32**, 113-129.

Ritsema R., Laane R.W.P.M. & Donard O.F.X. (1991) Butyltins in marine waters of the Netherlands in 1988 and 1989; concentrations and effects. *Mar. Environ. Res.* **32**, 243-260.

Salazar M.H. & Salazar S.M. (1991) Assessing site-specific effects of TBT contamination with mussel growth rates. *Mar. Environ. Res.* **32**, 131-150.

Sericano J.L., Wade T.L., Garcia-Romero B. & Brooks, J. (Submitted) Environmental Accumulation and Depuration of Tributyltin by the American Oyster, *Crassostrea virginica. Marine Biology.*

Seligman P.F., Valkirs A.O. & Lee R.F. (1986a) Degradation of tributyltin in San Diego Bay, California, waters. *Environ. Sci. Technol.* **20**, 1229-1235.

Seligman P.F., Valkirs A.O. & Lee R.F. (1986b) Degradation of tributyltin in marine and estuarine waters. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1189-1195.

Seligman P.F., Valkirs A.O., Stang P.M. & Lee R.F. (1988a) Evidence for rapid degradation of tributyltin in a marine. *Mar. Poll. Bull.* **19**, 531-534.

Short J.W. & Thrower F.P. (1986) Tri-n-butyltin caused mortality of chinook salmon, *Oncorhynchus tshawytcha*, on transfer to TBT-treated marine net pen. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1202-1205.

Short J.W. & Sharp J.L. (1989) Tributyltin in bay mussels (*Mytilus edulis*) of the Pacific Coast of the United States. *Environ. Sci. Technol.* **23**, 740-743.

Thain J.E. (1986) Toxicity of TBT to bivalves: Effects on reproduction, growth and survival. *Proceedings of the Oceans '86 Organotin Symposium*, vol. 4. pp. 1306-1313.

Thompson J.A.J., Sheffer M.G., Pierce R.C., Chau Y.K., Cooney J.J., Cullen W.R. & Maguire R.J. (1985) *Organotin Compounds in the Aquatic Environment: Scientific Criteria for Assessing their Effects on Environmental Quality.* National Res. Council of Canada. NRCC. Associate Committee on Scientific Criteria for Environmental Quality. 284 p.

Uhler A.D., Coogan T.H., Davis K.S., Durell G.S., Steinhauer W.G., Freitas S.Y. & Boehm P.D. (1989) Findings of tributyltin in bivalves from selected U.S. coastal waters. *Environ. Toxic. Chem.* **8**, 971-979.

Valkirs A.O., Davidson B., Kear L.L., Fransham R.L., Grovhoug J.G. & Seligman P.F. (1991) Long-term monitoring of tributyltin in San Diego Bay California. *Mar. Environ. Res.* **32**, 151-167.

Wade T.L., Garcia-Romero B. & Brooks J.M. (1988) Tributyltin contamination in bivalves from U.S. coastal estuaries. *Environ. Sci. Technol.* **22**, 1488-1492.

Wade T.L. & Garcia-Romero B. (1989) Status and trends of tributyltin contamination of oysters and sediments from the Gulf of Mexico. *Proceedings of the Oceans '89*, vol. 2, pp. 550-553.

Wade T.L., Garcia-Romero B. & Brooks J.M. (1991a) Bioavailability of Butyltins. In *Organic Geochemistry. Advances and Applications in the natural environment.* (Ed. D.A.C. MANNING). pp 571-573. Manchester University Press.

Wade T.L., Garcia-Romero B. & Brooks J.M. (1991b) Oysters as biomonitors of butyltins in the Gulf of Mexico. *Mar. Environ. Res.* **32**, 233-241.

Waite M.E., Waldock M.J., Thain J.E., Smith D.J. & Milton S.M. (1991) Reductions in TBT concentrations in UK estuaries following legislation in 1986 and 1987. *Mar. Environ. Res.* **32**, 89-111.

Table IIa. Arithmetic, geometric means and medians (ng Sn/g). Numbers in parenthesis indicate percentage of samples below MDL.

	TBT	DBT	MBT
1989			
Mean			
Arith.	176	32	13
Geom.	85	14	8
Median	77(2%)	12(26%)	5(60%)
1990			
Mean			
Arith.	96	17	6
Geom.	30	8	6
Median	24(17%)	5(72%)	5(90%)
1991			
Mean			
Arith.	150	25	8
Geom.	43	13	6
Median	42(17%)	8(40%)	5(66%)

Table IIb. Geometric mean plus or minus one standard deviation of the log butyltin concentrations (ng Sn/g).

	TBT	DBT	MBT
1989			
Plus	293	44	18
Minus	25	5	3
1990			
Plus	141	21	8
Minus	6	3	4
1991			
Plus	233	37	10
Minus	8	4	4

Table I. Sampling locations and site designators.

Desig.	Site	Location	Latitude	Longitude
		TEXAS		
LMSB	South Bay	Lower Laguna Madre	26° 02.58'	97° 10.49'
LMAC*	Arroyo Colorado	Laguna Madre	26 16.80	97 17.30
CCBH*	Boat Harbor	Corpus Christi	27 50.00	97 23.00
CCNB*	Nueces Bay	Corpus Christi	27 51.70	97 21.00
CCIC	Ingleside Cove	Corpus Christi	27 50.30	97 14.25
ABLR	Long Reef	Aransas Bay	28 03.30	96 57.50
CBCR*	Copano Reef	Copano Bay	28 08.20	97 07.58
MBAR	Ayres Reef	Mesquite Bay	28 10.30	96 49.70
SAPP*	Panther Pt. Reef	San Antonio Bay	28 13.20	96 43.00
SAMP*	Mosquito Point	San Antonio Bay	28 19.00	96 42,20
ESSP*	South Pass Reef	Espiritu Santo Bay	28 17.83	96 37.50
ESBD*	Bill Days Reef	Espiritu Santo Bay	28 25.00	96 27.00
MBGP*	Gallinipper Pt.	Matagorda Bay	28 35.00	96 34.00
MBLR	Lavac River Mouth	Matagorda Bay	28 39.30	96 35.00
MBCB*	Carancahua Bay	Matagorda Bay	28 40.00	96 23.20
MBTP	Tres Palacios Bay	Matagorda Bay	28 39.00	96 15.50
MBEM	East Matagorda	Matagorda Bay	28 42.30	95 53.00
BRCL*	Cedar Lakes	Brazos River	28 51.50	95 27.90
BRFS	Freeport River	Brazos River	28 55.00	95 20.50
GBCR	Confederate Reef	Galveston Bay	29 15.75	94 50.50
GBOB	Offatts Bayou	Galveston Bay	29 16.70	94 50.70
GBTD	Todd's Dump	Galveston Bay	29 30.10	94 54.00
GBYC	Yacht Club	Galveston Bay	29 37.00	94 59.50
GBSC*	Ship Channel	Galveston Bay	29 42.50	94 59.50
GBHR	Hanna Reef	Galveston Bay	29 29.50	94 42.50
SLBB	Blue Buck Point	Sabine Lake	29 48.00	93 54.42

*Sites that were not sampled consecutively from 1989 to 1991.

Table I. Continuation.

Desig.	Site	Location	Latitude	Longitude
		LOUISIANA		
CLSJ	St. Johns Island	Calcasieu Lake	29° 50.00'	93° 32.00'
CLLC	Lake Charles	Calcasieu Lake	30 03.50	93 17.50
JHJH	Joseph Harbor Bayou	Joseph Harbor Bayou	29 37.75	92 45.75
VBSP	Southwest Pass	Vermillion Bay	29 34.70	92 04.00
ABOB	Oyster Bayou	Atchafalaya Bay	29 13.00	91 08.00
CLCL	Caillou Lake	Caillou Lake	29 15.25	90 55.50
TBLB	Lake Barre	Terrebonne Bay	29 15.00	90 36.00
TBLF	Lake Felicity	Terrebonne Bay	29 16.00	90 24.50
BBSD	Bayou St. Denis	Barataria Bay	29 24.10	89 59.80
BBMB	Middle Bank	Barataria Bay	29 17.20	89 56.60
MRTP	Tiger Pass	Mississippi River	29 08.69	89 25.67
MRPL*	Pass a Loutre	Mississippi River	29 04.30	89 04.60
BSSI	Sable Island	Breton Sound	29 24.70	89 28.70
BSBG	Bay Garderne	Breton Sound	29 35.87	89 38.50
LBMP	Malheureux Point	Lake Borgne	29 52.30	89 40.70
LPGO*	Gulf Outlet	Lake Ponchartrain	30 02.20	89 03.00
		MISSISSIPPI		
MSPC	Pass Christian	Mississippi Sound	30 19.75	89 19.58
MSBB	Biloxi Bay	Mississippi Sound	30 23.38	88 15.42
MSPB	Pascagoula Bay	Mississippi Sound	30 21.05	88 37.00
		ALABAMA		
MBCP	Cedar Point Reef	Mobile Bay	30 19.40	88 07.30
MBHI	Harbor Island	Mobile Bay	30 33.59	88 02.80
MBDR*	Dog River	Mobile Bay	30 35.50	88 02.72

*Sites that were not sampled consecutively from 1989 to 1991.

Table I. Continuation.

Desig.	Site	Location	Latitude	Longitude
		FLORIDA		
PBPH	Public Harbor.	Pensacola Bay	30° 34.80'	87° 11.50'
PBIB*	Indian Bayou	Pensacola Bay	30 30.83	87 04.00
PBSP*	Sabine Point	Pensacola Bay	30 20.80	87 08.10
CBJB	Joes Bayou	Choctawhatchee Bay	30 24.70	86 29.55
CBSP	Shirk Point	Choctawhatchee Bay	30 28.95	86 28.60
CBSR	Off Santa Rosa	Choctawhatchee Bay	30 23.50	86 10.60
PCLO	Little Oyster Bay	Panama City	30 15.00	85 40.87
PCMP*	Municipal Pier	Panama City	30 08.20	85 37.50
SAWB	Watson Bayou	St. Andrew Bay	30 08.50	85 37.58
APDB	Dry Bar	Apalachicola Bay	29 41.50	85 05.00
APCP	Cat Point Bar	Apalachicola Bay	29 43.00	84 52.50
AESP	Spring Creek	Apalachee Bay	30 30.50	84 19.38
CKBP	Black Point	Cedar Key	29 10.25	83 03.00
TBNP	Navarez Park	Tampa Bay	27 48.30	82 45.28
TBMK	Mullet Key Bayou	Tampa Bay	27 37.17	82 43.62
TBPB	Papys Bayou	Tampa Bay	27 50.72	82 36.75
TBOT	Old Tampa Bay	Tampa Bay	28 01.48	82 37.95
TBKA	K. Airport	Tampa Bay	27 54.46	82 27.29
TBCB	Cockroach Bay	Tampa Bay	28 40.55	82 30.56
CBBI	Bird Island	Charlotte Harbor	26 31.00	82 02.60
CBFM	Fort Meyers	Charlotte Harbor	26 38.64	81 52.48
NBNB	Naples Bay	Naples Bay	26 00.00	81 32.00
RBHC*	Henderson Creek	Rookery Bay	26 01.83	81 43.75
EVFU	Faka Union Bay	Everglades	25 54.27	81 30.60
BHKF*				

*Sites that were not sampled consecutively from 1989 to 1991.

Table IIa. Arithmetic, geometric means and medians (ng Sn/g).

	TBT	DBT	MBT
1989			
Mean			
Arith.	176	32	13
Geom.	85	14	8
Median	77	12	5
1990			
Mean			
Arith.	96	17	6
Geom.	30	8	6
Median	24	5	5
1991			
Mean			
Arith.	150	25	8
Geom.	43	13	6
Median	42	8	5

Table IIb. Geometric mean plus or minus one standard deviation of the log butyltin concentrations (ng Sn/g).

	TBT	DBT	MBT
1989			
Plus	293	44	18
Minus	25	5	3
1990			
Plus	141	21	8
Minus	6	3	4
1991			
Plus	233	37	10
Minus	8	4	4

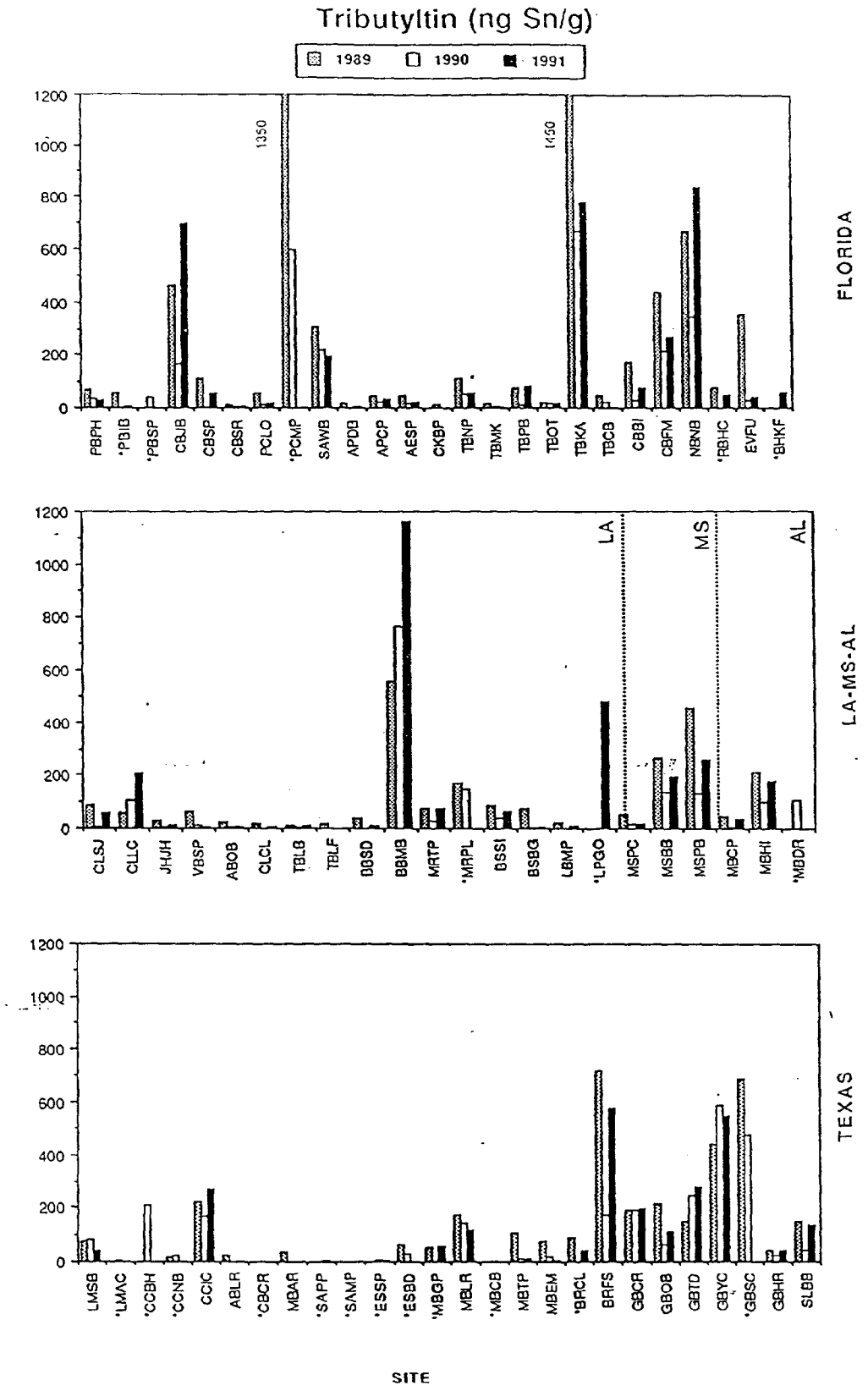


FIGURE 1. Geographical distribution of tributyltin concentrations in oysters (Crassostrea virginica) from the Gulf of Mexico coast. Stars indicate those sites that were not sampled in consecutive years.

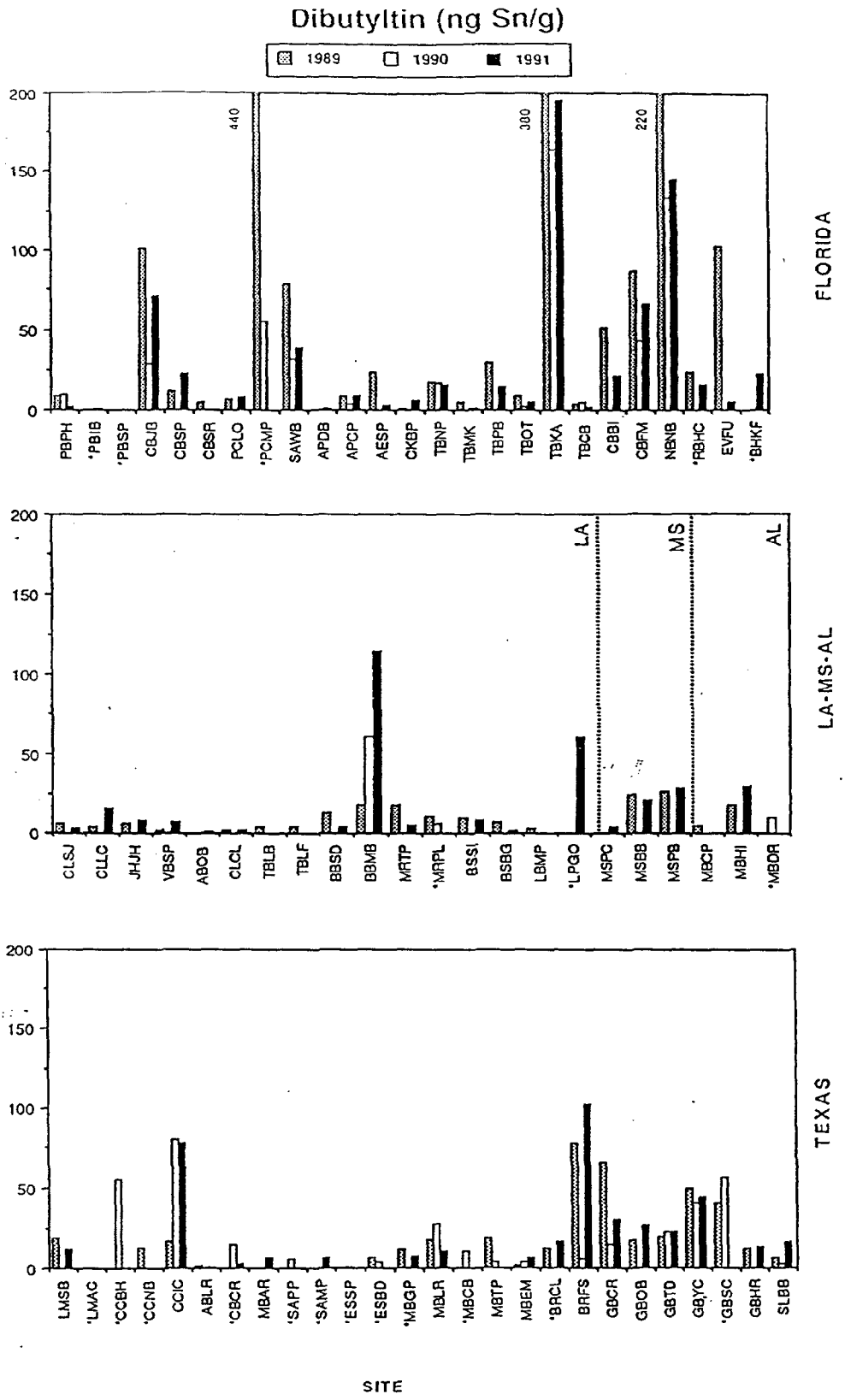


FIGURE 2. Geographical distribution of dibutyltin concentrations in C. virginica from the Gulf of Mexico coast. Stars indicate those sites that were not sampled in consecutive years.

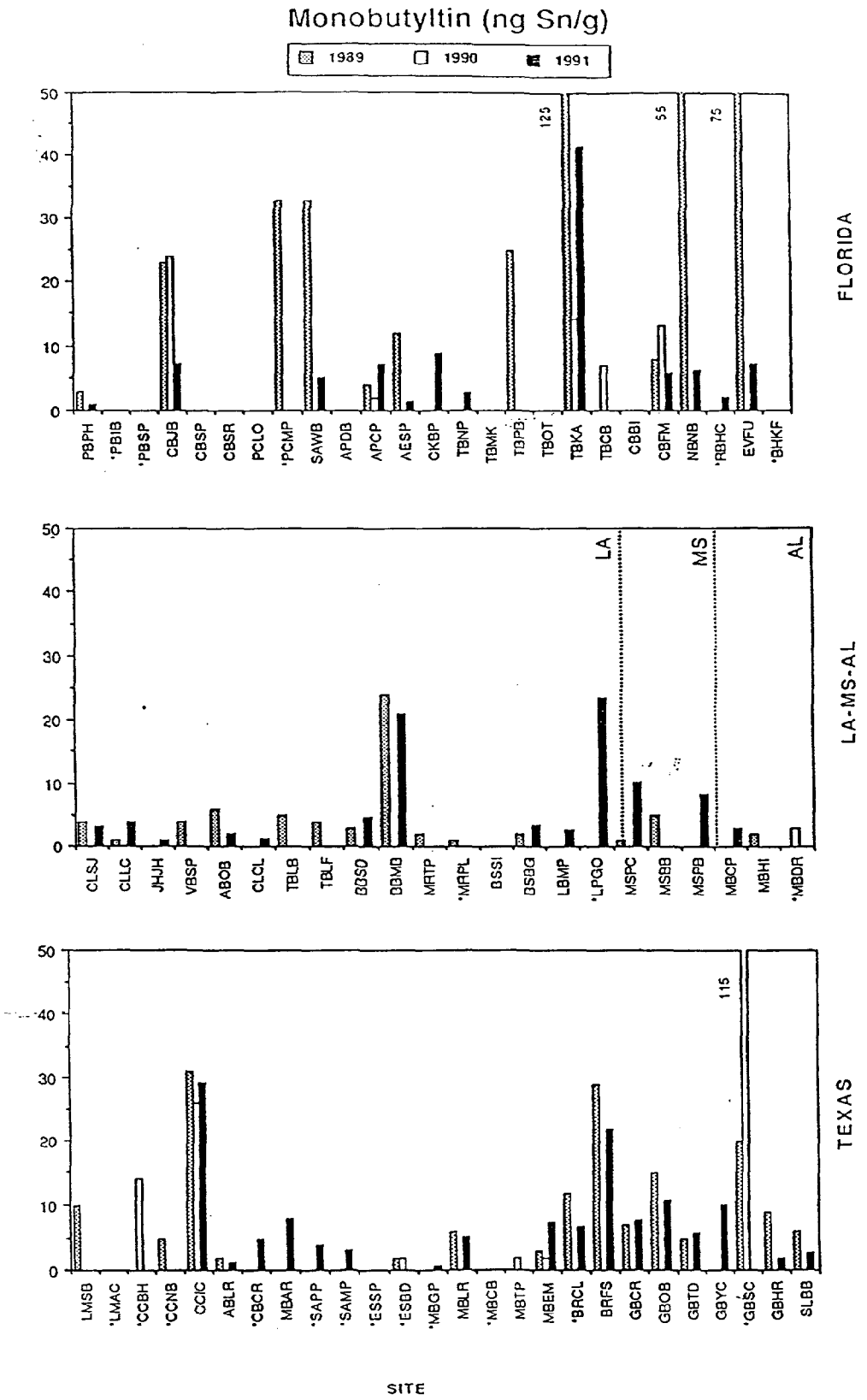


FIGURE 3. Geographical distribution of monobutyltin concentrations in C. virginica from the Gulf of Mexico coast. Stars indicate those sites that were not sampled in consecutive years.

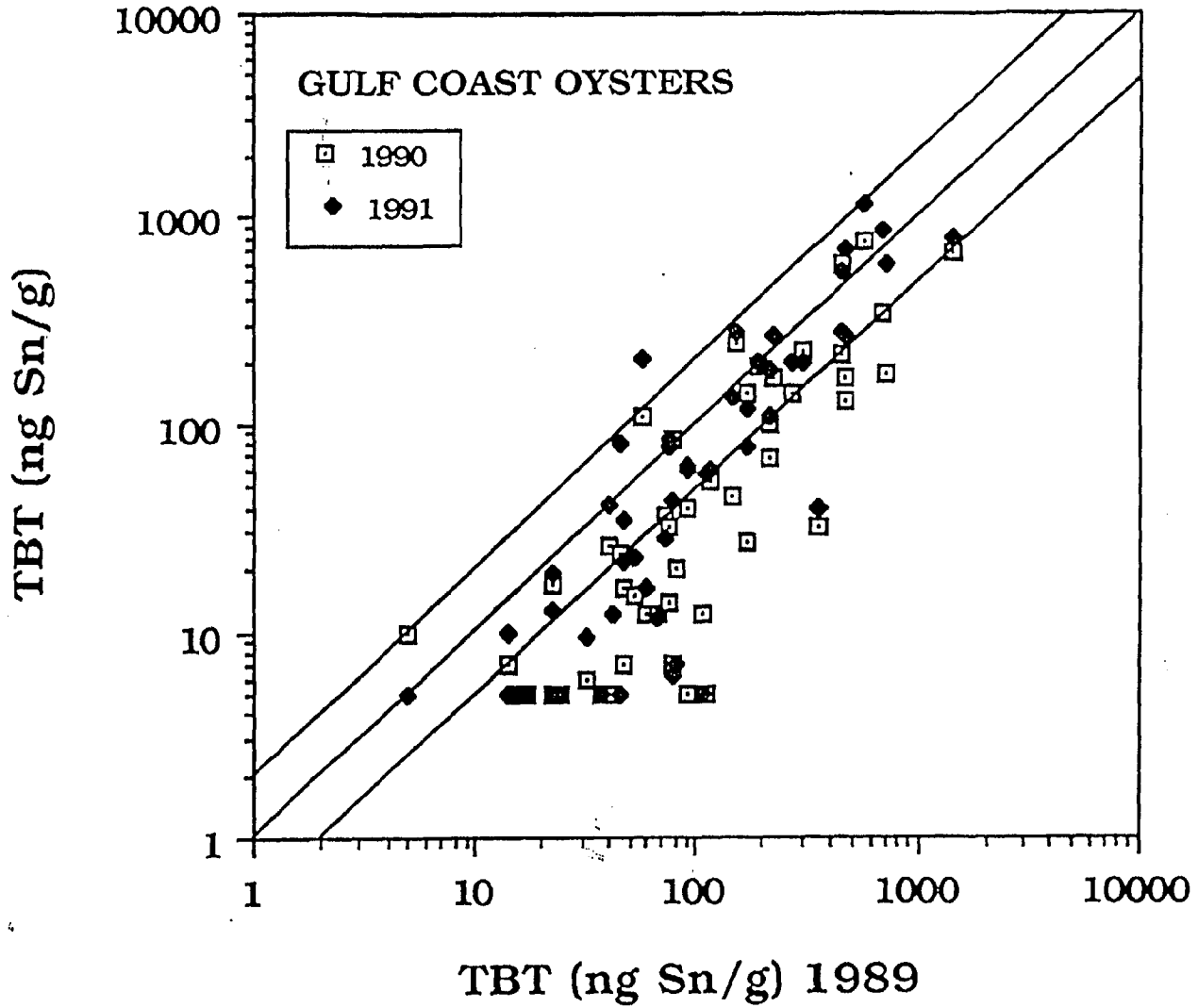


FIGURE 4. Tributyltin concentrations determined in 1989 versus the tributyltin concentrations determined in 1990 and 1991. Points falling along the center line have equal concentrations, colateral lines indicate a factor of two greater or lower than the concentrations determined in 1989.

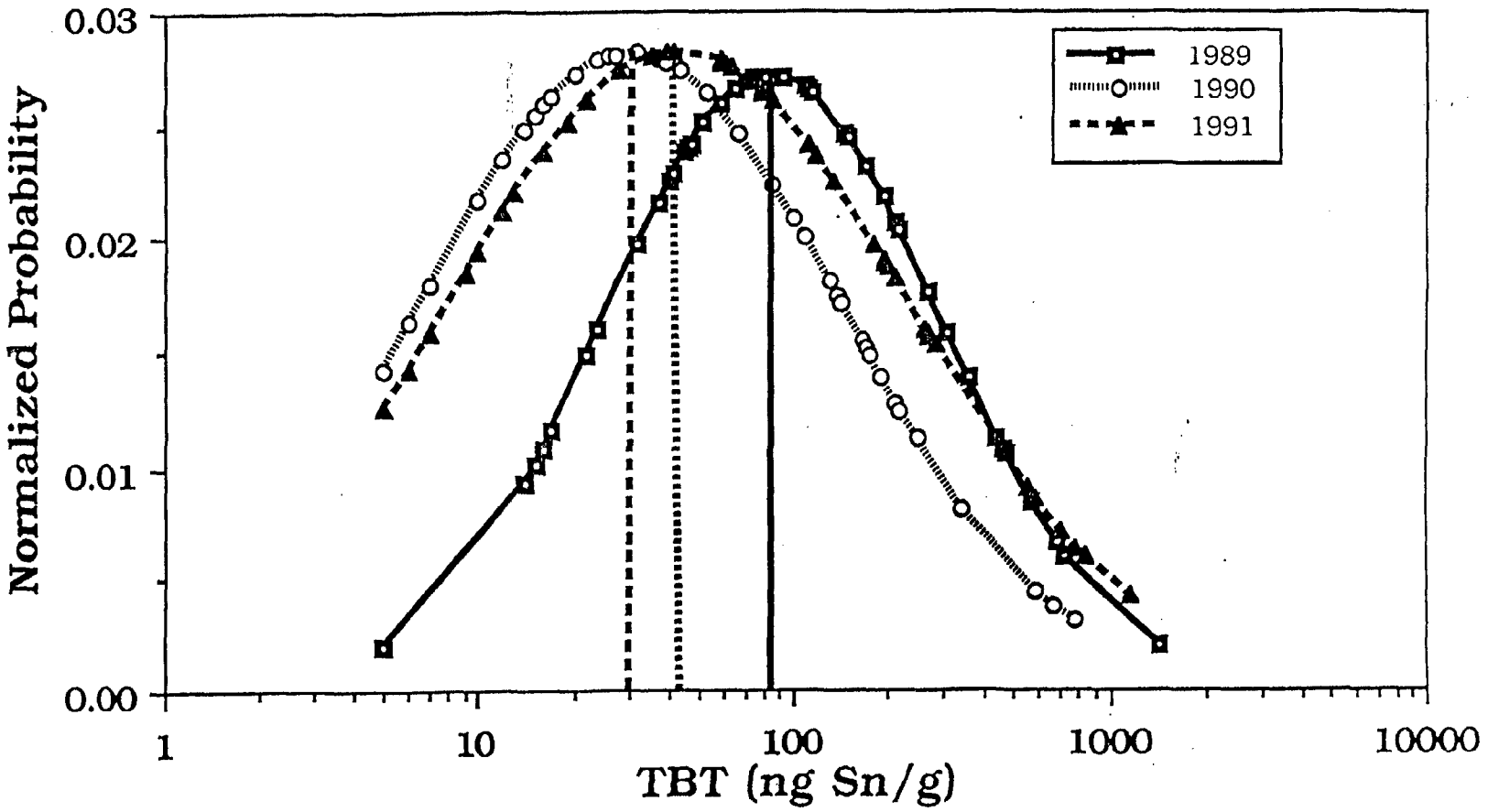


FIGURE 5. Log normal distribution of tributyltin concentrations determined in oysters in 1989, 1990, and 1991.

7.0 Biological Results

7.1 Condition Index/Shell Length/Condition Code

Condition code was rated as described in the Analytical Methods volume. Higher numbers indicate oysters in better condition. Most sites averaged 3 or 4. The highest median condition was 2 at five sites, the lowest was 7 at three sites.

Condition index is an estimate of the relative health of oysters. Healthy oysters, generally have more tissue dry weight compared to the cavity volume of their shells. Condition index is calculated by dividing the tissue dry weight of oysters by their shell volumes. The higher the number, the healthier the oyster.

Condition index varied from 0.062 gm dry wt • ml^{-1} at Brazos River, Freeport Surfside (BRFS) to 0.197 gm dry wt • ml^{-1} at Aransas Bay, Long Reef (ABLR). Condition index usually varies with the reproductive cycle being higher during the period of gonadal development and decreasing after spawning. Lower condition indices have also been associated with pollutant stress. Condition index varied concordantly from year to year among the Gulf sites. Condition index was relatively high in years 1987 and 1988 and was low in 1986 and 1989. *P. marinus* infection intensity followed the exact opposite trend. In 1990, condition index fell between these two extremes, as did *P. marinus* infection intensity. Length decreased steadily from 1986 to 1989 and declined at lower latitude sites. Length was unusually low in the panhandle of Florida relative to that expected from other sites in the equivalent latitude range. Both trends continued in 1990.

7.2 Gonadal/Somatic Index

Assessment of the physiological state of an oyster population requires an analysis of the state of gonadal development. Typically, oysters are undifferentiated in the winter, the gonads begin to develop in early spring, and spawning occurs during late spring through early fall. Most Gulf coast oysters spawn at least twice during that time period. The state of gonadal development is determined by observation of histological sections after staining. Oysters are sexed and assigned to a semiquantitative state of reproductive development as detailed in the Analytical Methods volume. Four stages of gonadal state were used as detailed below. This scale has been further refined as described in the Analytical Methods volume last year.

Stage 1. Undifferentiated/Mid Development Gonad:

Little or no gonadal tissue visible. Sex cannot be determined.

Early Development: Follicles beginning to expand, no ripe gametes visible. Primary and secondary gametocytes present. Sex can be determined.

Mid-Development: Follicles expanded and beginning to coalesce; no mature gametes present.

Stage 2. Undifferentiated/Mid-Development Gonad:

Late Development: Follicles greatly expanded, coalesced, but considerable connective tissue remaining; gametocytes and some mature gametes present.

Stage 3. Fully Developed Gonad:

Follicles packed with mature gametes. Most gametes mature; little connective tissue remaining within the gonadal tissue.

Stage 4. Spawning/Spent gonad:

Spawning: Gametes visible in gonoducts.

Spawned: Reduced number of gametes; some mature gametes still remaining; evidence of renewed reproductive activity.

Spent: Few or no gametes visible, gonadal tissue atrophying.

Gonadal index is calculated as the mean stage obtained from a minimum of 15 individuals per site. This gonadal index in oysters is a qualitative estimation of the state of reproductive development. It does not allow direct comparison (or normalization) of other data (e.g. hydrocarbon content) with reproductive development because a histological determination of reproductive state does not measure the total volume of gonadal material consistently present at all stages and all individual sizes.

Most collected oysters that could be assigned to a sex were female, as expected. Large oysters are usually female and large oysters were preferentially collected. There has been no consistent relationship among the years in sex ratio. Sites with more males in any one year were not necessarily sites with more males in another

year. For example, the site (BBSD) which had the most males (6) in 1989 had few males in 1990.

In Year I, except extreme south Texas, nearly all oysters collected west and south of Joseph Harbor, Louisiana and east and south of Lake Borgne were in the earliest stages of gonadal development. Little gonadal tissue was present. Oysters from sites between Joseph Harbor and Lake Borgne were collected later in the season. Nearly all individuals were in late stages of development or ready to spawn. A few individuals had already spawned and appeared to be developing new gonadal material again. In Years 2 and 3, collections were made earlier. Louisiana oysters were for the most part in early development. Sites in Texas and South Florida, however, provided some oysters in late development, full development or spawning. Sites having the most oysters in late development or ready to spawn were in the Laguna Madre and Corpus Christi Bay areas of Texas (>20%), and in Tampa Bay and Charlotte Harbor, where 50% or more of the oysters were ready to spawn at some sites. Elsewhere in Texas and south Florida values averaged below 15%. In Year 4, the majority of oysters in early development were in Florida, from the Brazos River South in Texas, and in the Mississippi River Delta area. Sites having >50% of the oysters in late development or ready to spawn occurred mostly in South Texas (Corpus Christi Bay, Matagorda Bay, and Laguna Madre Bay), along with some isolated sites in the Mississippi River Delta (Barataria Bay) and the Tampa Bay area. In Year 5, South Texas and South Florida again accounted for most of the sites. In Year 6, sites were generally higher around the Gulf, particularly in the northern Gulf: many more reproductive/advanced individuals were collected.

7.3 Long-Term Changes

We reported the Gulf-wide distributions of certain parameters in Preprint 7. In the following figures (7.1-7.14), we have extended this analysis to six years. The statistical approach is detailed in Preprint 7. In the first set of figures (7.1-7.7), we asked the question "How far apart must bays be before the similarity in yearly changes in certain parameters no longer exists?" We expect nearby bays to behave similarly. We expect bays far apart to behave differently. The evidence present so far suggests that large-scale climatic shifts dictate year-to-year changes so that bays within 500 to 1000km of each other may behave similarly.

Figure 7.1 shows the situation for gonadal stage. The y-axis is a plot of the log of the p value. Any value below -1 indicates a significant similarity. In this graph, nearby bays are very similar (PL .000001 at 200km). Similarity disappears at about 1000km (logP >-1 at 1000km). Accordingly, year-to-year changes in the frequency of reproductive/active individuals were similar in bays up to 1000km

apart. On the average, bays within 1000km rose or fell in unison in the biological attribute from one year to the next. This would be expected if climatic conditions, such as the average winter temperature, controlled by climate cycles, like El Niño, were responsible for year to year shifts.

The following is clear from these graphs: *P. marinus* prevalence and sex ratio were affected minimally by local conditions. Nearby bays were as dissimilar as bays far apart. Like gonadal stage, condition index, length, and *P. marinus* infection intensity all showed scales of similarity in the range of 1000km. So, the primary indicators of population health were impacted significantly by climate change on a scale of 1000km over the six years of study.

In the second set of graphs (Fig. 7.8-7.14), we look at groups of ten adjacent bays. This analysis searches for the pattern of regional similarity around the Gulf of Mexico. Preprint 7 has a detailed description of the method. In this analysis, values above the dark line indicate significant similarity. The biological attributes show the following pattern: Length, *P. marinus* prevalence, gonadal stage, and sex show similarities in the southern Gulf, east or west or both. This similarity suggests a subtropical climate control like El Niño. Condition index shows the opposite trend: similarity in the northern Gulf of Mexico from approximately Galveston Bay to Tampa Bay. *P. marinus* infection intensity reacts similar throughout the Gulf. These latter two indicate that temperate weather patterns are also important. Overall, the data clearly show that weather patterns exert control on oyster population health throughout the Gulf of Mexico and can account for the assessed pattern of year-to-year changes in population health over the six years of the study.

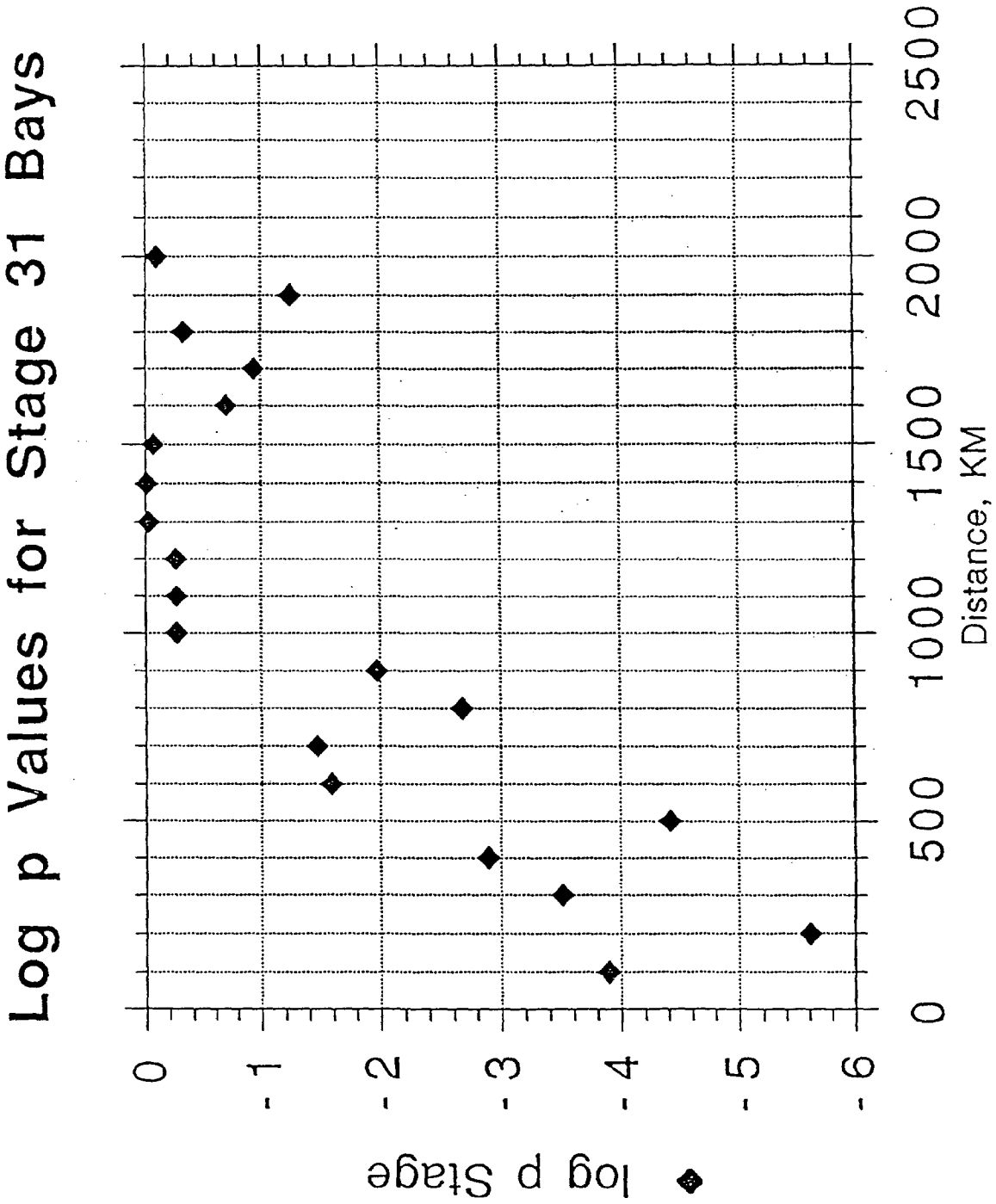


Figure 7.1 Gonadal stage logP values for Stage 31 Bays.

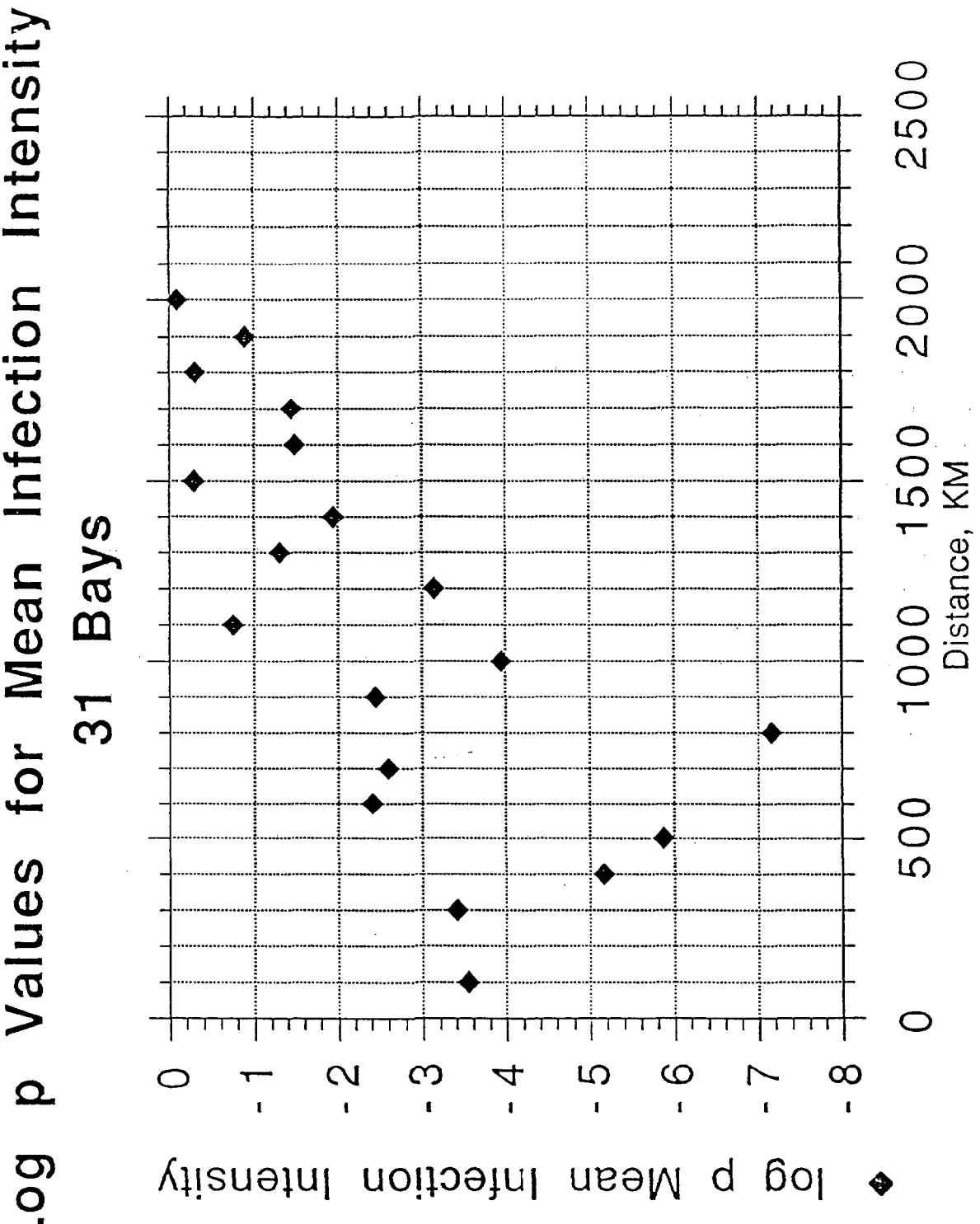


Figure 7.2 LogP values for Mean Infection Intensity 31 Bays.

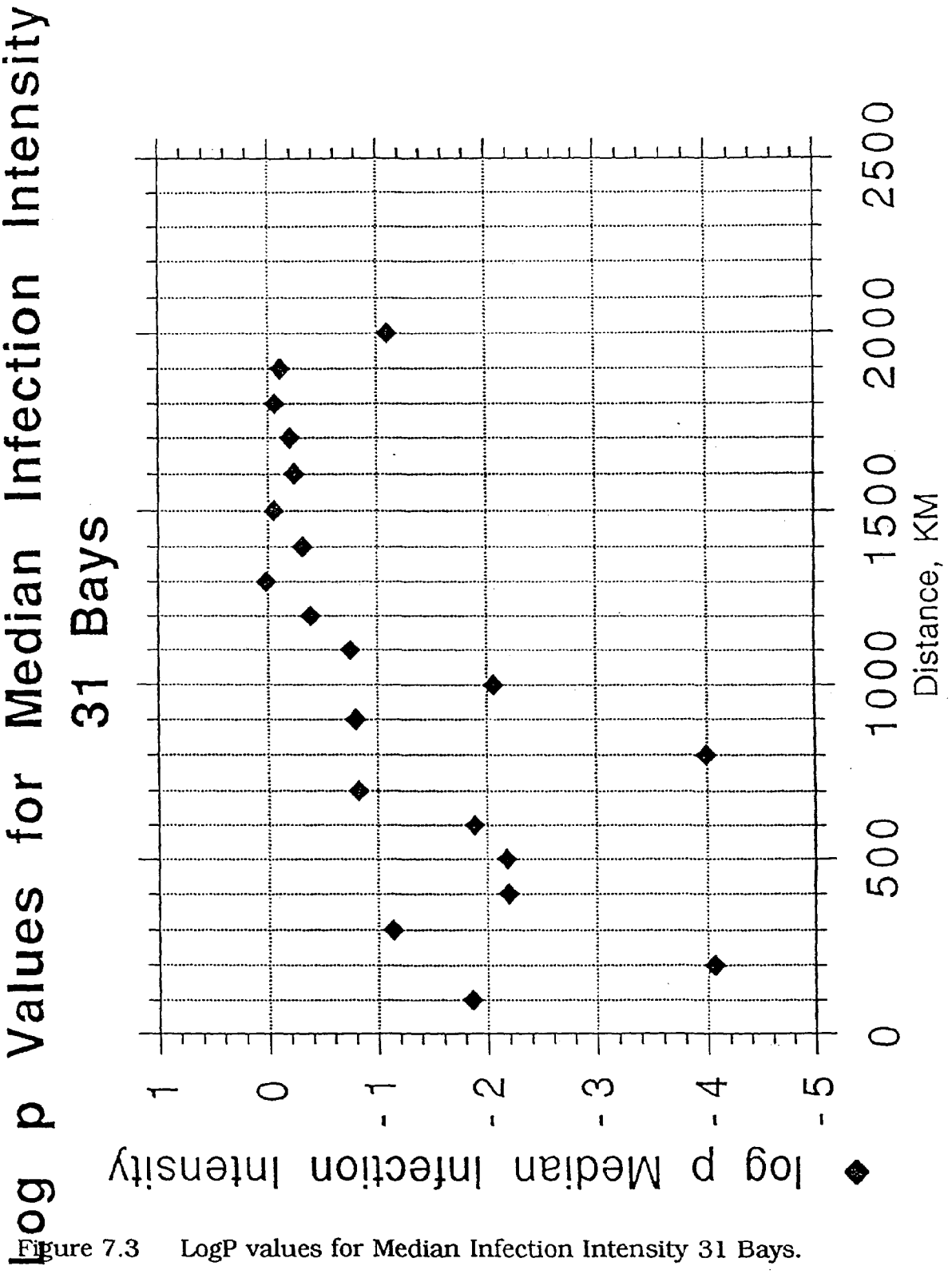


Figure 7.3 LogP values for Median Infection Intensity 31 Bays.

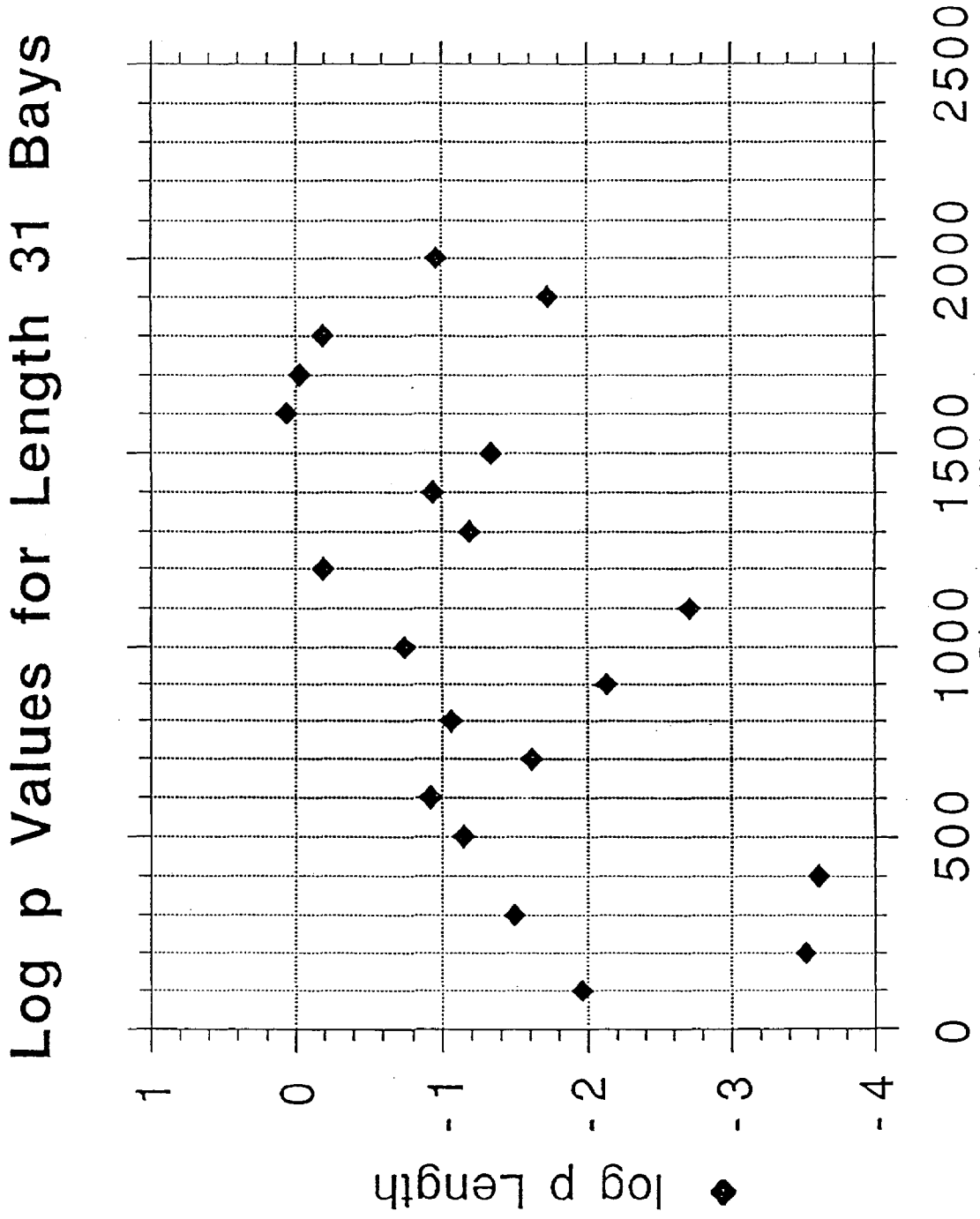


Figure 7.4 LogP values for Length 31 Bays.

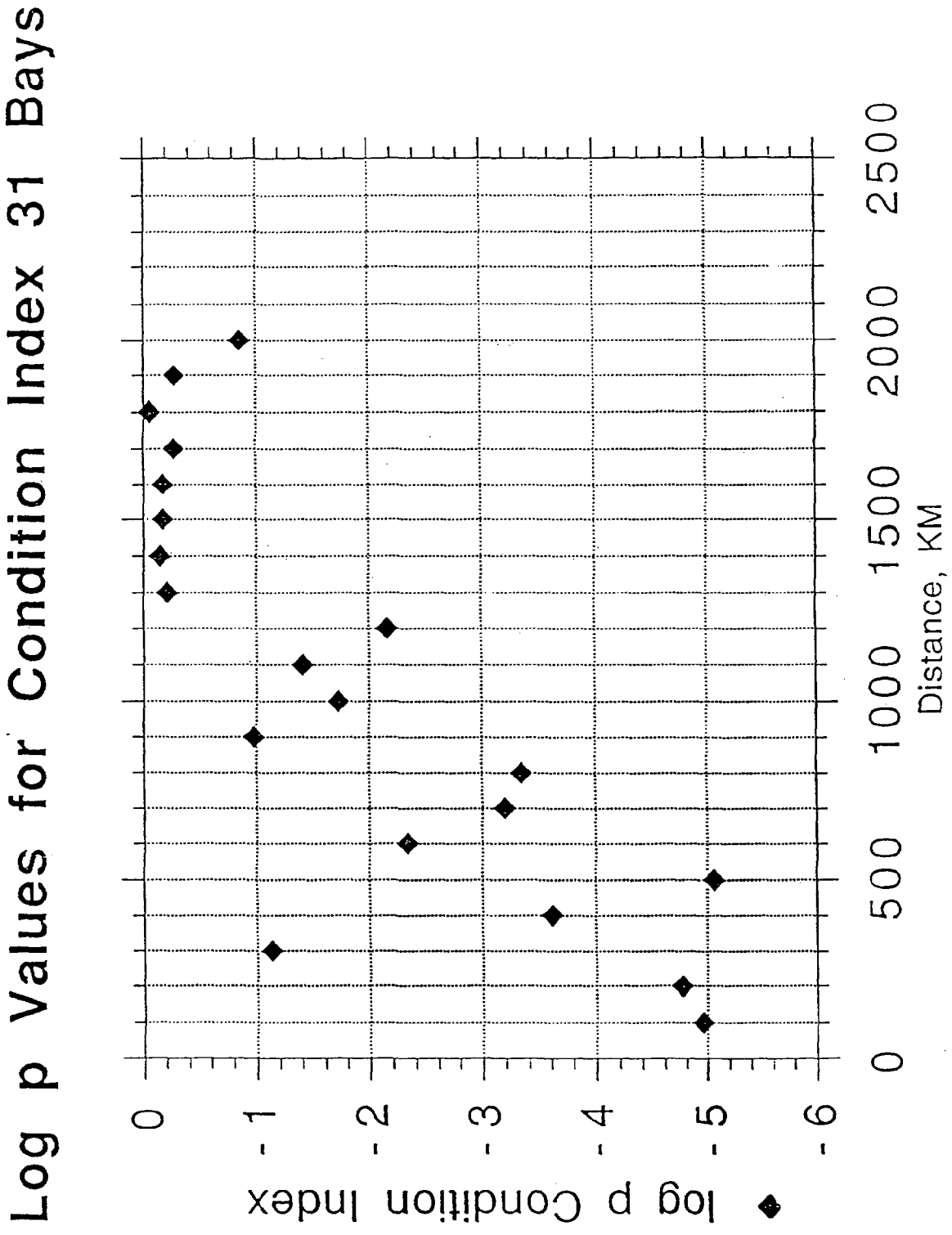


Figure 7.5 LogP values for Condition Index 31 Bays.

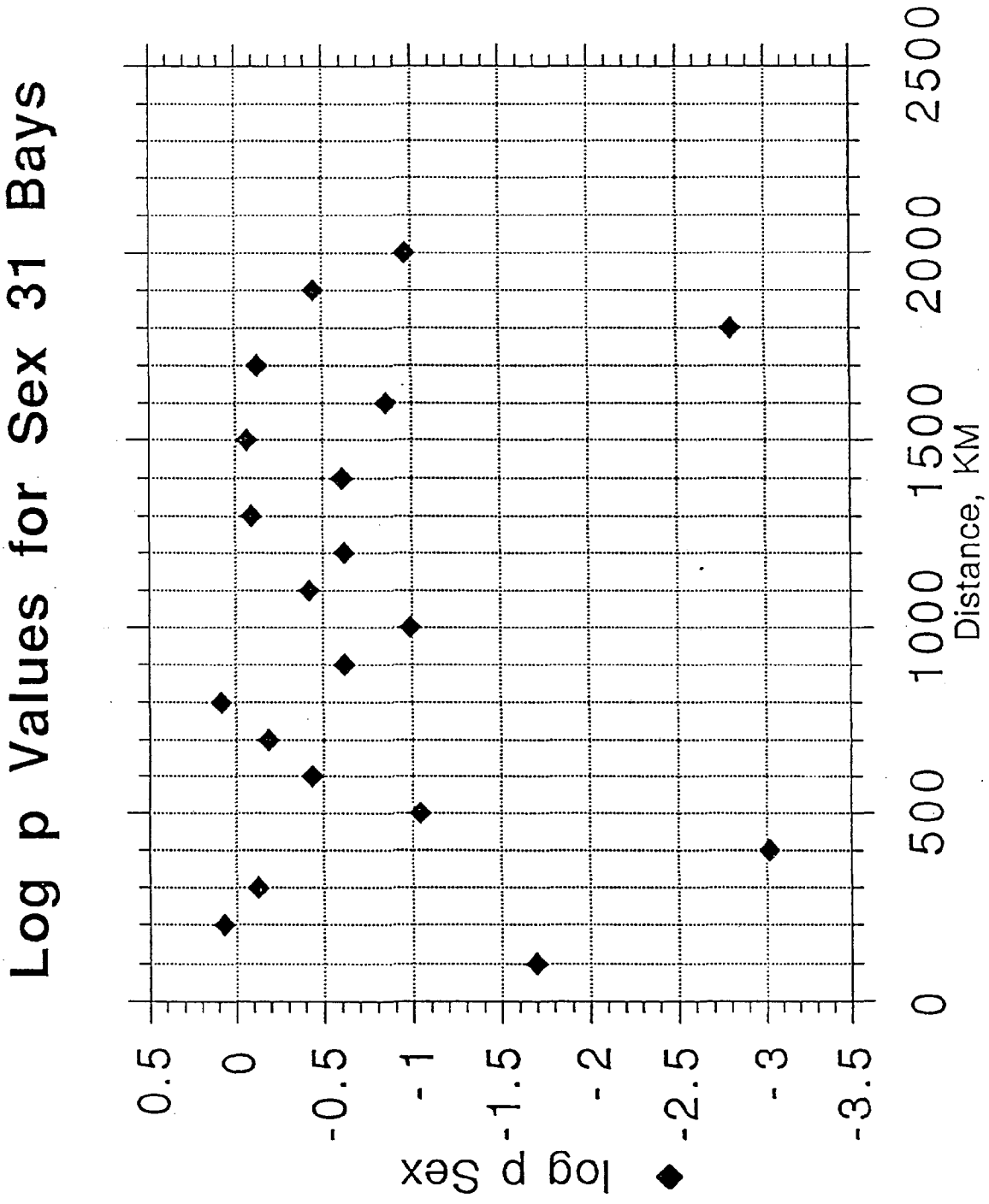


Figure 7.6 LogP values for Sex 31 Bays.

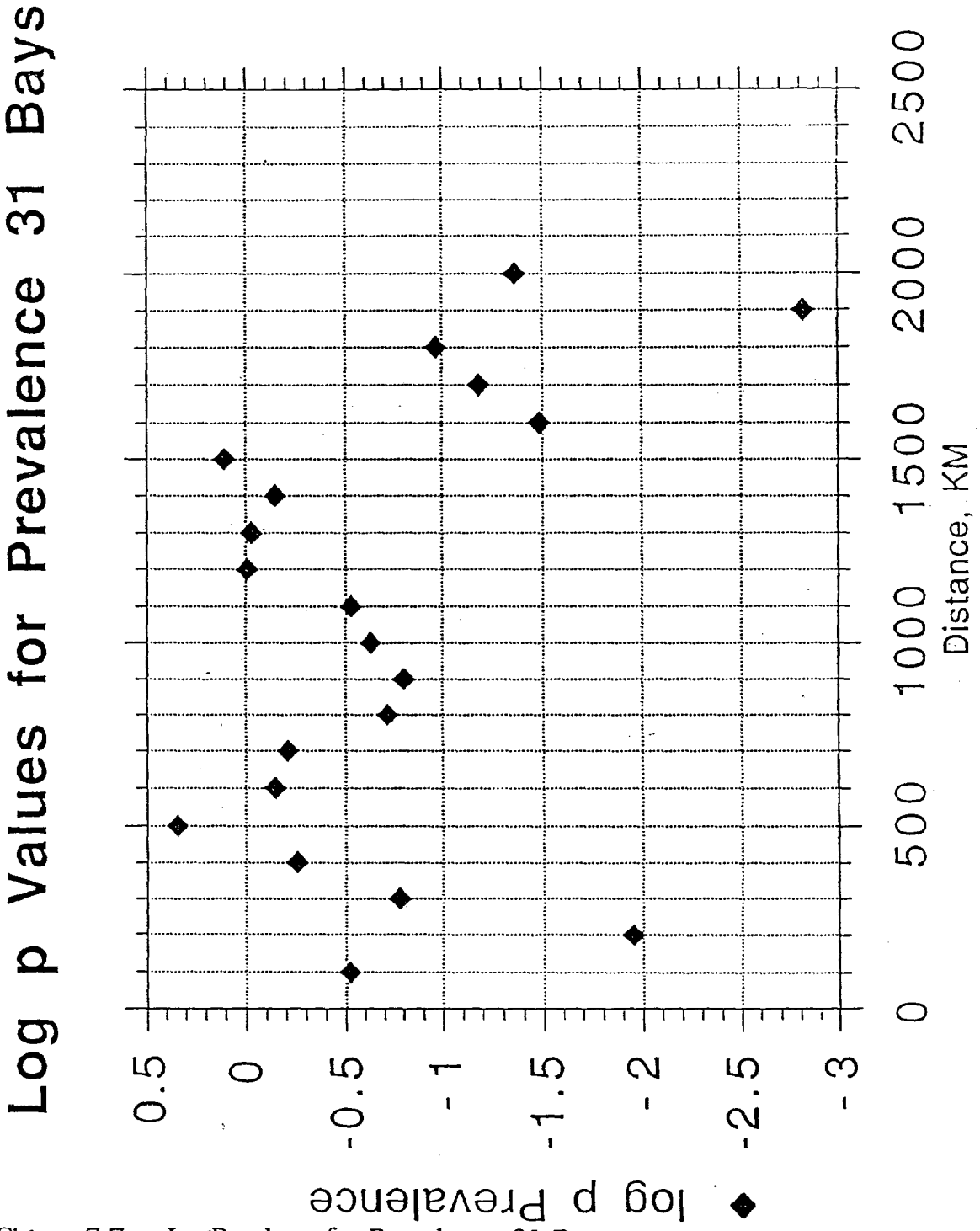


Figure 7.7 LogP values for Prevalence 31 Bays.

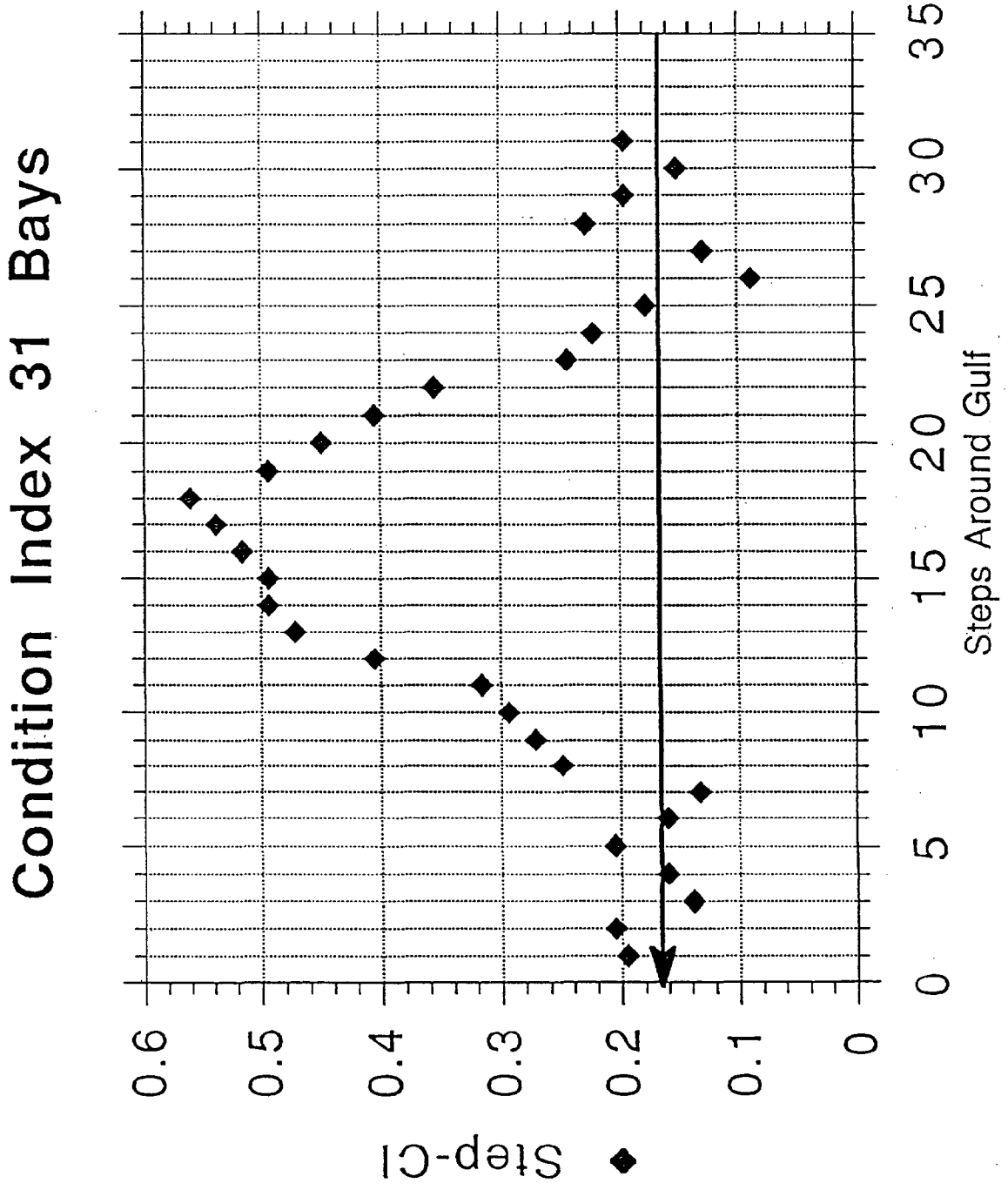


Figure 7.8 Condition Index 31 Bays.

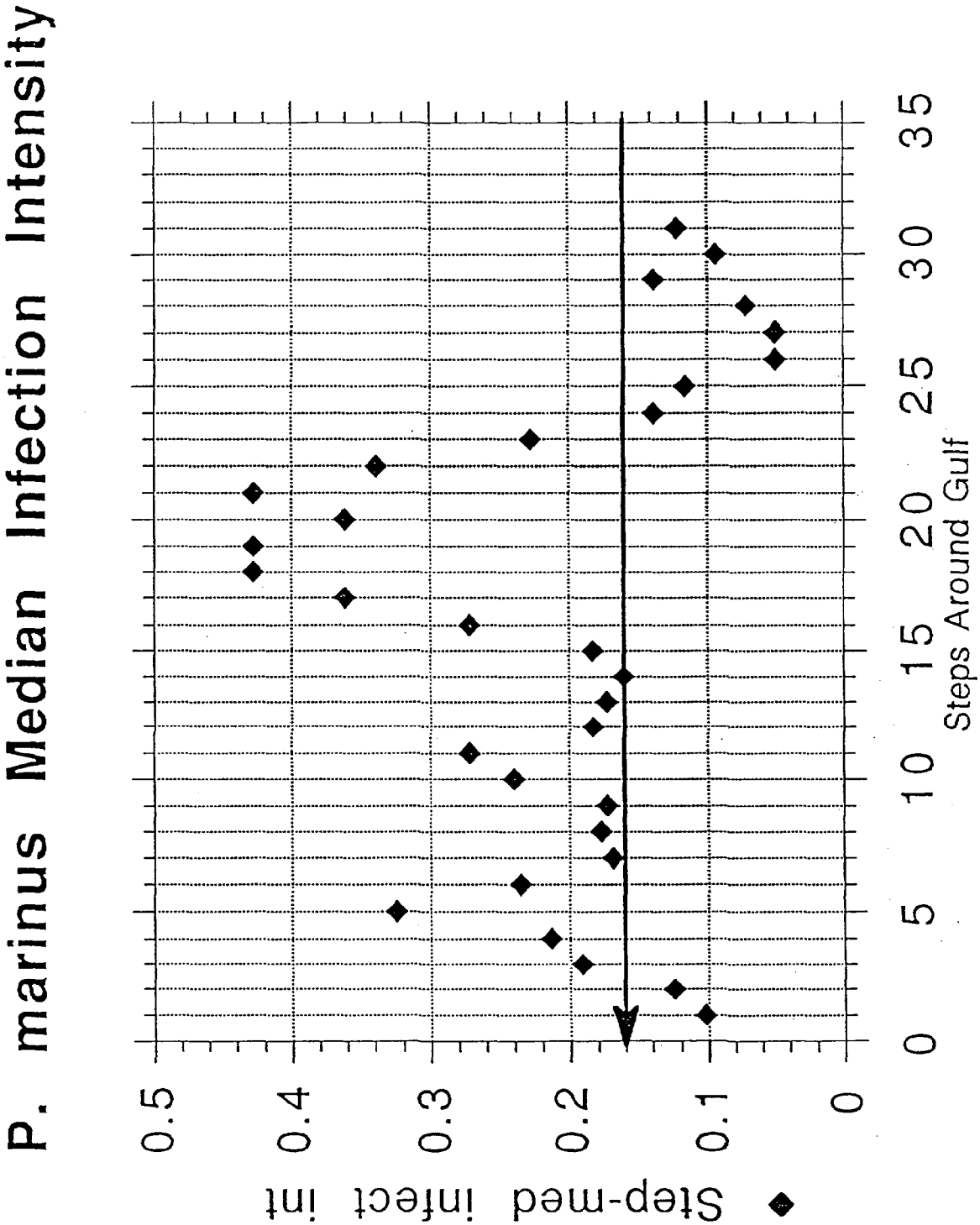


Figure 7.9 *P. marinus* Median Infection Intensity 31 Bays.

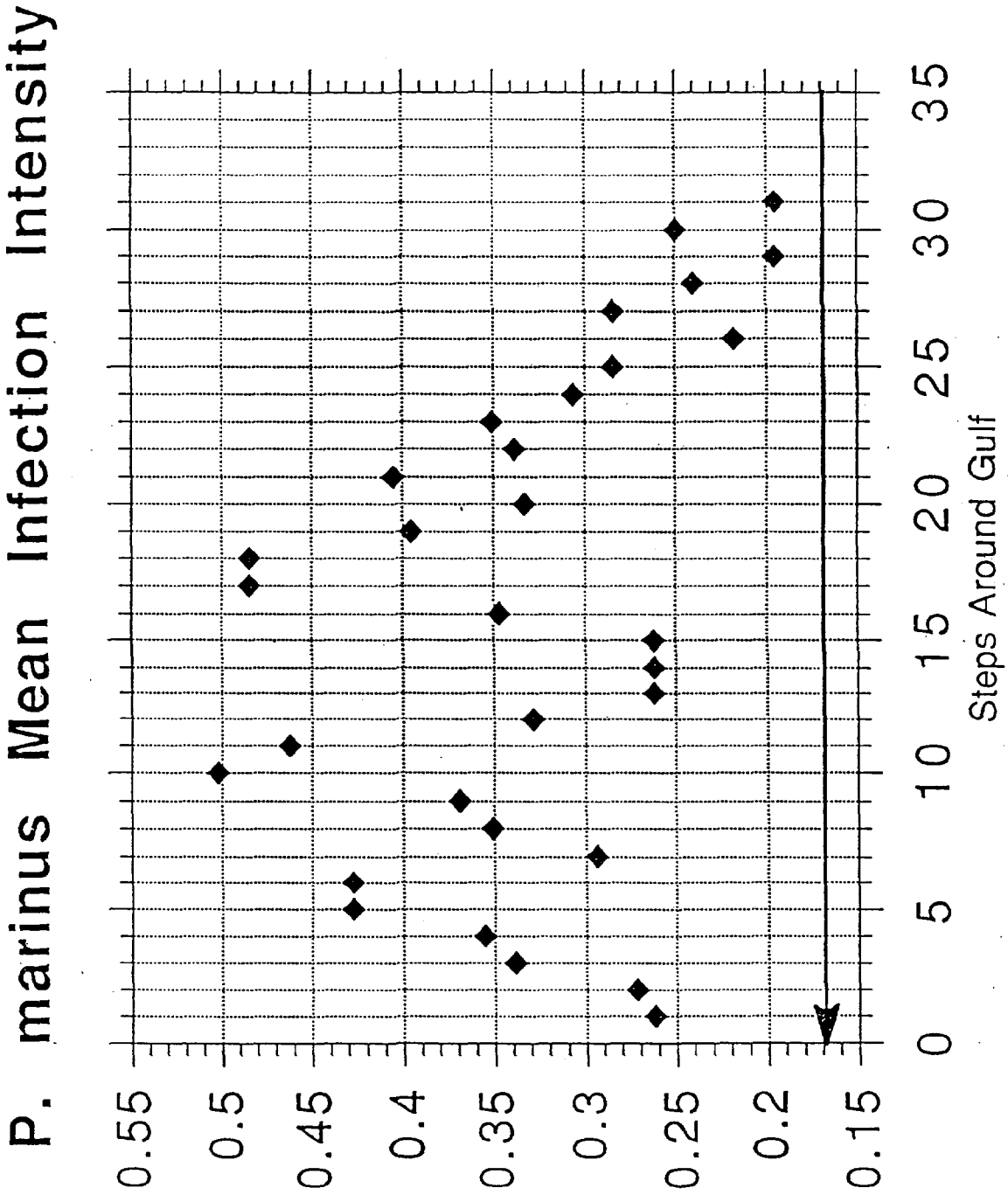


Figure 7.10 *P. marinus* Mean Infection Intensity 31 Bays.

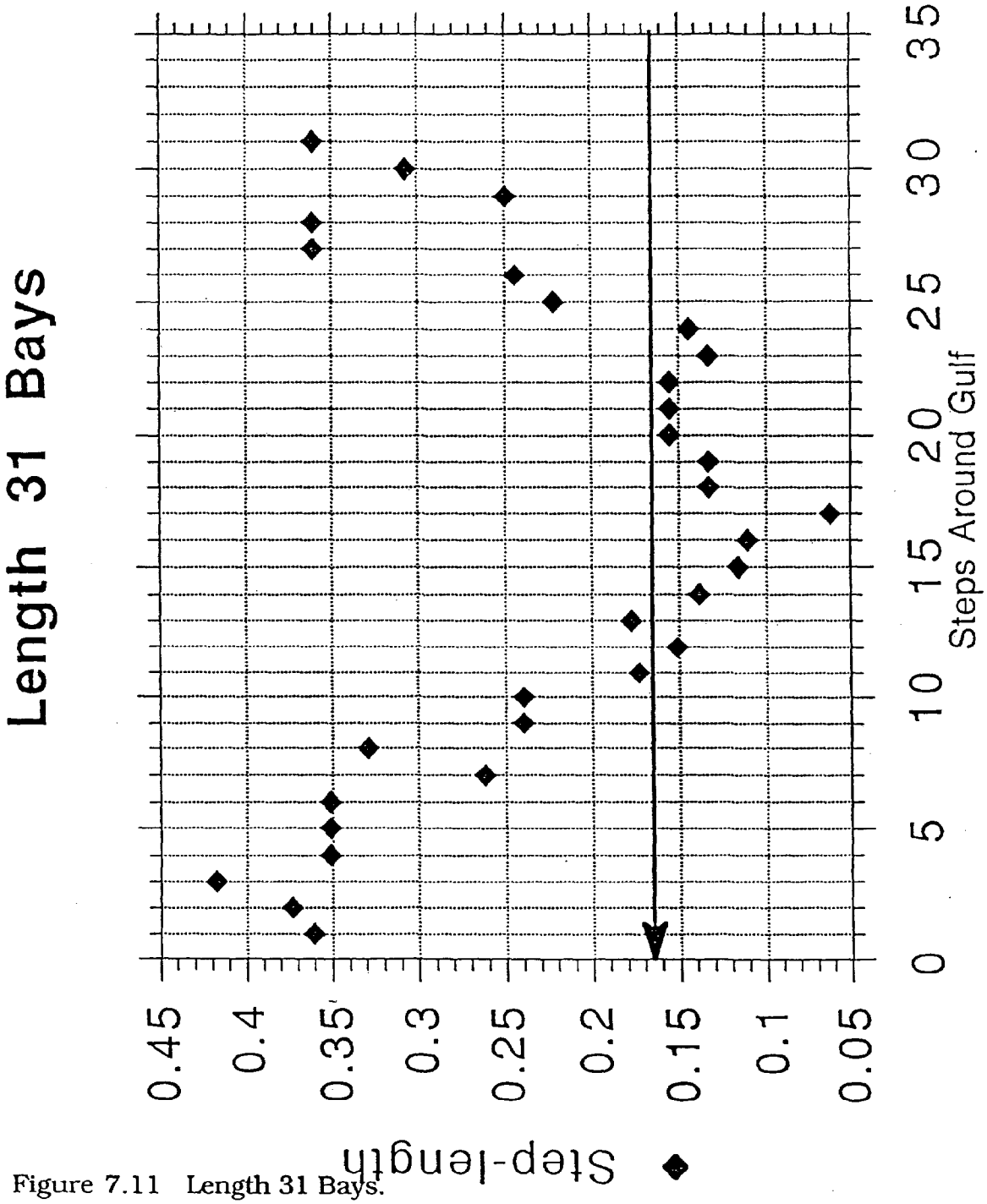


Figure 7.11 Length 31 Bays.

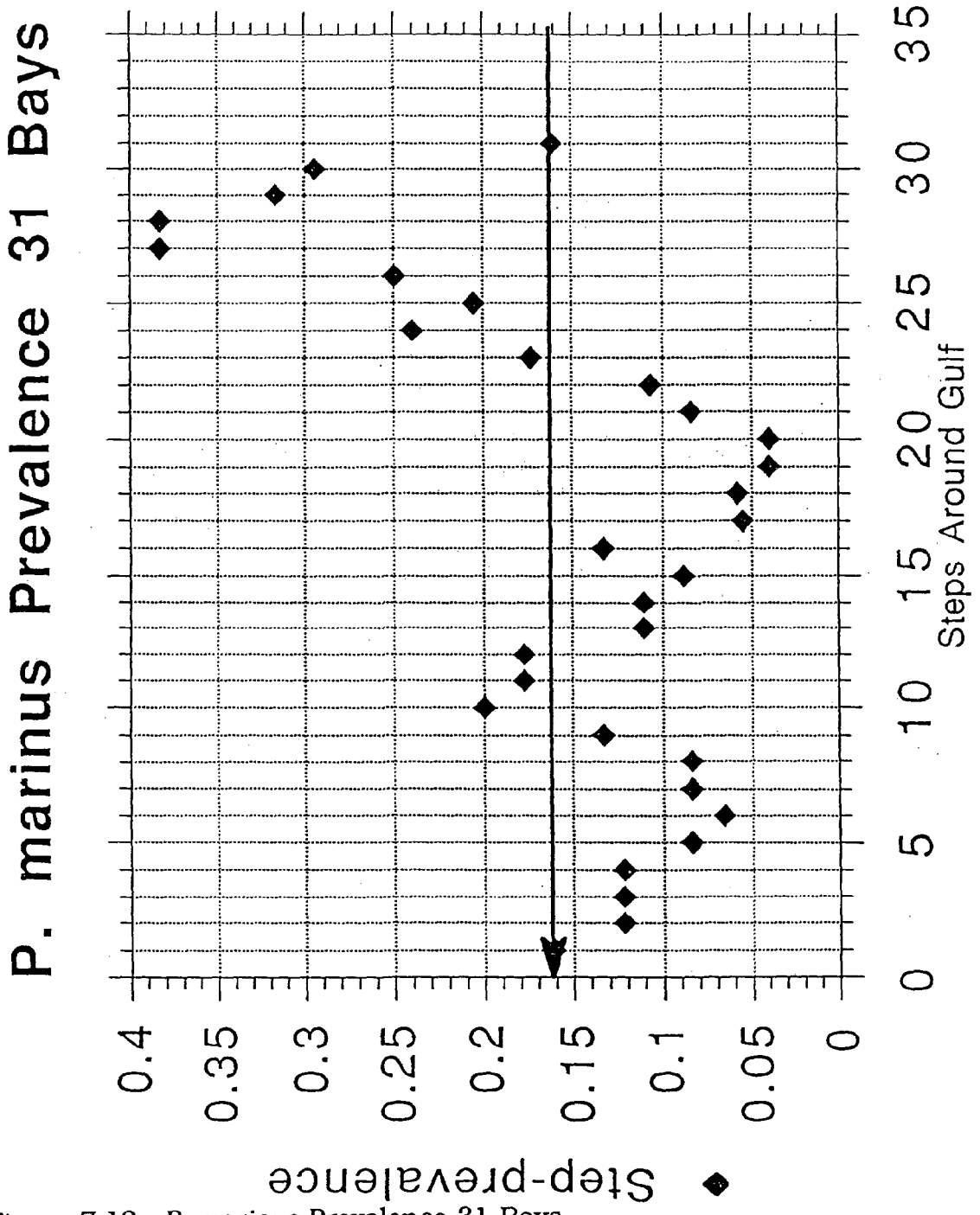


Figure 7.12 *P. marinus* Prevalence 31 Bays

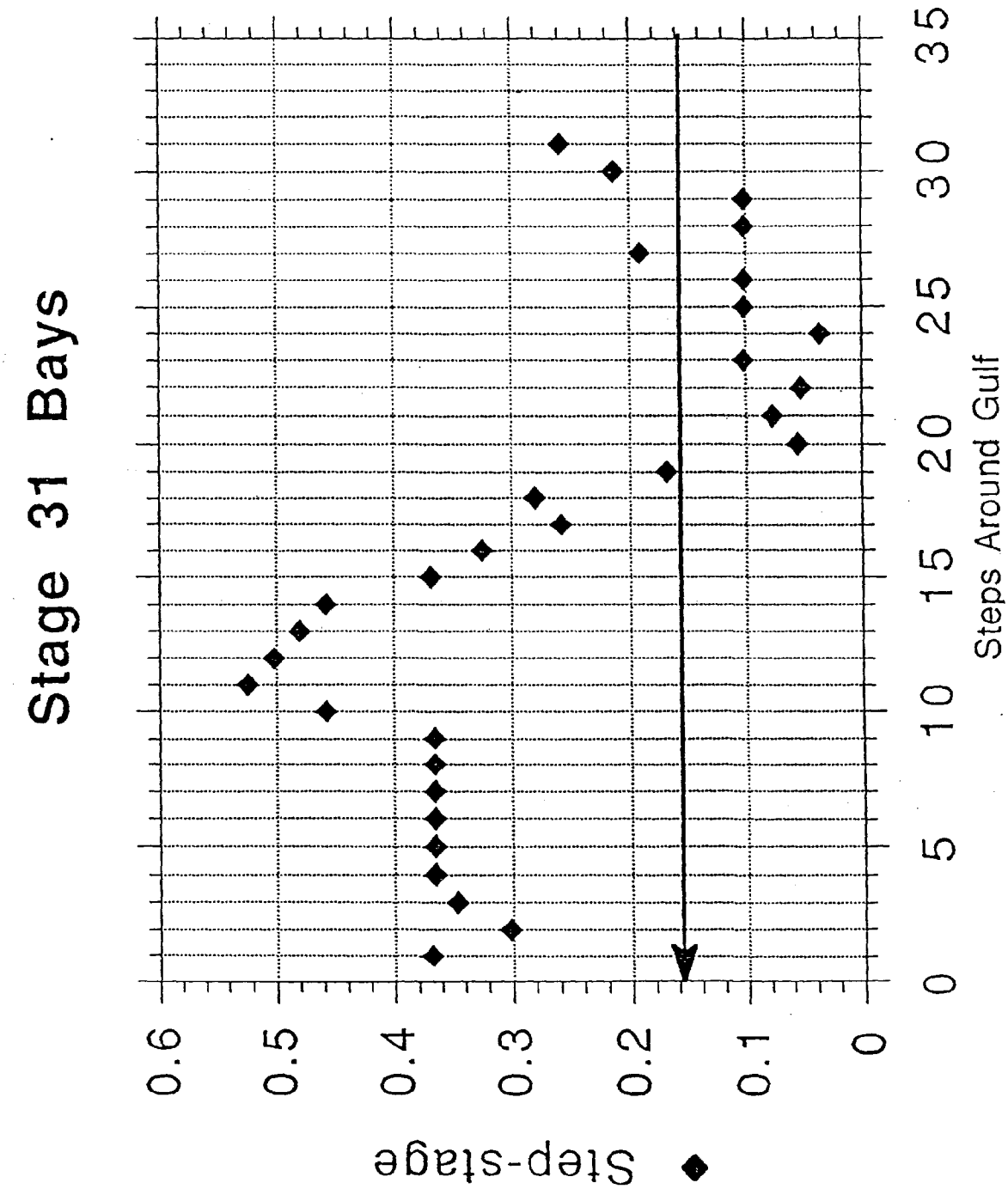


Figure 7.13 Stage 31 Bays.

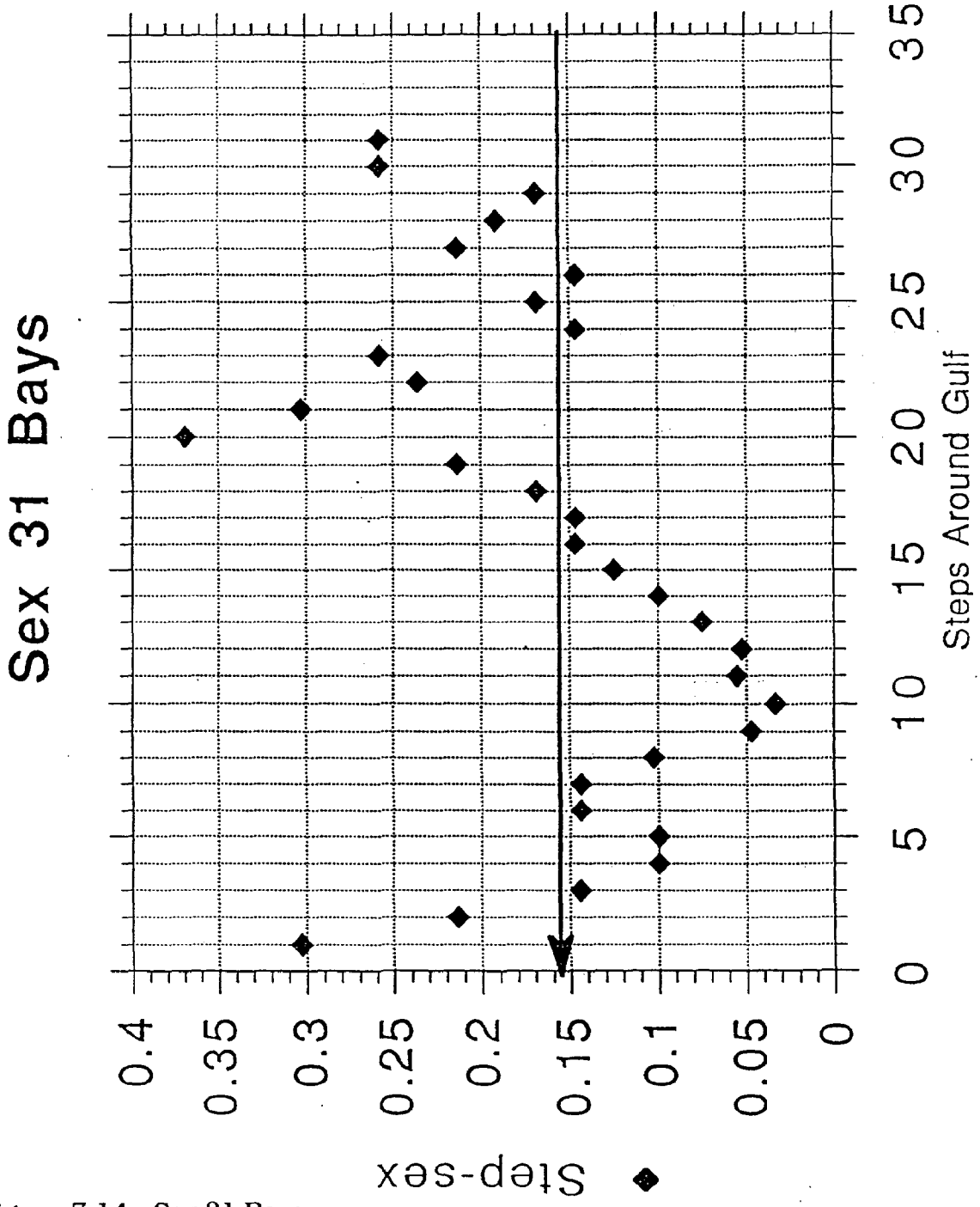


Figure 7.14 Sex 31 Bays.

Preprint 7

Spatial and Temporal Distributions of Contaminant Body Burden and disease in Gulf of Mexico Oyster Populations: The Role of Local and Large-Scale Climatic Controls

E.A. Wilson, E.N. Powell, T.L. Wade,
R.J. Taylor, B.J. Presley, and J.M. Brooks

HELGOLÄNDER MEERESUNTERSUCHUNGEN
Helgoländer Meeresunters. 46, ■-■ (1992)

Spatial and temporal distributions of contaminant body burden and disease in Gulf of Mexico oyster populations: The role of local and large-scale climatic controls

E. A. Wilson, E. N. Powell, T. L. Wade, R. J. Taylor, B. J. Presley & J. M. Brooks

Department of Oceanography, Texas A & M University; College Station, TX 77843, USA

ABSTRACT: As part of NOAA's Status and Trends Program, oysters were sampled from 43 sites throughout the Gulf of Mexico from Brownsville, Texas, to the Florida Everglades from 1986 to 1989. Oysters were analysed for body burden of a suite of metals and petroleum aromatic hydrocarbons (PAHs), the prevalence and intensity of the oyster pathogen, *Perkinsus marinus*, and condition index. The contaminants fell into two groups based on the spatial distribution of body burden throughout the Gulf. Arsenic, selenium, mercury and cadmium were characterized by clinal reduction in similarity with distance reminiscent of that followed by mean monthly temperature and precipitation. Zinc, copper, PAHs and silver showed no consistent geographic trend. Within local regions, industrial and agricultural land use and *P. marinus* prevalence and infection intensity frequently correlated with body burden.

Contaminants and biological attributes followed one of three temporal trends. Zinc, copper and PAHs showed concordant shifts over 4 years throughout the eastern und southern Gulf. Mercury and cadmium showed concordant shifts in the northwestern Gulf. Selenium, arsenic, length, condition index and *P. marinus* prevalence and infection intensity showed concordant shifts throughout most of or the entire Gulf. Concordant shifts suggest that climatic factors, the El Niño/Southern Oscillation being one example, exert a strong influence on biological attributes and contaminant body burdens in the Gulf. Correlative factors are those that probably affect or indicate the rate of tissue turnover and the frequency of reproduction; namely, temperature, disease intensity, condition index and length.

INTRODUCTION

Bivalve molluscs have frequently been used as indicator organisms in studies monitoring levels of contaminants in the environment. These organisms are preferred because of their ability to accumulate and concentrate both metal and organic contaminants enabling them to serve as long-term integrators of their environment (Phillips, 1977a). However, many biological and environmental factors affect the rate and extent of bioaccumulation. Biological factors including differential growth rate (Cunningham & Tripp, 1975a; Boyden, 1977), reproductive stage (Cunningham & Tripp, 1975a; Frazier, 1975; Martinčič et al., 1984), and general physiological condition, stress and disease (Shuster & Pringle, 1969; Sindermann, 1983) affect incorporation and depuration rates. Similarly, changes in environmental parameters such as salinity (Denton & Burdon-

Jones, 1981; Wright & Zamuda, 1987), freshwater runoff (Windom & Smith, 1972; Phillips, 1976a; Zaroogian & Cheer, 1976), duration of exposure to contaminants (Shuster & Pringle, 1969; Scott & Lawrence, 1982), temperature (Shuster & Pringle, 1969; Zaroogian & Cheer, 1976; Denton & Burdon-Jones, 1981), resuspension of sediments (Uncles et al., 1988) and proximity to point sources (Farrington & Quinn, 1973; Ratkowsky et al., 1974; Phillips, 1976b) can affect the bioavailability of environmental contaminants.

Although these local environmental and biological controls on variability in pollutant body burden are important; they, themselves, may be affected by long-term, large-scale phenomena such as climatic cycles. Such phenomena may override local controls and impose large-scale, concordant oscillations in environmental and biological parameters. Seasonal, climatic and other longterm cycles have been used in predicting harvests of commercially important fish and shellfish, including oysters (Dow, 1977; Ulanowicz et al., 1980; Allen & Turner, 1989), and have been implicated in the distribution and intensity of oyster disease (Powell et al., in press). By imprinting themselves on the local environment, these long-term cycles may also alter the bioavailability of contaminants and therefore, contaminant body burden. Accordingly, explaining spatial and temporal variability in contaminant body burden may require understanding both local and large-scale environmental phenomena.

The NOAA Status and Trends Program ("Mussel Watch") is an environmental monitoring program designed to monitor changes in environmental quality along the Atlantic, Pacific and Gulf coasts of the United States by measuring levels of chemical contaminants in fish, bivalves, and sediments and identifying biological responses to those contaminants. As part of the program, pollutant body burden of trace metals and polynuclear aromatic hydrocarbons (PAHs) were measured in oysters (*Crassostrea virginica*) collected from sites along the Gulf of Mexico coast from Brownsville, Texas to the Florida Everglades. The biological component of this study included determining the prevalence and intensity of infection by the endoparasitic protozoan *Perkinsus marinus* in these oyster populations. Over four years (1986–1989), this program has produced the most extensive spatial and temporal data set on contaminant body burden and disease prevalence and intensity available for natural oyster populations in the Gulf of Mexico and has implicated the El Niño/Southern Oscillation cycle as an important factor controlling large-scale temporal variability in oyster disease. The goal of this paper is to integrate the biological and chemical data to determine: (1) the spatial and temporal distributions of contaminant body burden as they compare to *P. marinus* in oyster populations; (2) the biological and environmental factors important in determining these distributions and (3) the role of local and long-term controls on contaminant body burdens.

MATERIALS AND METHODS

Sample collection

Oysters were collected from natural populations along the coast of the Gulf of Mexico during December to February of each year from 1986 to 1989. In all, 75 sites were sampled; 43 sites were sampled in all 4 years. Forty oysters were collected at each of three stations at each site; twenty for trace metal analysis and twenty for biological and trace organic analysis. Temperature and salinity were recorded at the time of collection.

The maximum anterior-posterior length was measured for each oyster (Morales-Alamo & Mann, 1989). Displacement volume of the 20 oysters collected for the biological and trace organic analyses was determined before and after shucking. Each oyster was sampled for the presence and intensity of infection by *Perkinsus marinus* (Ray, 1966). Prevalence of infection was calculated as: (the number of infected oysters/number of oysters sampled). Infection intensity was ranked on the 0-to-5-point scale of Mackin (1962) as modified by Craig et al. (1989). After the biological sample was removed, the remainder of the oyster tissue was placed in precombusted jars, sealed with Teflon lids, weighed and frozen for trace organic analysis. Tissue dry weight and displacement volume were used to calculate condition index = dry weight of tissue/internal volume of shell cavity (Lawrence & Scott, 1982). The twenty oysters collected for trace metal analysis were scrubbed, frozen in the shell and returned to the laboratory. Further sample preparation and the analytical techniques employed for both trace organic and trace metal analyses were described in Brooks et al. (1989).

Statistical analysis

Data reduction

Within-site variability was typically low (Craig et al., 1989; Wilson et al., 1990), so the three stations were combined for the following statistical analyses. Statistical analysis of the data was limited to the 43 sites sampled in each of the 4 years. Each site was assigned to one of 26 bay systems as slightly modified from Broutman & Leonard (1988) (Table 1) [see Craig et al. (1989) or Presley et al. (1990) for site maps, and Wilson et al. (1990) and Powell et al. (in press) for further information on the sites]. Salinity and temperature data for the sites are given in Brooks et al. (1989). Powell et al. (in press) list mean *P. marinus* prevalences and infection intensities for the bay groups. Mean values of condition index and length for the bay groups are presented in Table 2.

Seven of the 13 metals analysed as part of the Status and Trends protocol were chosen for further consideration: arsenic, cadmium, copper, silver, mercury, zinc and selenium. These metals were selected because they generally were present in highest concentration among the metals measured and because they exhibited some of the most dramatic differences in body burdens in populations around the Gulf of Mexico and among the 4 years of the study. Wade et al. (1988) and Brooks et al. (1989) list the individual PAHs analysed, but, for this study, body burdens of the individual PAHs were summed and a total value was used for statistical analysis. Contaminant data are presented as the geometric mean of all sites included in each bay group (Tables 3, 4 and 5).

Values for mean monthly precipitation and mean monthly temperature were obtained from NOAA (1985–1989). The values used were averages of several stations around each bay system. Average monthly stream flow from gauged streams – Rio Grande (IBWC, 1985–1989), the Mississippi and Atchafalaya Rivers (Army Corps of Engineers, personal communication) and the remaining gauged rivers and streams (USGS, 1985–1989) – and estimated freshwater runoff (from precipitation data) for areas downstream of gauges, estimated from the total watershed area (NOAA, 1987), were summed to estimate total freshwater input to each bay system. Land use around the bay systems, classified as either industrial, agricultural or residential, was compiled from NOAA (1987).

Table 1. Site names, four-letter site designations, locations and assignments into bay groups for the 43 Status and Trends sites sampled in all 4 years of the study

Bay group	Site name	Site designation	Location Latitude		Longitude	
	Texas					
1	Laguna Madre, South Bay	LMSB	26	2.58	97	10.49
2	Corpus Christi Bay, Nueces Bay	CCNB	27	51.70	97	21.00
3	Aransas Bay, Long Reef	ABLR	28	3.30	96	57.50
	Copano Bay, Copano Reef	CBCR	28	8.20	97	7.58
	Mesquite Bay, Ayres Reef	MBAR	28	10.30	96	49.70
5	Matagorda Bay, Gallinipper Point	MBGP	28	35.00	96	34.00
	Matagorda Bay, Tres Palacios Bay	MBTP	28	39.00	96	15.50
6	East Matagorda Bay	MBEM	28	42.30	95	53.00
8	Galveston Bay, Yacht Club Reef	GBYC	29	37.00	94	59.50
	Galveston Bay, Todd's Dump Reef	GBTD	29	30.10	94	54.00
	Galveston Bay, Hanna Reef	GBHR	29	27.50	94	42.50
	Galveston Bay, Confederate Reef	GBCR	29	15.75	94	50.50
9	Sabine Lake, Blue Buck Point	SLBB	29	48.00	93	54.42
	Louisiana					
10	Lake Calcasieu, St. Johns Island	CLSJ	29	50.00	93	23.00
11	Joseph Harbor Bayou	JHJH	29	37.75	92	45.75
12	Vermillion Bay, Southwest Pass	VBSP	29	34.70	92	4.00
13	Atchafalaya Bay, Oyster Bayou	ABOB	29	13.00	91	8.00
	Caillou Lake	CLCL	29	15.25	90	55.50
14	Lake Barre	TBLB	29	15.00	90	36.00
	Lake Felicity	TBLF	29	16.00	90	24.50
15	Barataria Bay, Bayou St. Denis	BBSD	29	24.10	89	59.80
	Barataria Bay, Middle Bank	BBMB	29	17.20	89	56.50
18	Breton Sound, Bay Garderne	BSBG	29	35.87	89	38.50
	Breton Sound, Sable Island	BSSI	29	24.70	89	28.70
19	Lake Borgne, Malheureux Point	LBMP	29	52.30	89	40.70
	Mississippi					
20	Mississippi Sound, Pass Christian	MSPC	30	19.75	89	19.58
	Biloxi Bay	MSBB	30	23.38	88	55.42
	Pascagoula Bay	MSPB	30	21.05	88	37.00
	Alabama					
21	Mobile Bay, Cedar Point Reef	MBCP	30	19.40	88	7.30
	Florida					
22	Pensacola Bay, Indian Bayou	PBIB	30	30.83	87	4.00
23	Choctawhatchee Bay, Santa Rosa	CBSR	30	23.50	86	10.60
	Choctawhatchee Bay, Shirk Point	CBSP	30	28.95	86	28.60
24	St. Andrew Bay, Watson Bayou	SAWB	30	8.50	85	37.58

GJN)18620 (KLJ)50 (SW)Wilson

Table 1 (Continued)

Bay group	Site name	Site designation	Location Latitude		Location Longitude	
	Florida					
25	Apalachicola Bay, Dry Bar	APDB	29	41.50	85	5.00
	Apalachicola Bay, Cat Point Bar	APCP	29	43.00	84	52.50
27	Cedar Key, Black Point	CKBP	29	10.25	83	3.00
28	Tampa Bay, Papys Bayou	TBPB	27	50.72	82	36.75
	Tampa Bay, Cockroach Bay	TBCB	27	40.55	82	30.56
	Tampa Bay, Mullet Key Bayou	TBMK	27	37.28	82	43.62
29	Charlotte Harbor, Bird Island	CBBI	26	31.00	82	2.60
30	Naples Bay	NBNB	26	7.00	81	47.10
	Rookery Bay, Henderson Creek	RBHC	26	1.83	81	43.75
31	Everglades, Faka Union Bay	EVFU	25	54.27	81	30.60

GJN)1863 (KLJ)50 (SW)Wilson

Table 2. Arithmetic means for condition index (g cm $^{-3}$) and length (cm) for the 26 bay groups for each of the 4 years of the study. Means are determined from all sites within each designated bay group

Bay group	Condition index 1986	1987	1988	1989	Length 1986	1987	1988	1989
1	0.086	0.076	0.122	0.115	8.163	6.953	6.035	6.028
2	0.087	0.131	0.110	0.065	7.407	5.673	5.521	7.042
3	0.092	0.141	0.104	0.098	8.470	8.197	8.187	6.383
5	0.099	0.137	0.114	0.075	9.378	8.299	6.916	7.071
6	0.087	0.119	0.100	0.099	10.132	8.370	6.717	6.292
8	0.106	0.119	0.109	0.052	9.032	8.556	8.546	8.332
9	0.075	0.130	0.088	0.043	10.440	9.648	9.655	8.395
10	0.100	0.108	0.135	0.054	11.477	8.265	7.988	9.323
11	0.120	0.126	0.081	0.112	8.358	8.787	8.187	7.062
12	0.108	0.097	0.088	0.081	8.715	9.658	9.908	9.057
13	0.105	0.112	0.122	0.051	9.731	10.360	8.182	8.197
14	0.096	0.106	0.107	0.071	8.961	9.223	7.178	7.488
15	0.088	0.114	0.125	0.068	10.079	9.566	7.040	6.861
18	0.165	0.103	0.113	0.058	9.657	8.504	7.708	8.465
19	0.097	0.128	0.125	0.053	8.942	7.270	7.524	5.682
20	0.119	0.112	0.144	0.093	8.399	7.153	7.104	7.204
21	0.144	0.105	0.113	0.096	8.622	9.003	6.033	6.663
22	0.109	0.135	0.144	0.081	9.090	4.558	6.017	6.456
23	0.119	0.122	0.091	0.063	7.747	4.949	6.673	5.974
24	0.141	0.150	0.140	0.089	6.008	4.810	6.528	6.347
25	0.149	0.109	0.119	0.102	8.433	7.347	8.286	6.637
27	0.159	0.104	0.106	0.089	7.438	5.515	6.714	5.390
28	0.087	0.120	0.117	0.086	6.578	5.898	6.373	6.443
29	0.081	0.109	0.179	0.061	6.517	5.295	6.466	6.640
30	0.116	0.108	0.162	0.078	6.702	5.262	4.673	5.469
31	0.123	0.125	0.022	0.054	8.060	6.560	6.558	5.835

(JN)1864 (KL)150 (SW)Wilson

Table 3. Geometric means of pollutant body burden for the metals silver, arsenic, cadmium and selenium for each bay group and each year of the study. Values given are parts per million (ppm)

	Pollutant body burden															
Bay	Silver				Arsenic				Cadmium				Selenium			
group	1986	1987	1988	1989	1986	1987	1988	1989	1986	1987	1988	1989	1986	1987	1988	1989
1	1.73	1.14	0.88	1.68	21.67	15.10	18.67	14.84	3.83	2.72	2.13	2.64	2.87	2.13	4.66	2.49
2	1.38	1.80	1.91	1.48	10.95	10.63	10.66	8.20	6.30	11.88	4.74	4.32	2.68	2.35	5.15	4.03
3	4.05	2.08	2.79	2.63	7.47	6.36	6.37	7.14	7.63	5.14	6.34	5.73	4.43	2.79	5.61	3.87
5	1.87	1.62	3.10	2.65	8.24	5.88	7.99	9.37	4.57	3.90	4.84	5.16	2.63	2.45	4.82	3.07
6	4.67	5.14	2.50	2.59	8.33	5.77	5.93	6.84	7.33	6.61	4.70	5.38	4.07	3.50	4.57	3.95
8	1.76	2.39	2.24	2.35	4.81	4.88	3.48	4.44	4.33	4.11	3.16	5.00	3.04	3.07	4.04	3.34
9	2.69	7.13	4.60	3.29	5.50	4.10	5.50	5.80	4.17	7.16	4.36	5.13	2.53	2.68	4.07	2.84
10	2.00	2.19	2.45	1.74	10.33	6.60	4.37	5.91	4.33	5.08	3.49	3.88	2.30	2.25	3.66	3.31
11	2.57	3.16	2.90	2.29	8.00	7.97	4.80	5.63	4.83	3.61	4.57	5.09	2.13	3.48	3.52	4.37
12	5.00	4.55	3.47	5.08	9.67	8.50	4.67	6.13	9.67	9.25	6.43	10.39	2.23	4.37	5.43	4.44
13	1.04	1.61	2.01	1.67	10.09	7.14	7.20	5.69	3.34	5.28	4.84	5.55	1.78	2.61	3.27	3.17
14	0.43	0.51	0.93	0.68	10.25	8.78	7.39	4.98	1.98	2.29	3.69	3.13	1.54	2.22	5.16	2.21
15	0.36	0.73	1.23	0.77	9.75	10.77	7.89	7.55	1.44	1.46	2.17	2.17	1.03	1.75	3.58	2.24
18	1.07	0.95	1.49	0.91	6.63	9.28	10.22	6.34	2.99	6.76	7.04	5.10	1.85	2.75	3.89	2.36
19	1.83	0.78	1.49	1.47	6.33	4.27	4.90	4.46	5.43	5.26	5.68	6.13	2.57	2.32	4.29	2.59
20	3.29	2.03	2.12	2.79	14.96	9.66	14.30	8.14	4.14	3.79	3.87	4.07	2.09	2.61	3.41	2.43
21	2.20	1.84	1.98	2.01	15.66	6.33	7.04	6.45	2.50	2.38	3.56	3.75	1.63	1.78	2.34	1.77
22	1.33	2.80	1.66	1.50	11.33	17.20	12.14	8.84	3.87	2.83	2.86	2.74	2.40	2.13	3.42	2.29
23	3.99	2.49	2.79	1.69	6.71	8.01	6.21	9.35	3.57	2.51	4.29	3.09	3.27	3.62	5.87	4.25
24	1.70	1.67	1.64	1.85	15.67	12.97	17.48	12.42	1.13	1.16	1.06	1.35	1.20	1.68	2.76	2.34
25	1.72	2.62	1.76	1.26	10.05	11.93	11.54	9.89	2.87	2.59	2.55	2.12	1.61	1.99	3.27	2.06
27	0.33	0.45	0.42	1.12	39.00	23.70	18.86	18.90	2.10	1.72	2.44	3.11	1.37	2.43	2.05	3.89
28	0.90	1.15	0.85	1.08	7.32	6.66	8.66	7.26	2.29	2.24	2.97	2.34	1.36	2.09	2.39	1.71
29	1.53	3.38	1.27	2.13	38.67	31.13	11.67	14.76	3.90	4.06	3.01	2.86	1.70	2.63	1.77	1.91
30	2.51	2.98	3.26	2.18	24.52	27.97	28.16	19.28	2.02	1.38	1.81	1.43	1.49	2.20	2.09	1.61
31	0.70	1.39	0.77	1.29	8.83	7.63	7.47	7.97	3.20	1.83	2.20	2.27	1.87	1.97	2.37	2.51

(JN)1865 (KL)150 (SW)Wilson

Table 4. Geometric means of pollutant body burden for the metals copper, zinc and mercury for each bay group and each year of the study. Values are given in parts per million (ppm)

	Pollutant body burden											
Bay	Copper				Zinc				Mercury			
group	1986	1987	1988	1989	1986	1987	1988	1989	1986	1987	1988	1989
1	120.00	120.33	169.84	130.08	1633.33	1257.67	4945.86	1621.72	130.00	196.67	100.14	184.16
2	110.55	162.33	191.61	111.18	3343.98	3260.00	4346.31	4157.87	79.86	22.00	136.25	122.36
3	172.61	98.45	197.68	113.84	1211.01	724.60	1304.14	1286.43	105.69	54.11	142.06	104.43
5	116.01	106.22	162.32	233.00	1137.03	941.69	1382.09	1700.61	173.69	106.27	148.58	223.72
6	190.00	127.67	214.00	238.39	883.33	3127.00	1072.67	1292.25	67.67	77.33	69.00	78.57
8	121.57	161.71	156.76	193.06	2186.77	2544.19	3186.98	3438.99	56.93	92.16	47.31	90.61
9	90.00	493.67	272.33	220.52	8000.00	5989.00	4146.67	3370.99	143.33	146.67	108.33	98.29
10	183.33	180.00	276.58	185.80	2600.00	1899.33	3093.52	2345.64	111.67	89.33	98.20	96.19
11	173.33	182.67	170.00	192.22	1200.00	1324.33	1625.67	2129.86	47.67	50.33	70.00	92.56
12	353.33	509.33	248.67	611.67	2300.00	2896.67	1435.33	4089.20	39.00	77.67	38.33	61.51
13	105.28	186.84	163.18	148.00	1264.91	2353.74	2143.93	1783.13	32.44	46.47	45.34	55.75
14	63.89	75.52	114.35	86.43	1568.44	1578.96	2104.39	2388.97	43.72	77.81	87.49	77.66
15	39.28	86.93	134.88	71.44	916.67	2097.02	2593.78	2625.89	43.72	77.81	87.49	77.66
18	92.49	105.32	95.16	67.39	1052.78	1491.33	1205.69	993.89	32.79	108.78	52.28	64.36
19	293.33	116.00	281.99	228.19	3400.00	1285.00	3433.15	3169.99	33.33	153.33	124.37	89.47
20	128.52	146.79	172.63	208.14	3215.66	3229.64	2762.19	4555.68	130.52	136.36	113.76	156.95
21	100.00	59.33	133.86	132.51	916.67	956.00	1891.42	1957.27	70.00	67.67	73.71	61.09
22	75.00	43.33	65.60	53.19	2133.33	470.00	1572.89	1356.49	243.33	84.33	119.58	121.23
23	77.28	54.89	99.86	114.03	2178.69	1983.16	3331.22	2170.02	256.48	296.37	233.33	267.79
24	416.67	279.67	210.71	139.86	5400.00	3316.00	3150.72	3657.02	71.67	43.00	71.40	132.34
25	54.21	41.16	49.67	71.32	530.72	333.13	356.36	529.04	117.36	100.43	74.09	113.84
27	14.67	20.33	18.07	38.64	300.00	483.00	916.73	488.35	106.67	138.00	139.02	91.03
28	68.06	52.83	67.33	74.37	1666.15	1211.38	1859.14	2026.37	230.97	196.97	239.87	213.37
29	85.00	153.00	103.36	153.71	1300.00	1679.67	1706.11	2695.68	313.33	296.67	188.36	234.87
30	149.40	237.02	202.52	189.99	1302.35	1827.04	2089.41	1963.47	187.08	164.92	137.15	151.44
31	44.67	40.33	48.33	69.88	933.33	864.67	1167.67	1133.40	246.67	190.00	155.00	181.52

Table 5. Geometric means of pollutant body burdens for total polynuclear aromatic hydrocarbons (PAH). Values are given for each bay group and each year of the study in parts per billion (ppb)

Bay group	PAH body burden			
	1986	1987	1988	1989
1	24.50	20.00	314.22	48.00
2	57.89	417.00	969.89	408.85
3	52.54	24.10	106.16	29.67
5	41.25	79.48	93.97	75.12
6	90.67	64.67	29.67	202.67
8	248.19	233.97	275.68	319.59
9	219.33	58.33	171.67	172.67
10	385.33	63.33	252.46	364.41
11	41.33	162.00	28.00	82.67
12	68.67	88.67	51.67	30.00
13	153.33	61.65	57.04	81.99
14	213.92	29.77	354.18	204.29
15	257.28	241.21	147.85	2670.34
18	203.63	65.41	263.38	131.48
19	74.00	34.67	89.63	101.50
20	471.57	687.38	468.32	453.26
21	72.67	333.33	535.73	214.97
22	435.67	187.67	313.99	139.03
23	298.80	940.56	1121.29	398.74
24	13277.67	2709.67	2533.99	961.36
25	52.02	22.33	1334.12	1025.00
27	38.33	44.33	380.11	72.50
28	144.93	72.73	111.36	176.94
29	20.67	201.67	84.85	509.35
30	100.18	51.77	68.90	134.98
31	98.00	20.00	196.67	108.00

Spatial Distribution

The spatial distribution of each contaminant was examined using a spatial autocorrelation method described by Cliff & Ord (1973). We used Moran's I as the test statistic, where:

$$I = (n/W) \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} z_i z_j}{\sum_{i=1}^{n} z_i^2}$$

and

$$W = \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij}; \; z = x_i - \bar{x};$$

$i \neq j$

n = number of samples, x_i = value of each sample and w_{ij} = a weighting measure as described below.

Moran's I is sensitive to the location of extreme departures from the mean $(x_i - \bar{x})$. For example, in a patchy distribution, adjacent samples would both tend to be much above or below the mean more frequently than would be expected by chance. Significance levels were calculated after Jumars et al. (1977) under the assumption of randomization. Cliff & Ord (1973) showed, for samples that are spatially randomly distributed, that the expected value of I is $-(n-1)^{-1}$. Hence, values of I below $-(n-1)^{-1}$ indicate negative spatial autocorrelation (an even distribution) and values above $-(n-1)^{-1}$ positive spatial autocorrelation (a patchy distribution).

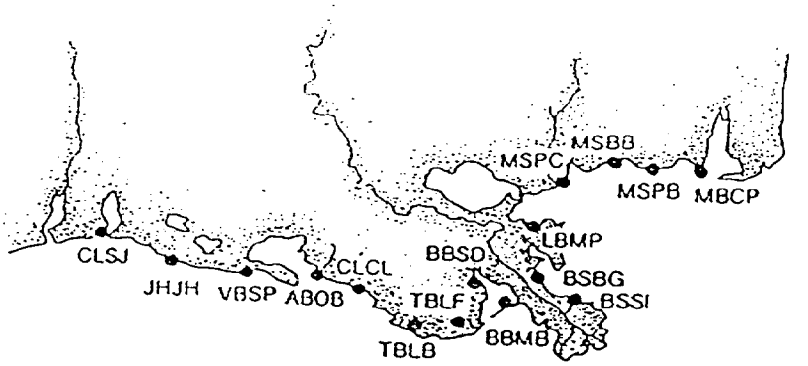
The use of this technique depends upon the choice of a weighting system (w_{ij}) which is a mathematical expression of the spatial relationship between the sampled sites. Factors involved in the choice of a weighting system were discussed by Jumars et al. (1977), Sokal & Oden (1978a) and Cliff & Ord (1973). We constructed a Gabriel-connected graph (Gabriel & Sokal, 1969) for the bays sampled in all 4 years. In this case, two sites (AB) were connected if no third site (C) existed that formed an obtuse angle when connected between the other two (< ACB). Gabriel-connected pairs were given a weight (w_{ij}) of 1.0 and all other pairs $w_{ij} = 0$ (Fig. 1).

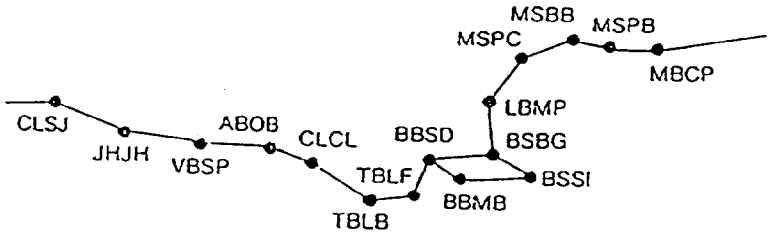
The change in spatial relationship among samples at varying distances can be used to identify the scale of spatial variation. For example, in a patchy population, samples closer than patch size will be more similar than expected by chance (e.g. Moran's $I > -(n-1)^{-1}$), whereas samples further apart than patch size will be less similar (e.g. Moran's $I < -(n-1)^{-1}$). We examined the change in spatial relationship with distance using correlograms (plots of sample similarity versus distance between samples) calculated as discussed by Sokal & Oden (1978a, b). Distances were calculated along the Gabriel network by Marble's method (1967). Bays within a given distance from one another when joined along the Gabriel network were given $w_{ij} = 1.0$; for all others $w_{ij} = 0$. Therefore, our correlograms were distance-corrected using the terminology of Sokal & Oden (1978a).

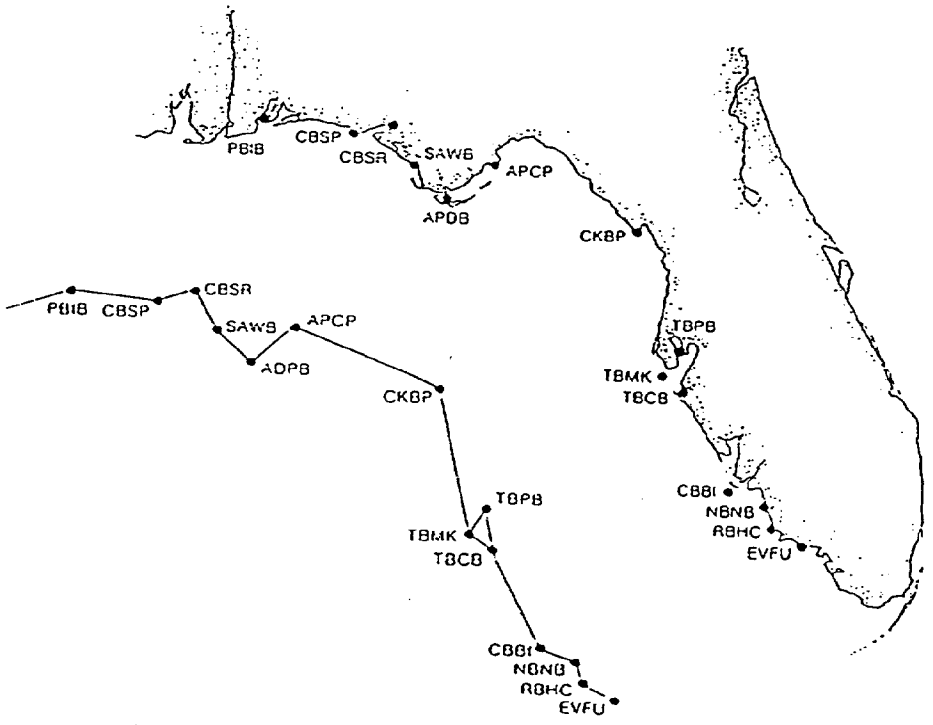
Temporal changes in spatial distribution

To examine the spatial scale of yearly changes in the biological variables and contaminant body burdens and to determine whether concordant changes occurred among several variables, we used the analytical approach of Powell et al. (1984) as adapted by Powell et al. (in press). First, we ranked each of the 4 years for each bay group from 1 (highest) to 4 (lowest) for prevalence and mean infection intensity of *P. marinus*, length, condition index and each of the eight contaminants. Two bays or two parameters were compared by subtracting each year's rank for one from the corresponding rank for the other and summing the absolute value of the 4 differences. As an example, if the data for bay group 1 were ranked 1, 2, 3, and 4 for the 4 years and the data for bay group 2 were ranked 1, 3, 2, and 4, then the differences would be 0, −1, 1, 0 and the absolute value of the sum would be 2. The values of the sums obtained in this way can only take the values 0, 2, 4, 6, and 8. The frequency spectrum of occurrences of the possible sums between site pairs having randomly distributed ranks is 0 (.042), 2 (.125), 4 (.292), 6 (.375) and 8 (.167). A frequency spectrum of sums calculated from the data in this way was compared to the frequency expected by chance combinations of the rankings using Kolmogorov-Smirnow (K-S) one-sample two-sided tests. Significance of the K-S statistic was judged using Conover's (1972) method for calculating exact P-values for discrete

(JN)1853 (KL)150 (SW)Wilson


MSBB
MSPC
MSPB
MBCP
LBMP
BBSD
BSBG
BSSI
CLSJ
JHJH
VBSP
ABOB
CLCL
TBLF
BBMB
TBLB


MSBB
MSPB
MSPC
MBCP
LBMP
CLSJ
ABOB
CLCL
BBSD
BSBG
JHJH
VBSP
TBLF
BSSI
BBMB
TBLB


PBIB
CBSP
CBSR
SAWB
APCP
APDB
CKBP
TBPB
TBMK
TBCB
CBBI
NBNB
RBHC
EVFU

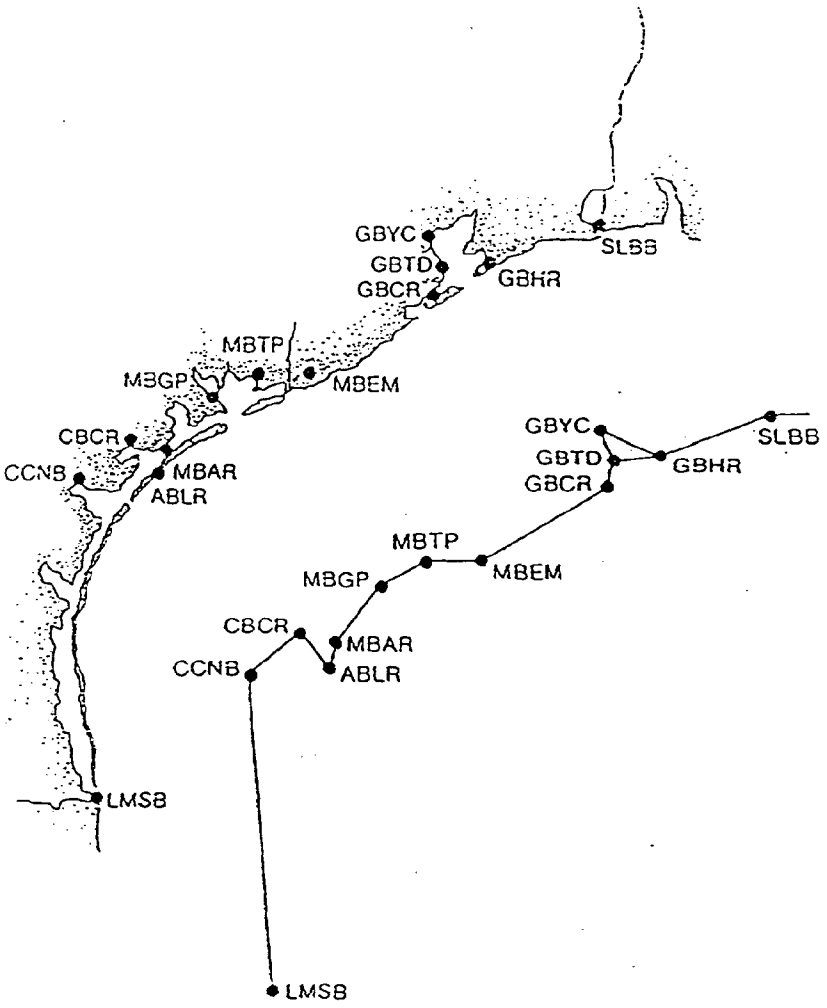


Fig. 1. Distance-corrected Gabriel graphs for the sites sampled around the Gulf of Mexico in each of the 4 years of the Status and Trends Program. Four-letter site designations correspond with those in Table 1. Dots indicate site locations. Graph is drawn opposite for clarity. See Powell et al. (in press) for more details

data. In this analysis, if the year-to-year change in any variable between bay systems tended to co-vary, most going up or most going down, then values of 0 or 2 in the previous example would occur more frequently than expected by chance. If the yearly changes tended to oppose one another (for example some bays going up, the others down) then values of 8 would be frequently obtained.

We utilized the preceeding approach on two geographical scales, the entire Gulf of Mexico (all bay systems) and sets of ten contiguous bay systems, contiguous being defined along the Gabriel graph. In the latter case, 10 bay systems were chosen because the number of bay systems in Texas and extreme western Louisiana, as defined here, was

10. To determine the extent of regional similarity, sets of nine bay systems were examined step by step around the Gulf of Mexico in the following manner. Step one always compared bays from the Laguna Madre in south Texas through Vermillion Bay in western Louisiana. Step 2 was generated by deleting the most southern bay system (Laguna Madre) and adding the next bay system to the east (Atchafalaya Bay and Caillou Lake). Consecutive steps followed the same protocol with one exception. Steps originating on the eastern Gulf coast were allowed to wrap around the Gulf. For example, step 22 compared the five southernmost Florida bay systems (Cedar Key to the Everglades) and the 5 southernmost bay systems in Texas (Laguna Madre through East Matagorda Bay). We examined all possible pair-wise combinations within the set of 10 bay systems. This generated 45 sums. The frequency of these sums was compared against the expected frequency of sums using K-S tests as previously described. A non-significant value for the K-S statistic among the 10 bay systems making up one step indicates local control of the temporal variation in the variable (e.g. pollutant body burden, disease). In other words, coincident oscillations (two bay systems simultaneously going up or down in value) from one year to the next did not occur among the 10 bay systems more frequently than expected by chance over the spatial scale encompassing the 10 bay systems. This result would suggest no regional imprint on local control of variability. Similarly, a significant value of the K-S statistic would suggest that regional influences overrode local controls so that temporal variation, in contaminant body burden or disease for example, was substantially affected by climatic, as well as local, factors. Plots of the K-S statistic as a function of steps around the Gulf give a graphical representation of the spatial extent of this similarity.

Within those geographic regions where yearly changes in pollutant body burden were concordant using the K-S test, R^2 improvement tests were conducted to determine the factors that might have produced the observed concordancy. Factors tested in most models included length, condition index, mean temperature, mean precipitation, *P. marinus* prevalence and infection intensity, and agricultural and industrial land use. The parameters that produced the best R^2 model were then used in regression analysis, again only in regions of yearly concordancy as defined by consecutive significant results of the K-S test. Not knowing the response times for pollutant body burdens to shifts in environmental regimes in most cases, analyses were conducted using the average precipitation and temperature values for the 5 months prior to sampling and the 2 months prior to sampling. Prevalence and infection intensity of *P. marinus* were used in R^2-improvement and regression analyses only with the average precipitation and temperature data for the 2 months prior to sampling because *P. marinus* responds so rapidly to changes in the environmental regime.

RESULTS

Spatial distribution, Gulf-wide

Correlograms calculated along the Gabriel network for the distribution of contaminant body burden with distance around the Gulf of Mexico are given in Figures 2 to 5. The contaminants can be divided into two distinct groups. For mercury, selenium, arsenic and cadmium, site-to-site similarity gradually declines with distance over the first approxi-

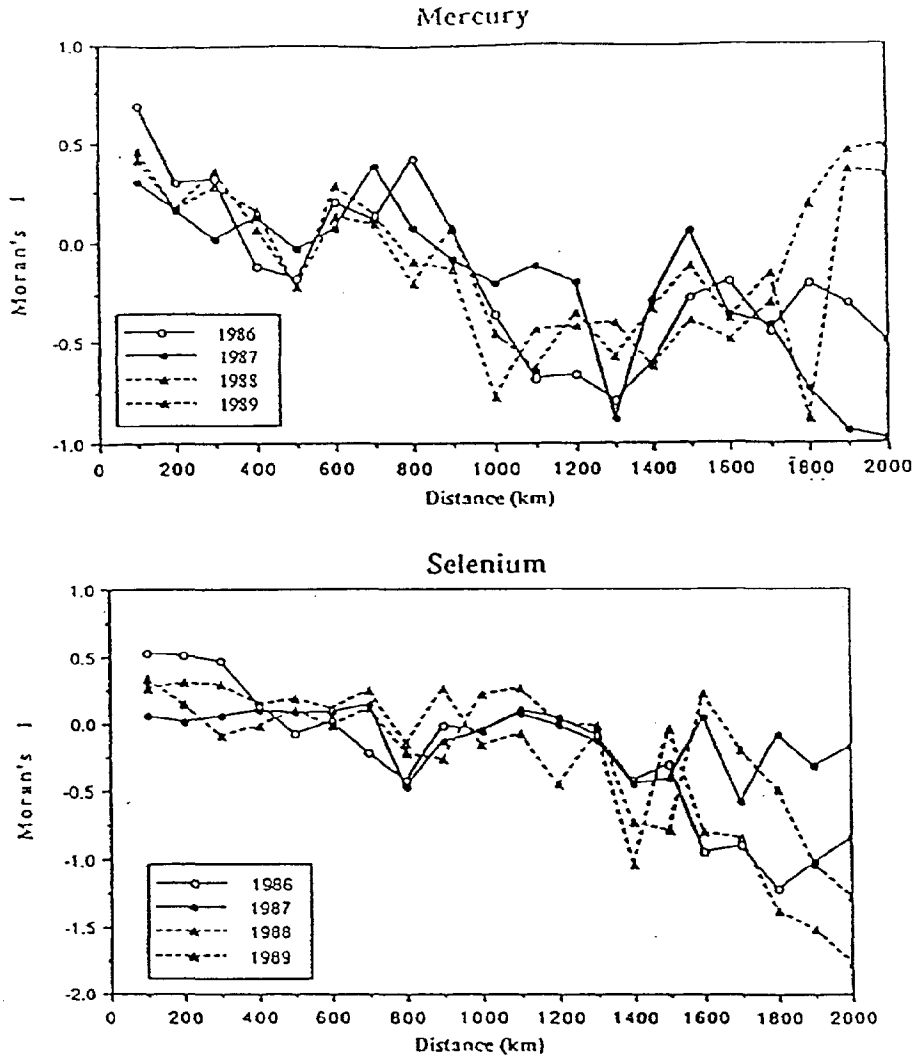


Fig. 2. Correlograms relating distance (km) to Moran's I obtained using body burden of mercury and selenium for all sites sampled in each year. Distances were calculated along the Gabriel network, where stations separated by, for example, 101 and 200 km were used to generate the 200-km point. The ideal random value for Moran's I is approximately -0.04

mately 1600 km in each of the 4 years. The correlograms pass through $I = -(n-1)^{-1}$ at about 400 km. That is, bay groups less than 400 km apart are more similar in body burden of these metals than expected by chance and sites become less and less similar at larger and larger spatial scales. Another characteristic of the distribution of group 1 contaminants is the close association between the first 2 years (1986 and 1987) and the last 2 years (1988 and 1989) at the longest spatial scales. Body burden of oysters from the east and west coasts of the Gulf varied similarly among the bays within both pairs of years and

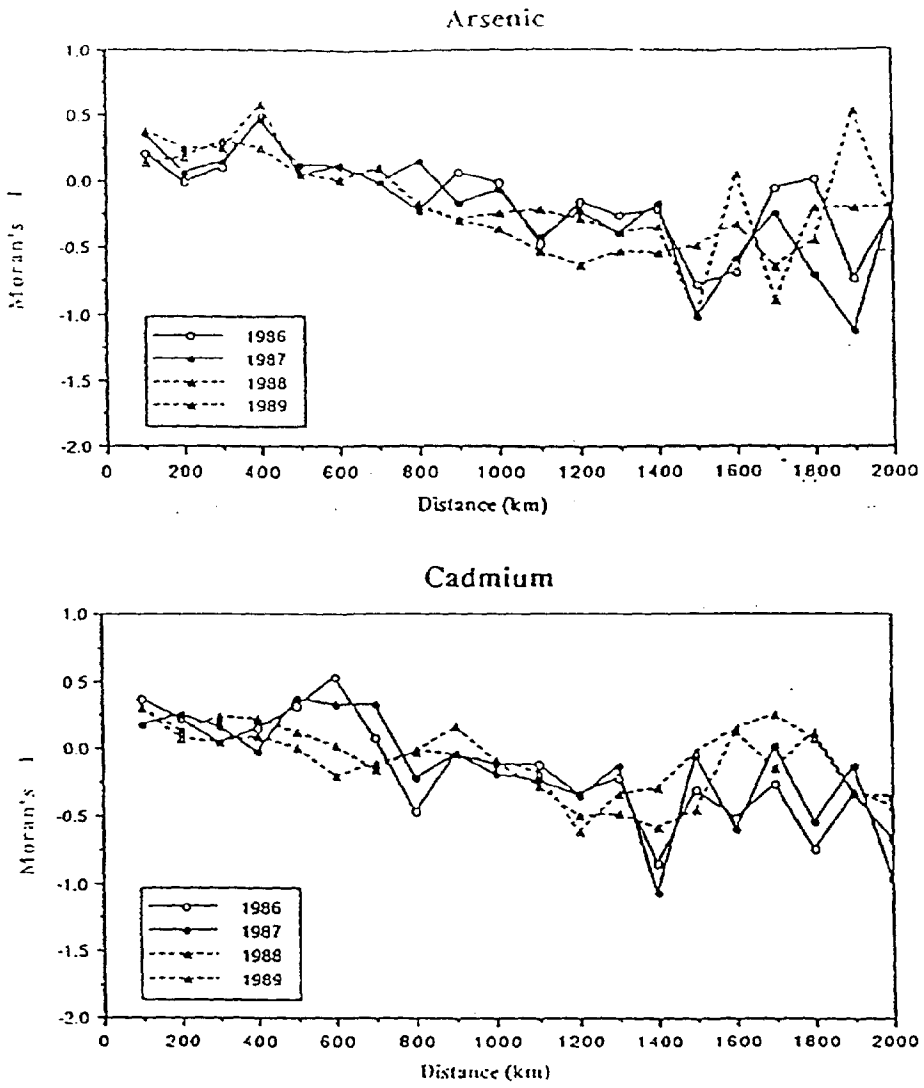


Fig. 1. Correlograms relating distance (km) to Moran's I obtained using body burden of arsenic and cadmium for all sites sampled in each year. Distances were calculated along the Gabriel network, where stations separated by, for example, 101 and 200 km were used to generate the 200-km point. The ideal random value for Moran's I is approximately −0.04

were affected similarly by some large-scale event which occurred between 1987 and 1988.

In contrast to group 1 contaminants, the distribution of group 2 contaminants, copper, zinc, silver and total PAHs, shows no overall trend; rather the pattern tends to oscillate about the ideal random value at most spatial scales for all 4 years. This pattern also does not clearly show the distinct break between 1986–1987 and 1988–1989.

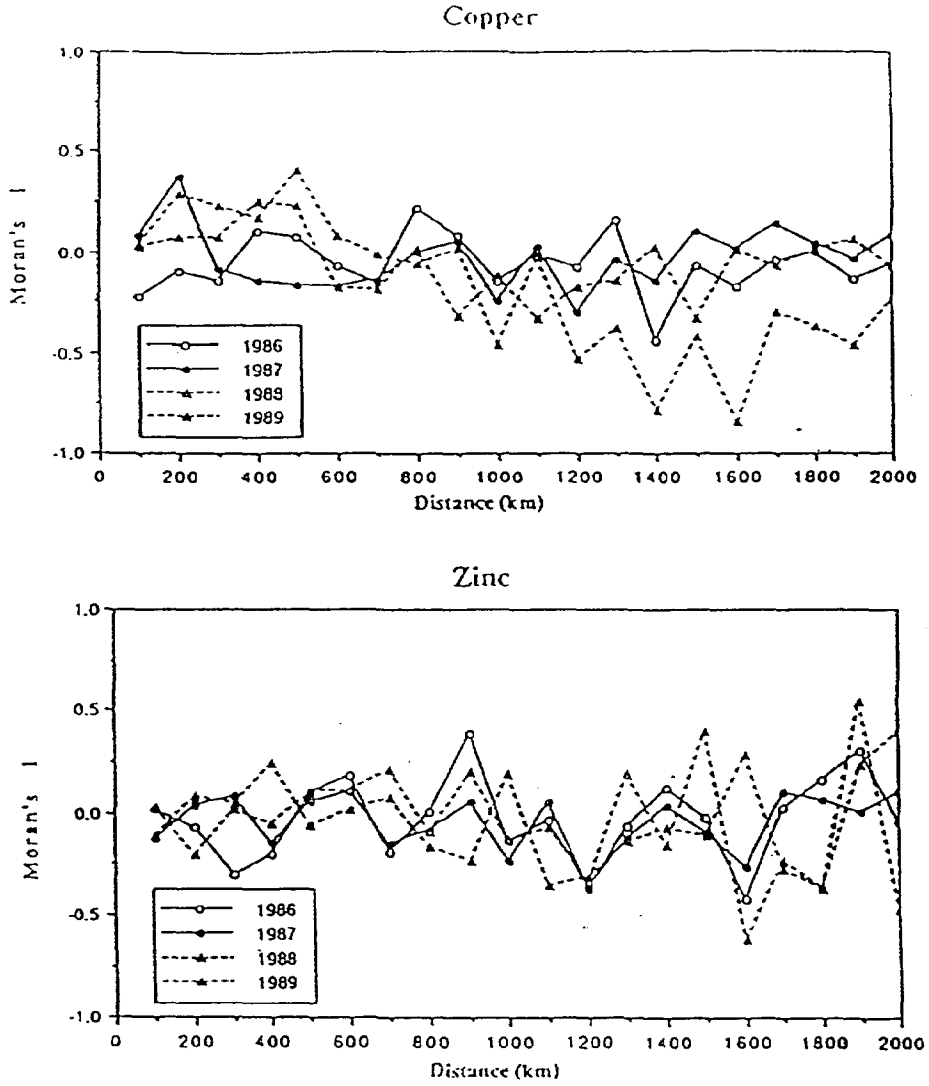


Fig. 4. Correlograms relating distance (km) to Moran's I obtained using body burden of copper and zinc for all sites sampled in each year. Distances were calculated along the Gabriel network, where stations separated by, for example, 101 and 200 km were used to generate the 200-km point. The ideal random value for Moran's I is approximately −0.04

Perkinsus marinus is an important pathogen in oyster populations in the Gulf of Mexico, and is responsible for high mortality in most years (Hofstetter, 1977). Correlograms for mean prevalence and mean infection intensity of *P. marinus* for each of the 4 years are given in Powell et al. (in press). Overall, the spatial distribution of *P. marinus* prevalence and infection intensity, while not identical, retains many of the spatial characteristics of group 1 contaminants. Correlograms for *P. marinus* prevalence and

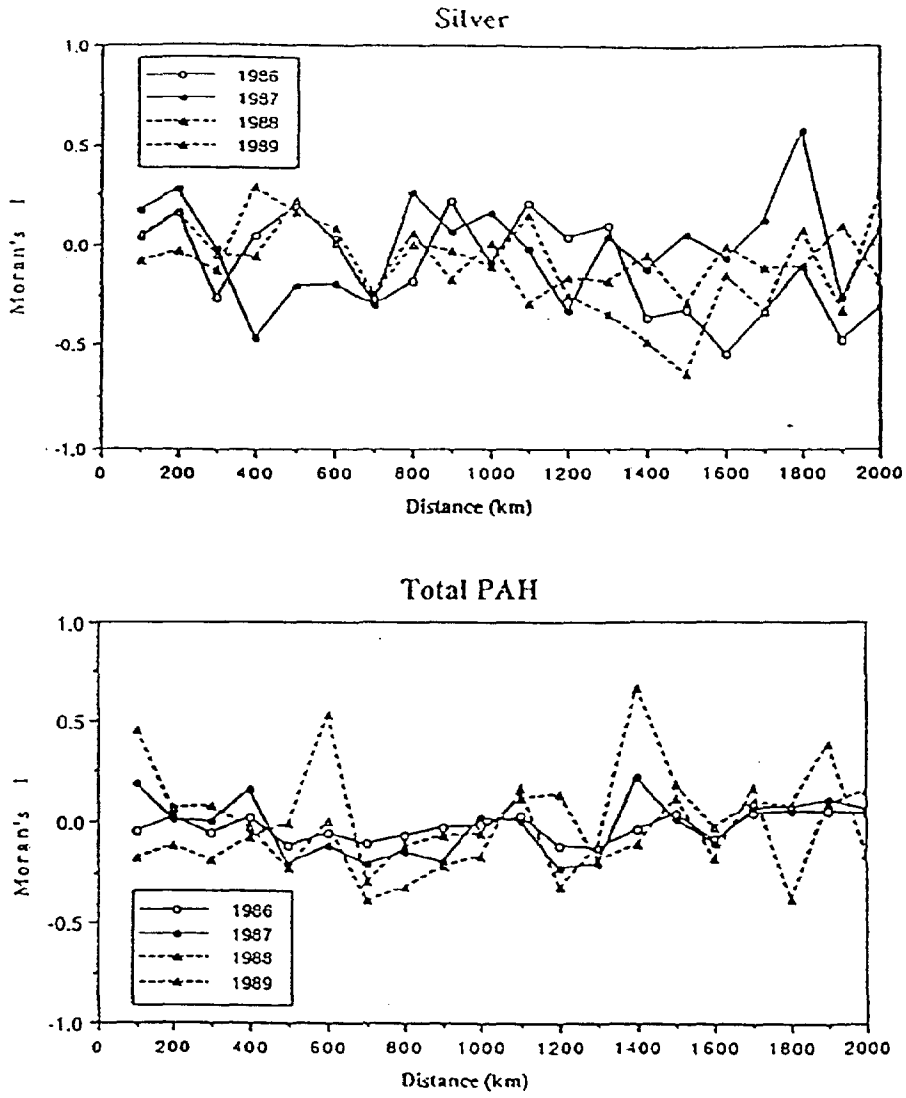


Fig. 5. Correlograms relating distance (km) to Moran's I obtained using body burden of silver and total PAH for all sites sampled in each year. Distances were calculated along the Gabriel network, where stations separated by, for example, 101 and 200 km were used to generate the 200-km point. The ideal random value for Moran's I is approximately −0.04

infection intensity demonstrate a very strong relationship between year pairs 1986/87 and 1988/89. Again, the break between 1987 and 1988 results in two very different distributional patterns at certain spatial scales. Similarity declines over the first approximately 1400 km, although more rapidly than it does for the contaminants, and returns again at longer spatial scales.

(JN)1867 (KL)150 (SW)Wilson

Temporal changes in spatial distribution

Utilizing first the spatial scale of the Gulf of Mexico (~2000 km along the Gabriel network), few contaminants had significantly concordant shifts (Table 6). For selenium, and to a lesser extent zinc, copper and arsenic, however, many more bay systems in the Gulf tended to vary similarly year-to-year than would be expected by chance. That is, the tissue concentration of these contaminants tended to increase or decrease uniformly from

Table 6. Results of analyses to detect concordant temporal shifts among all 26 bay systems in the Gulf of Mexico. A significant result indicates that temporal shifts of the measured variable were of the same sign (values increasing or decreasing) in most of the bay systems around the Gulf

Parameter	KS Statistic	P Value
Silver	0.11218	0.3833
Arsenic	0.17949	0.0840
Cadmium	0.11859	0.3744
Copper	0.19551	0.0601
Mercury	0.11859	0.3744
Selenium	0.50321	6.0×10^{-8}
Zinc	0.21795	0.0313
Total PAH	0.05128	1.00
Condition index	0.38782	5.9×10^{-5}
Length	0.34936	3.4×10^{-4}
P. marinus mean infection	0.48718	6.0×10^{-8}
P. marinus mean prevalence	0.21795	0.0313

one year to the next in all or a significant portion of the bay systems. Such coincident shifts in body burden would indicate some regional or Gulf-wide control on body burden. Among the biological indices, condition index, length, *Perkinsus marinus* prevalence and *P. marinus* infection intensity all were characterized by nearly Gulf-wide coincident oscillations in yearly values.

Meteorological data (Trenberth et al., 1988; Ropelewski & Halpert, 1986; Douglas & Englehart, 1981) suggest that the eastern and southern Gulf are dissimilar from the western Gulf. Powell et al. (in press) found that *P. marinus* prevalence followed this trend. Consequently, substantial geographic areas of similarity might go unrecognized at a spatial scale encompassing the entire Gulf of Mexico. Accordingly, we also looked at groups of 10 bay systems covering approximately 600 km of coastline (range 500–900 km, excepting those that "wrap around" the Gulf, thereby including the eastern and western portions of the southern Gulf).

Average length of the oysters sampled tended to decrease in each year throughout the study. The largest oysters were always preferentially sampled at each site. The decrease in length could represent the depletion of the largest individuals over time due to this sampling strategy; however, the decrease occurred in fished and unfished populations and, in many areas, most collected oysters were no more than 2 years old. Accordingly, collections the previous year would not have sampled the same cohort. Trends in size, then, are probably a natural phenomenon. Yearly trends in length are coincident over most of the Gulf, except the Galveston Bay/Sabine Lake area of Texas.

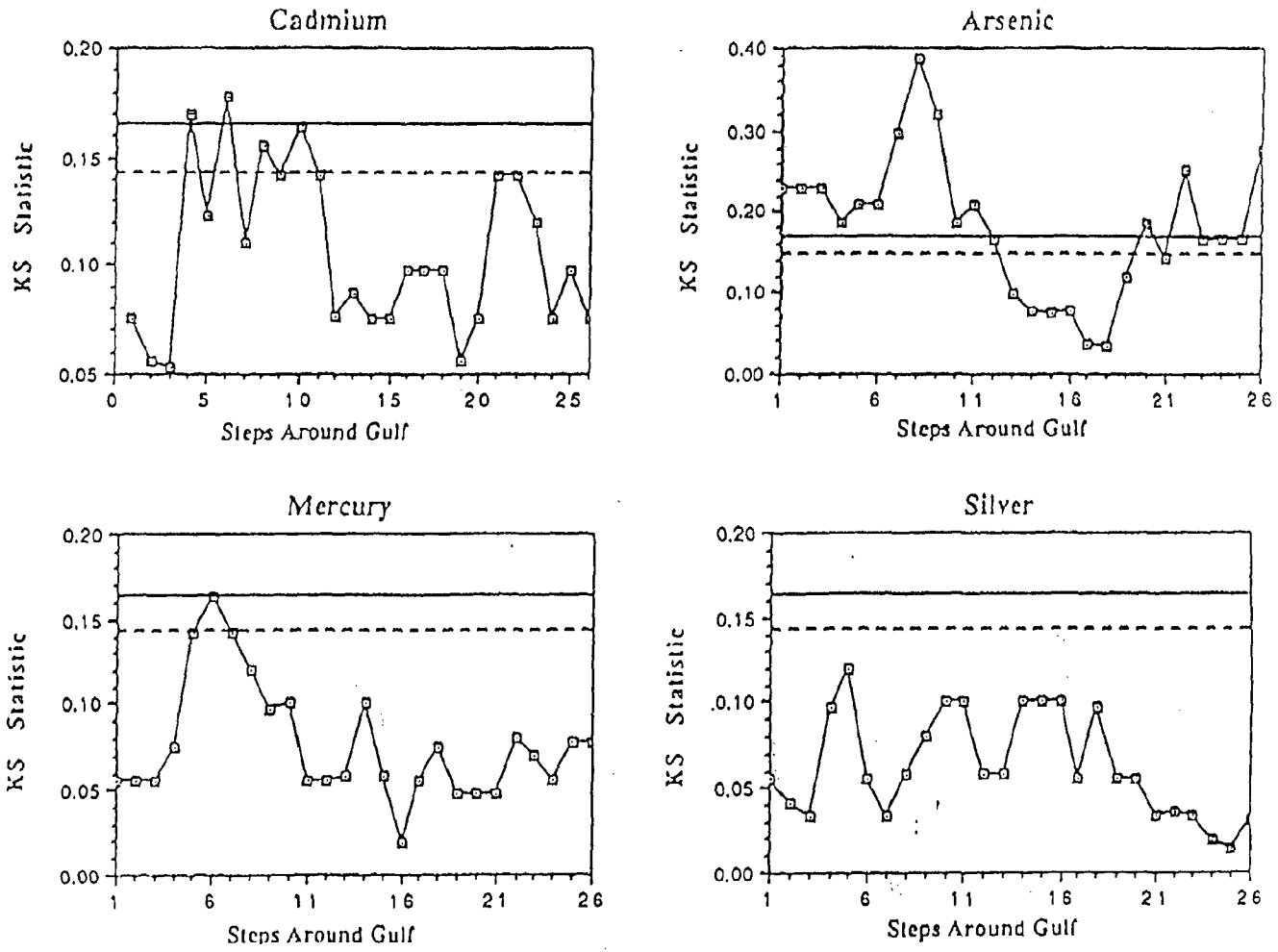


Fig. 6. Graphical representation of results of the Kolmogorov-Smirnov test for arsenic, silver, cadmium, and mercury using all bay pairs in each group of 10 bay systems (one step). The two lines indicate the $\alpha = 0.05$ (solid) and 0.10 (dashed) significance levels for an n of 45 (number of site pairs used)

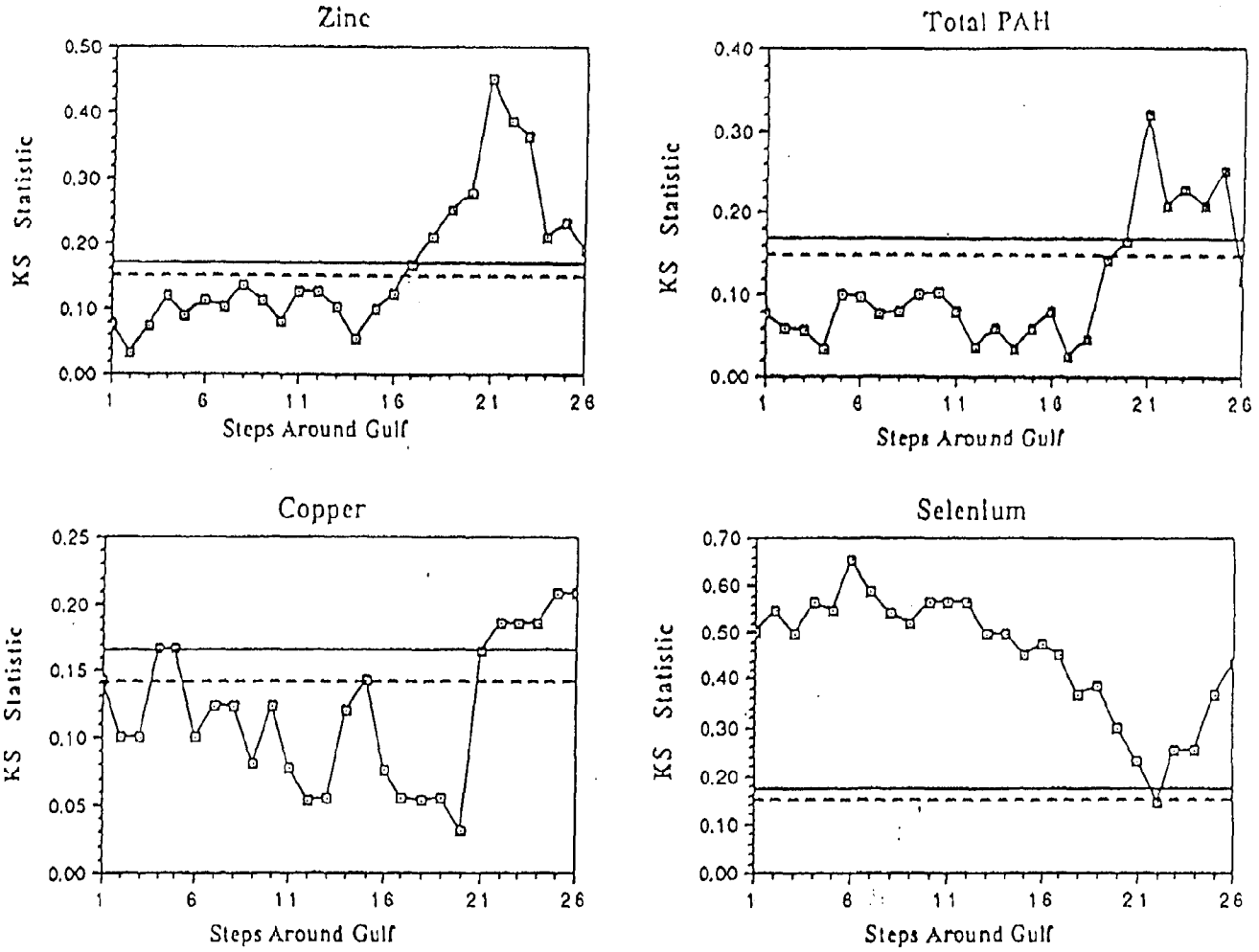


Fig. 7. Graphical representation of results of the Kolmogorov-Smirnov test for selenium, copper, zinc, and PAH using all bay pairs in each group of 10 bay systems (one step). The two lines indicate the $\alpha = 0.05$ (solid) and 0.10 (dashed) significance levels for an n of 45 (number of site pairs used)

length increasing or decreasing from one year to the next coincidentally in most bays within a contiguous group of 10 (Figure 8). Of particular note are the highly significant concordancies in the eastern and southern Gulf of Mexico. Yearly trends in length in southern Texas and southern Florida were nearly identical, small oysters being collected in certain years and large oysters in other years.

Low values of condition index typically indicate a stressed or unhealthy population. Condition index also varies with the reproductive cycle. In 1989, 3 of the 26 bay systems had condition indices greater than 0.1, while 1986 had 15, 1987 24 and 1988 21. The two years with lower mean prevalences of *P. marinus* had higher average condition indices, as might be expected. Similar year-to-year variations in condition index occurred throughout the Gulf of Mexico (Figure 8). As the time of sampling of the populations was similar in all cases, except the Louisiana bays in 1986 (Craig et al., 1989; Wilson et al., 1990), this trend indicates that a Gulf-wide variation in climatic conditions probably controls condition index in Gulf oysters.

Similar year-to-year variations in prevalence of *P. marinus* occurred throughout the Gulf with the exception of the central-northern region represented by bays on both sides of the Mississippi River (Figure 8). Powell et al. (in press) noted that the Mississippi River represents an important boundary in *P. marinus* infection. The only uninfected populations in the Gulf of Mexico are regularly found on the Mississippi delta. Concordant year-to-year variations in mean infection intensity of *P. marinus* occurred throughout the Gulf of Mexico, as was the case for condition index and nearly so for length, suggesting a similar relationship with climatic variables, if not a causal process. *P. marinus* infection intensity could be a controlling factor in both length and condition index. Again, in both prevalence and infection intensity, the similarity in yearly trends on both sides of the southern Gulf is noteworthy.

The pollutants divide into 3 groups based on their temporal variations. (1) Like condition index, length and *P. marinus* infection intensity, year-to-year variations in selenium coincided throughout the Gulf. The similarity between selenium and condition index is particularly noteworthy. Year-to-year variations in arsenic were only slightly lower than selenium in their regional scale of concordancy; concordancy occurred over much of the eastern and western Gulf, only failing to encompass the Louisiana region.

(2) Mercury and cadmium varied similarly in the western Gulf, but not in the eastern Gulf. The degree of concordancy was low; large-scale control of body burden occurred only in the Texas region. This pattern, then, is different from all biological parameters.

(3) Year-to-year variations in copper, zinc, and PAH body burden were concordant in the eastern and southern Gulf, particularly Florida and southern Texas, but not in the northern and western Gulf, a trend exactly opposite of that noted for cadmium and mercury. The concordance of yearly variations in body burden on both sides of the southern Gulf similar to that noted previously for *P. marinus* prevalence and infection intensity and condition index. Again the region of concordancy begins in the Mississippi/Alabama area of the Gulf at a similar location as it does for *P. marinus*, suggesting a similar climatic control.

(4) Silver failed to show regional concordancy anywhere in the Gulf.

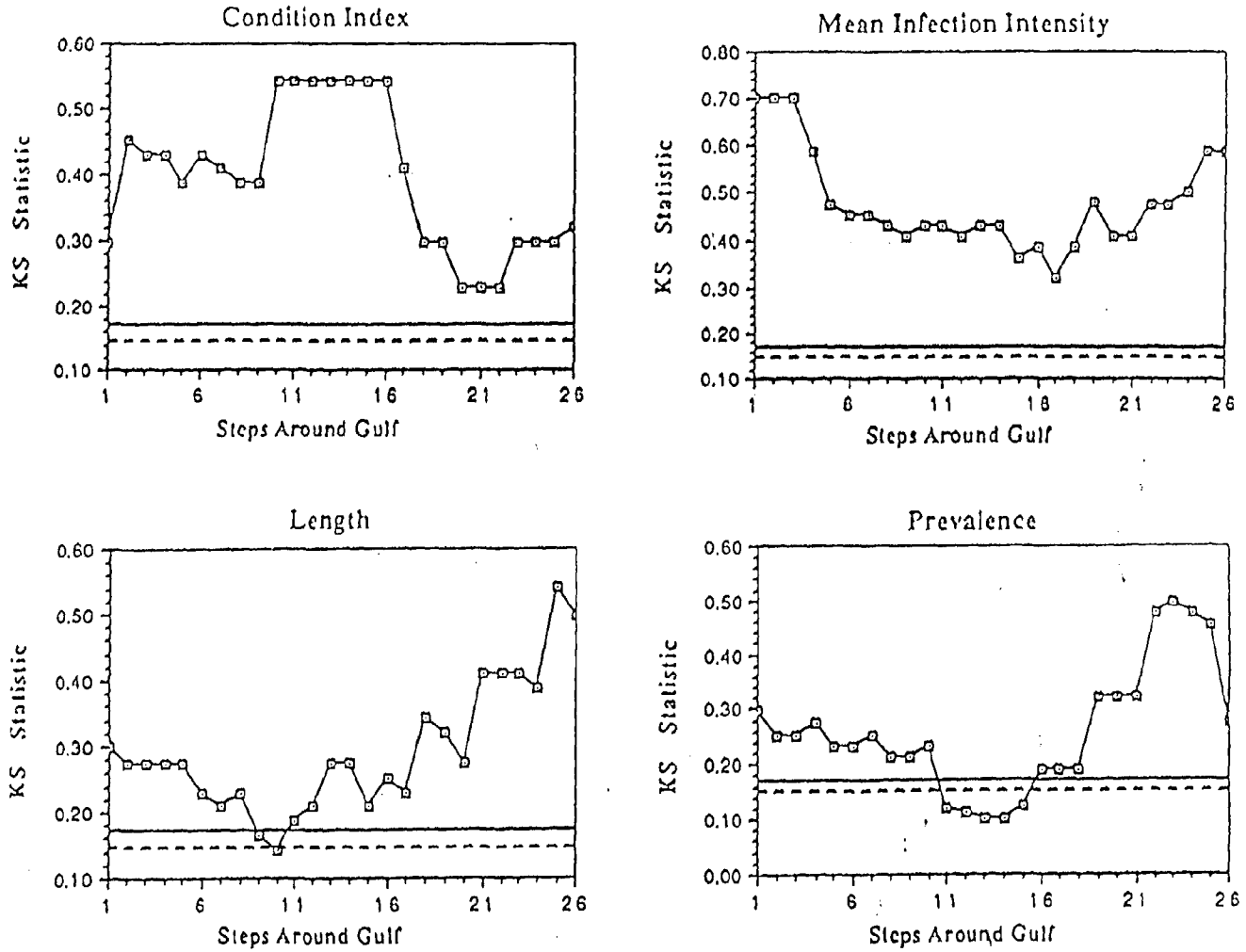


Fig. 8. Graphical representation of results of the Kolmogorov-Smirnov test for condition index, length and the mean infection intensity and prevalence of infection of *Perkinsus marinus* for all pairs in each group of 10. The two lines indicate the $\alpha = 0.05$ (solid) and 0.10 (dashed) significance levels for an n of 45 (number of site pairs used)

(JN)1855 (KL)150 (SW)

DISCUSSION

Explaining the temporal and spatial variation in contaminant body burdens is complicated because body burden may be affected by so many environmental and biological factors (Farrington et al., 1983). Oysters can incorporate metal and organic contaminants either through direct absorption from water or ingestion with food particles (Ehrhardt, 1972; Stegeman & Teal, 1973). Therefore, any factor which affects the bioavailability of pollutants such as changes in environmental condition, physiological condition or food supply may ultimately affect contaminant body burden (Farrington et al., 1983). Factors controlling these conditions are, in the extreme, of two kinds: local and large-scale. These, in the extreme, offer two opposing expectations. In the local case, temporal variations in body burden should never occur simultaneously in adjacent bays more frequently than expected by chance. In the large-scale case, we should expect coincidental variations within some large geographic region. It may be true that local variations outweigh large-scale factors in some regions and not in others, depending upon the strength of the two signals. El Niño, for example, affects the southern and eastern Gulf. A contaminant significantly affected by environmental conditions associated with El Niño might show coincidental changes in the eastern and southern Gulf while local variables controlled its temporal variability in the northwestern Gulf. Understanding the variation in contaminant body burden, then, requires investigating the effects of biological and environmental factors on both the local and larger geographic scales.

Temporal distribution and climatic control on variability

The biological attributes and contaminants can be placed into three groups based on the regional scale of their concordant temporal changes in the Gulf. For selenium and arsenic body burden, condition index, length, and prevalence and mean infection intensity of *Perkinsus marinus*, year-to-year variations are similar in nearly every bay around the Gulf. This implies controlling factors Gulf-wide or nearly Gulf-wide in scope. The geographic scale is largest for selenium, *P. marinus* infection intensity, condition index and length, but encompasses all save the Louisiana bays for arsenic and *P. marinus* prevalence. The second group, including mercury and cadmium, varies concordantly from southern Texas to approximately the Mississippi delta, suggestive of some large-scale phenomenon in the northwestern Gulf producing similar changes in body burden for these contaminants. The last group, including copper, zinc, and total PAHs, varies concordantly in southern Texas and southern Florida, suggesting a subtropical control on body burden for these contaminants.

Of particular note are the geographic boundaries of these three groups. The boundaries between the northwestern and the southern/eastern Gulf are clear; the vicinity of the Mississippi River delta and the Matagorda/Aransas Bay area of Texas. The break in similarity for arsenic and *P. marinus* prevalence in the Mississippi River region also marks the eastern extent of similarity for mercury and cadmium and the western extent for copper, zinc and PAHs. The western extent of similarity for mercury and cadmium, Matagorda Bay, approximates the northern extent of similarity for PAHs, zinc and copper.

These groupings of contaminants and biological attributes require three levels of

explanation. First, if only climatic factors are of sufficient scale to explain the concordances observed, what climatic factors are ultimately responsible? Second, why are certain groups of pollutants climatically controlled only in one part of the Gulf; do geochemical similarities, for example, explain these groupings? Third, what factors mediate the climatic control on body burden?

Climatic controls. Large-scale concordances in temporal change can only be explained by climatic factors; only these operate on an appropriate geographic scale. Choices for the climatic factors ultimately responsible are relatively limited. (Of course, our data do not permit us to verify what the ultimate causative factors are. A 4-year time series is inadequate for statistical treatment.) The concordant shifts in the eastern and southern Gulf suggest a tropical or subtropical control. The El Niño/Southern Oscillation phenomena is of appropriate scale and location. El Niño occurs in the Pacific, but affects temperature and rainfall in the Gulf of Mexico region by altering dominant weather patterns (Trenberth et al., 1988; Philander, 1989) and has been implicated in temporal variations in *P. marinus* prevalence and infection intensity. El Niño/La Niña events typically affect the Gulf from the panhandle of Florida through southern Florida and southern Texas, where concordancy for selenium, arsenic, copper, zinc, PAHs and most biological variables occur. A strong El Niño/La Niña shift occurred between 1987 and 1988 and contributed to the North American drought that summer (Philander, 1989). We noted that the year groups 1986/87 and 1988/89 tended to be statistically similar in many analyses, as did Powell et al. (in press). A second large-scale meteorological phenomenon, the Pacific North American Teleconnection (PAMT), controls the number and severity of winter storms in the northwest Gulf region (Wallace & Gutzler, 1981) where concordancy for mercury and cadmium, as well as selenium, arsenic, and most biological variables occurs. Combined, these two weather patterns could explain the geographic scale of concordancy observed in each of the contaminants and biological variables.

Why groupings exist. Any of the contaminants of biological attributes might respond to two scales of environmental change. Local changes, originating for example from the nearness of urbanized areas, the presence of certain contaminants in particular drainage basins and the extent of agricultural development, should produce discordance between adjacent bay systems. Galveston Bay, for example, might drain a large geographic area whereas an adjacent bay, East Matagorda Bay, may receive only local precipitation. Contrasting with this are large-scale climatic trends which affect weather patterns at least regionally. Changes in the precipitation regime during El Niño cycles are a good example. All watersheds in regional areas may be affected in the same way. Depending upon the competing strengths of local and climatic variability, biological attributes or contaminants might respond most strongly to one or the other. In our case, the body burdens of copper, zinc and PAHs would appear to be predominantly under local control in the northwestern Gulf and under climatic control in the eastern/southern Gulf. Mercury and cadmium have the opposite distinction. Selenium, arsenic and many of the biological attributes respond regionally in both areas. Silver seems generally to be under local control.

For the first two groups, the reason why local controls are relatively more important in one region than another is unclear, nor is it clear why cadmium and mercury behave similarly, as do copper, zinc and PAHs. To the extent that copper and zinc often behave similarly in bivalves (Phillips, 1976a, b; Phelps et al., 1985; Roesijadi & Klerks, 1989) and

quite differently from cadmium (Brooks & Rumsby, 1965; Boyden, 1974; Cheng, 1988a, b; but see Roesijadi et al., 1989). These data fit an expected scenario.

Mediating factors. We used R^2-improvement and regression analyses on the yearly rankings for those locations in the Gulf demonstrating concordant yearly shifts to examine what biological and climatic parameters might contribute most to the observed concordancy. Inasmuch as the biological parameters certainly were also influenced by climatic parameters, the set of independent variables were not in themselves completely independent; thus the analyses serve as a guide to the mediating factors responsible only in this context. Moreover, we expected factors to differ between the northwestern and the eastern and southern Gulf regions.

In determining which variables might affect *P. marinus*, we considered condition index, monthly mean temperature, monthly mean precipitation, length, cadmium, selenium, zinc, copper, PAHs, silver and mercury (Table 7). *P. marinus* prevalence was positively correlated with mercury body burden and negatively correlated with temperature and condition index in the western Gulf. Negative correlations existed for zinc, selenium, copper and cadmium and positive correlations for PAHs and arsenic in the eastern and southern Gulf (Table 7). *P. marinus* infection intensity responded negatively to selenium, condition index, and length and positively to copper in the western Gulf; condition index and selenium demonstrated negative correlations in the eastern and southern Gulf. These correlations demonstrate several important trends. (1) Biological variables were the most important correlates of the distribution of *P. marinus* in the western Gulf where concordance in contaminant body burden was least well developed;

Table 7. Results of regression analyses within regions of concordancy of yearly changes for *Perkinsus marinus* prevalence and infection intensity. Possible significant results represent the number of steps or groups of 10 bay systems tested individually. Number given indicates the number out of that possible number significant at $\alpha = 0.10$. N, a negative correlation; P, a positive correlation

P. marinus prevalence			
Western Gulf		Eastern/southern Gulf	
(10 possible)		(11 possible)	
Temperature	9 N	Arsenic	6 N
Condition index	9 N	Copper	4 P
Mercury	7 P	Cadmium	5 N
		PAH	4 P
		Zinc	6 N
		Selenium	8 N
P. marinus infection intensity			
Western Gulf		Eastern/southern Gulf	
(15 possible)		(10 possible)	
Copper	7 P	Condition index	10 N
Length	12 N	Selenium	5 N
Condition index	15 N		
Selenium	8 N		

(JN)1868 (KL)150 (SW)Wilson

contaminants were most important in the eastern and southern Gulf where the El Niño signal was strongest. (2) Most correlations were negative for biological and environmental variables and contaminants. Mercury, copper and PAHs were important exceptions. (3) The negative relationships with condition index and length are, perhaps, expected; that with temperature is a surprise as is the absence of an effect of precipitation. (4) The most consistent Gulf-wide signals were negative correlations with selenium body burden and condition index. Both of these responded concordantly throughout the Gulf as did *P. marinus* prevalence and infection intensity.

The parameters used for the analyses of the contaminants were length, condition index, *P. marinus* prevalence and mean infection intensity, and mean monthly temperature and mean monthly precipitation for the two months prior to sampling. Cadmium, mercury and arsenic varied concordantly in the northwestern Gulf (Table 8). Temperature (negative), length and condition index (positive) generally explained about 35 % of the variation for arsenic; temperature, length (negative) and mean infection intensity (positive) explained 25–35 % of the variation for cadmium. Mercury responded positively with temperature and *P. marinus* prevalence.

Copper, zinc, arsenic and PAHs generally varied concordantly in the eastern and southern Gulf. Temperature (negative), mean infection intensity (positive) and preva-

Table 8. Results of regression analyses within regions of concordancy of yearly changes in contaminant body burden. Possible significant results represent the number of steps or groups of 10 bay systems tested individually. Number given indicates the number out of that possible number significant at $\alpha = 0.10$. N, a negative correlation; P, a positive correlation

Arsenic (Western Gulf)		**Arsenic (Eastern/southern Gulf)**	
(8 possible)		(7 possible)	
Temperature	6 N	Precipitation	4 N
Condition index	5 P	Temperature	3 N
Mercury (Western Gulf)		**PAH (Eastern/southern Gulf)**	
(3 possible)		(6 possible)	
Temperature	3 P	Temperature	3 N
Precipitation	3 P	Length	3 N
Cadmium (Western Gulf)		**Copper (Eastern/southern Gulf)**	
(9 possible)		(6 possible)	
Perkinsus marinus intensity	4 N	*Perkinsus marinus* prevalence	3 N
Length	3 N	Length	6 N
		Condition index	2 N
Selenium (Entire Gulf)		**Zinc (Eastern/southern Gulf)**	
(26 possible)		(10 possible)	
Length	24 N	*Perkinsus marinus* prevalance	8 N
Condition index	15 N	Temperature	6 N
Temperature (only southern sites)	7 N	*Perkinsus marinus* intensity	6 P
Precipitation (only northern sites)	11 N		

(JN)1809 (KL)150 (SW)Wilson

hence (negative) explained 31 to 50% of the yearly variation in zinc. Prevalence (negative), condition index (negative) and length (negative) explained 35 to 55% of the variation in copper. For PAHs, temperature (negative) and length (negative) were most important. Temperature and precipitation were most important for arsenic.

Selenium body burden varied concordantly over most of the Gulf. In the northwestern Gulf, precipitation (negative), length (negative) and condition index (negative) explained 35 to 75% of the variation. In the eastern and southern Gulf, condition index (negative), length (negative) and temperature (negative) explained 25 to 45% of the variation.

Overall, then, a few trends were evident. (1) Regressions with condition index and length were generally negative; higher body burdens occurred in smaller oysters, which is a general phenomenon (Boyden, 1977; and others referenced previously). Lower condition index suggests that small size was not just indicative of young oysters, but in fact indicates oysters in poorer health (less biomass per length or mantle cavity volume). The relationship between *P. marinus* infection intensity and condition index corroborates this view. (2) Temperature was usually negatively correlated. The exception was mercury, where temperature was a positive factor. Although temperature might directly affect body burden, we would suggest that temperature probably controls the frequency of fall spawning and spawning generally results in lower pollutant body burdens (e.g. Frazier, 1975, 1976; Boyden & Phillips, 1981; Wilson et al., 1990), hence the higher body burdens at lower temperatures. (3) Precipitation was generally negatively correlated, suggesting higher salinities corresponded to higher body burdens, but precipitation was only important in selenium and arsenic. For the most part, temperature and precipitation were not themselves correlated, the exception being the north-central Gulf. (4) Arsenic is taken up primarily from food (Sanders, 1980; Sanders et al., 1989); accordingly, it is likely that the response of body burden to climatic factors was biologically mediated in at least this case. (5) *P. marinus* prevalence and mean infection intensity were important in copper, zinc, mercury and cadmium. Correlations were generally negative with prevalence but positive with infection intensity. Again, mercury was the exception. Lower prevalence would correspond with lower temperatures (Soniat & Gauthier, 1989). Prevalence includes many light infections which probably are meaningless with respect to body burden. High infection intensities, on the other hand, probably slow reproduction (White et al., 1988; Wilson et al., 1988; Wilson et al., 1990) and are likely responsible for the observed reductions in condition index and length.

Again, we emphasize the intimate relationship between *P. marinus* and the other biological and environmental variables; consequently, the analyses can only provide a rough estimate of the relative importance of these variables without the actual processes being more completely understood. Overall, the factors affecting the rate of tissue turnover, particularly the gametogenic cycle and general health, determined in part by the temperature regime and disease intensity, would seem to be of primary importance in determining yearly trends in contaminant body burden (see also Wilson et al., 1990). Generally, higher contaminant body burdens were found in populations characterized by lower health.

Spatial distribution and climatic control on variability

Gulf-wide trends. As with *P. marinus*, most characteristics of the spatial distribution of the pollutants were conservative features; they were repeated in each of the 4 years. A general clinal relationship might be expected to dominate the spatial distribution of contaminants; bays farther and farther apart being less and less similar in body burden. Temperature and precipitation show this clinal relationship (Fig. 9). The farther sites are apart, the less similar the local weather regimes are likely to be. Many geographical variables related to contaminant source availability probably do so as well. River inflow does not (Fig. 9).

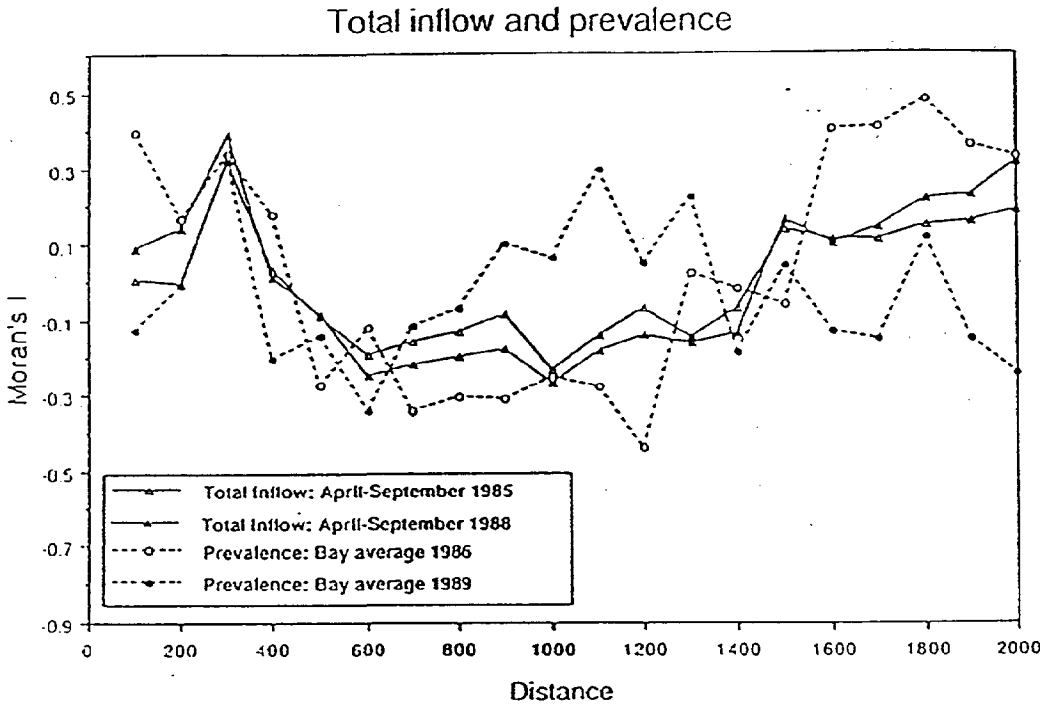


Fig. 9. Correlograms relating distance (km) to Moran's I obtained using temperature, precipitation and total freshwater inflow for all sites sampled in each year. Distances were calculated along the Gabriel network, where stations separated by, for example, 101 and 200 km were used to generate the 200-km point. See Powell et al. (in press) for more details

Arsenic, selenium, mercury and cadmium show gradually declining similarities with distance; the clinal variation predicted from the precipitation and temperature regime. The correlograms of copper, zinc, silver and PAHs do not; the spatial extent of regional similarity is of varying size throughout the Gulf so that no consistently significant spatial scale exists. Why these two groups differ can be related to the temporal trends previously described. From one year to the next, the body burden of selenium and arsenic varied concordantly throughout the Gulf; the body burden of cadmium and mercury was predominately affected by local factors throughout the Gulf. In both cases, the Gulf-wide

(JN)1857 (KL)150 (SW)Wilson

trends were sufficiently uniform that local factors of a clinal nature might successfully generate a strong spatial signal throughout the Gulf in any given year. In contrast, for those contaminants having a strong regional response in the year-to-year variability in the eastern Gulf, but which were locally-controlled in the western Gulf (copper, zinc and PAHs), fundamental differences in the controlling factors between the two regions probably prevented a general clinal relationship from being observed throughout the entire Gulf.

None of the correlograms mimic those of local agricultural or urban land use (Craig et al., 1989) or *P. marinus* prevalence and infection intensity (Powell et al., in press). It is tempting, therefore, to suggest that the precipitation and temperature regimes are important in controlling site-to-site trends in contaminant body burden over large geographic areas, whereas organism health modifies these bay-to-bay relationships on smaller regional scales. A correlation with latitude and the body burden of some contaminants does exist in Gulf coast oysters (Wilson et al., 1990). Temperature and freshwater inflow can change the supply of contaminants and therefore their bioavailability (Shuster & Pringle, 1969; Zaroogian & Cheer, 1976; Denton & Burdon-Jones, 1981). Cunningham & Tripp (1973, 1975b), Zaroogian & Cheer (1976), Zaroogian (1980), and Zaroogian & Hofmann (1982), for example, comment on the temperature dependence of body burdens in cadmium, mercury and arsenic either linked directly to temperature or a seasonal biological cycle, such as reproduction that correlates directly with temperature (Wilson et al., 1990), and Parizek et al. (1974) describe a relationship between selenium, cadmium and mercury. Several sources cite the co-occurrence of zinc, copper and silver, and that a common source for these metals is freshwater runoff (Windom & Smith, 1972; Frazier, 1975; Phillips, 1977b, c), as is also likely for PAHs (Wade et al., 1988). Body burdens of copper and zinc may also be related to salinity (Wright & Zamuda, 1987). Nevertheless, sufficient data is not now available to identify the primary controlling factors behind the large-scale distribution of contaminants in the Gulf.

Regional correlations. We examined the correlations between various environmental and biological variables and contaminant body burdens within the regions observed to have concordant yearly shifts in body burden; the reason being the expectation that the significant variables controlling body burden may be different in different areas of the Gulf and that the areas providing concordant temporal trends might offer some guidance in dividing the Gulf into regional areas.

Using the 5-month average for precipitation and temperature (no measures of *P. marinus* infection included) (Table 9) shows that precipitation and temperature are only significantly correlated with some contaminants and are only significant in 1986 and 1988. Whereas precipitation is always positively correlated, temperature is negatively correlated, indicating that high precipitation and low temperatures over long periods of time before sampling may influence body burden. Agricultural and industrial land use are also important for some contaminants. Including *P. marinus*, and necessarily using a shorter time scale (2 month) (Table 10), shows that *P. marinus* prevalence and mean infection intensity are often significantly correlated with contaminant body burdens within regions showing similar temporal responses to climatic variation.

Despite the seeming likelihood that large-scale trends in the Gulf must ultimately originate in Gulf-wide trends in temperature and freshwater inflow, few of the contaminants show consistent correlations with either temperature or precipitation on the regional

(JN)1870 (KL)150 (SW)Wilson

Table 9. Results of regression analyses within regions of similarity in pollutant body burden as determined using the K-S test. These results use the average of the 5 months prior to sampling for precipitation and temperature. * signifies a significant negative correlation. CI = Condition index; Industry refers to industrial land use; Agriculture refers to agricultural land use

Pollutant	1986	1987	1988	1989
Arsenic	....	....	Length P = 0.0099*	....
Cadmium	Precipitation P = 0.0457 Temperature P = 0.0167*	Industry P = 0.0473	Length P = 0.0057	....
Copper	....	....	....	....
Mercury	Industry P = 0.0441*	....	Industry P = 0.0150*	Industry P = 0.0274* Agriculture P = 0.0446
Selenium	Agriculture P = 0.0054	....	Agriculture P = 0.0021	Agriculture P = 0.0416
Silver	....	....	....	....
Zinc	Precipitation P = 0.0074 CI P = 0.0035	....	Precipitation P = 0.0145	....
PAH	....	....	....	....

Table 10. Results of regression analyses within regions of similarity in pollutant body burden as determined using the K-S test. These results utilize the average of the 2 months prior to sampling for precipitation and temperature. Mean and Median Infection and Prevalence refer to *Perkinsus marinus*. Other abbreviations and symbols as described in Table 9

Pollutant	1986	1987	1988	1989
Arsenic	Industry P = 0.0002* Prevalence P = 0.0472	Precipitation P = 0.0111*	Length P = 0.0061*	Precipitation P = 0.0183* CI P = 0.0098 Median Infection P = 0.0135*
Cadmium	CI P = 0.0162* Prevalence P = 0.0310 Mean Infection P = 0.0044*	Industry P = 0.0483	Precipitation P = 0.0087* Length P = 0.0037 Mean Infection P = 0.0132*	....
Copper	Temperature P = 0.0366	Mean Infection P = 0.0062 Median Infection P = 0.0053*	Temperature P = 0.0230	Industry P = 0.0402* Median Infection P = 0.0290 Prevalence P = 0.0208*
Mercury	Industry P = 0.0416* Prevalence P = 0.0116	Industry P = 0.0199* Mean Infection P = 0.0187	....	Temperature P = 0.0383* Agriculture P = 0.0187 Industry P = 0.0204*
Selenium	Agriculture P = 0.0023 Mean Infection P = 0.0159*	Agriculture P = 0.0285 Mean Infection P = 0.0159 Median Infection P = 0.0173*	Agriculture P = 0.0037	Agriculture P = 0.0254
Silver	Precipitation P = 0.0082* Prevalence P = 0.0429 Mean Infection P = 0.0042* Median Infection P = 0.0177	....	....	Length P = 0.0168 CI P = 0.0073 Prevalence P = 0.0006* Median Infection P = 0.0010
Zinc	Precipitation P = 0.0082 CI P = 0.0039*	....	Median Infection P = 0.0267*	Length P = 0.0257
PAH	....	....	Prevalence P = 0.0247 Mean Infection P = 0.0241*	Length P = 0.0101

level, and these regions typically cover a substantial range in latitudes (Tables 9, 10). In fact, from these analyses, local input from industrial and agricultural land use and levels of *P. marinus* infection appear to be more important. Most pollutants show a significant correlation with some measure of *P. marinus* infection in at least one instance. The relationship between *P. marinus* and temperature and salinity (again, related to precipitation) is well documented in the literature (Mackin, 1962; Soniat, 1985; Soniat & Gauthier, 1989). Correlations are more frequent using the average of the climatic data for the 2 months before sampling rather than for the 5 months before sampling, suggesting that response times to variations in environmental variables might be more nearly 2 than 5 months, and this is the response time expected if *P. marinus* was an important factor in body burden (Choi et al., 1989). The infrequent correlations with length, condition index or *P. marinus* prevalence (as opposed to infection intensity) are also noteworthy, particularly considering the frequent importance of these biological indices generally (Cossa et al., 1980; Scott & Lawrence, 1982; Lytle & Lytle, 1990; Páez-Osuna & Marmolejo-Rivas, 1990; and others referenced previously) and in the temporal trends we observed. Recall, however, that *P. marinus* infection intensity, condition index and length are correlated on most spatial scales.

Whether a cause and effect relationship exists between disease and contaminant body burden has not been demonstrated. *P. marinus* can produce physiologic abnormalities in its oyster host that in turn can affect the oyster's ability to feed (Mackin & Ray, 1955). Since feeding is one method of uptake for certain contaminants, such as arsenic (Sanders et al., 1989), body burden could be reduced with increased infection of *P. marinus*. Contaminant exposure and disease may also affect the health of the digestive gland (Bayne et al., 1979; Axiak et al., 1988). Cadmium has been shown to stimulate phagocytosis (Cheng, 1988a) which is one means of defense against disease (Fisher & Newell, 1986; Fisher & Tamplin, 1988). The negative correlation between cadmium body burden and *P. marinus* may be a reflection of this stimulatory action.

(JN)1858 (KL)150 (SW)Wilson

CONCLUSIONS

The results of environmental monitoring studies have long been looked upon with suspicion when the results have been compared over varied environmental conditions (Phillips, 1977a). Our results stress the variability of pollutant body burdens as they relate to variations in environmental and physiological conditions. Consideration of local controls are important, but so are large-scale geographic and climatic controls which can override the local controls. All biological variables responded regionally on a Gulf-wide scale. Local controls were relatively unimportant throughout the Gulf in explaining temporal trends, albeit of more importance in explaining the spatial relationships within any one year. Among the contaminants, local and regional controls were important in discrete geographic areas in most cases. Some contaminants responded primarily regionally (e.g. selenium), some primarily locally (e.g. silver). These regional differences affected not only the temporal trends, but also the spatial distribution of body burden within any one year. Accordingly, consideration of the spatial distribution of body burden, and particularly, consideration of the temporal trends in body burden must take into account that climatic factors may be more important in some regions than others and that the health of the population may contribute markedly to body burden; consequently,

factors controlling the health of populations may indirectly affect temporal trends by mediating the climatic response.

Variations in source content have not been included in the analysis. Inasmuch as arguably the most important parameter controlling body burden has not explicitly been included in the analysis, the fact that several environmental and biological parameters nevertheless demonstrated significant correlations with body burden is noteworthy. Among the biological parameters, factors related to disease, and among the environmental parameters, factors related to land use and meteorological conditions are likely to play an important role in determining pollutant body burden at least regionally. It is particularly important to recognize that regional factors of importance may go unrecognized at larger geographic scales because statistical analyses may be compromised by varying responses to selected variables in different regions. We should not expect selected variables to be consistently of paramount importance everywhere.

Both *P. marinus* prevalence and intensity, as well as the other biological variables, and contaminant body burdens must ultimately respond to temperature and rainfall. These latter two parameters should be the initial factors mediating the effect of climate on pollutant body burden. Whether they are the proximate causes, or whether biological parameters intercede, remains unclear. Certainly, however, factors like disease intensity and the gametogenic cycle play an important role in determining the health and condition of Gulf oyster populations. That the contaminant body burdens in the Gulf are almost uniformly relatively low suggests that the correlations observed between body burden and biology originate either in biological control of contaminant body burden or coincident control of both directly by climatic cycles rather than the impact of pollution on organism health (e.g. Khan, 1990) which might result at higher exposure levels.

Acknowledgements. The authors wish to thank the Status and Trends field and laboratory crews at Texas A & M University who collect and analyse the trace metal and PAH samples. This research was conducted under grants from the U.S. Department of Commerce, National Oceanic and Atmospheric Administration Ocean Assessments Division #50-DGNC-5-00262 and #46-DGNC-0-00047, the Sea Grant College Program #NA89AA-D-SG139 and the Center for Energy and Minerals Resources and a Sea Grant Marine Fellowship to E.W. Funds for computer analysis were provided by the TAMU College of Geosciences. We appreciate this support.

(JN)1859 (KL)150 (SW)Wilson

LITERATURE CITED

Allen, R. L. & Turner, R. E., 1989. Environmental influences on the oyster industry along the west coast of Florida. – J. Shellfish Res. *8*, 95–104.

Axiak, V., George, J. J. & Moore, M. N., 1988. Petroleum hydrocarbons in the marine bivalve *Venus verrucosa*: accumulation and cellular responses. – Mar. Biol. *97*, 225–230.

Bayne, B., Moore, M., Widdows, J., Livingstone, D. & Salkeld, P., 1979. Measurement of the responses of individuals to environmental stress and pollution, studies with bivalve molluscs. – Phil. Trans. R. Soc. Lond. (B) *286*, 563–581.

Boyden, C. R., 1974. Trace element content and body size in molluscs. – Nature, Lond. *251*, 311–314.

Boyden, C. R., 1977. Effect of size upon metal content of shellfish. – J. mar. biol. Ass. U.K. *57*, 675–714.

Boyden, C. R. & Phillips, D. J. H., 1981. Seasonal variation and inherent variability of trace elements in oysters and their implications for indicator studies. – Mar. Ecol. Prog. Ser. *5*, 29–40.

Brooks, J. M., Wade, T. L., Atlas, E. L., Kennicutt, M. C., Presley, B. J., Fay, R. R. & Wolff, G., 1989. Analysis of bivalves and sediments for organic chemicals and trace elements from the Gulf of Mexico estuaries. – A. Rep. NOAA (Office of Oceanography and Marine Assessment) ■, ■–■.

Brooks, R. R. & Rumsby, M. G., 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. – Limnol. Oceanogr. *10*, 521–527.

Broutman, M. A. & Leonard, D. L., 1988. The quality of shellfish growing waters in the Gulf of Mexico. National Oceanographic and Atmospheric Association, Office of Oceanography and Marine Assessment, National Ocean Services.

Cheng, T. C., 1988a. In vivo effects of heavy metals on cellular defense mechanisms of *Crassostrea virginica*: phagocytic and endocytotic indices. – J. Invertebr. Pathol. *51*, 215–220.

Cheng, T. C., 1988b. In vivo effects of heavy metals on cellular defense mechanisms of *Crassostrea virginica*: total and differential cell counts. – J. Invertebr. Pathol. *51*, 207–214.

Choi, K.-S., Wilson, E. A., Lewis, D. H., Powell, E. N. & Ray, S. M., 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. – J. Shellfish Res. *8*, 125–131.

Cliff, A. D. & Ord, J. K., 1973. Spatial autocorrelation. Pion, London, ◀ pp.

Conover, W. J., 1972. A Kolmogorov goodness-of-fit test for discontinuous distributions. – J. Am. statist. Ass. *67*, 591–596.

Cossa, D., Bourget, E., Pouliot, D., Piuze, J. & Chanut, J. P., 1980. Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis*. – Mar. Biol. *58*, 7–14.

Craig, A., Powell, E. N., Fay, R. R. & Brooks, J. M., 1989. Distribution of *Perkinsus marinus* in Gulf coast oyster populations. – Estuaries *12*, 82–91.

Cunningham, P. A. & Tripp, M. R., 1973. Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*. – Mar. Biol. *20*, 14–19.

Cunningham, P. A. & Tripp, M. R., 1975a. Factors affecting the accumulation and removal of mercury from tissues of the American oyster *Crassostrea virginica*. – Mar. Biol. *31*, 311–319.

Cunningham, P. A. & Tripp, M. R., 1975b. Accumulation, tissue distribution and elimination of $^{203}HgCl_2$ and $CH_3{}^{203}HgCl$ in the tissues of the American oyster *Crassostrea virginica*. – Mar. Biol. *31*, 321–334.

Denton, G. R. W. & Burdon-Jones, C., 1981. Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium, and lead by the black-lip oyster *Saccostrea echinata*. – Mar. Biol. *64*, 317–326.

Douglas, A. V. & Englehart, P. J., 1981. On a statistical relationship between autumn rainfall in the central equatorial Pacific and subsequent winter precipitation in Florida. – Mon. Weath. Rev. U.S. Dep. Agric. *109*, 2377–2382.

Dow, R. L., 1977. Effects of climatic cycles on the relative abundance and availability of commercial marine and estuarine species. – J. Cons. int. Explor. Mer *37*, 274–280.

Ehrhardt, M., 1972. Petroleum hydrocarbons in oysters from Galveston Bay. – Environ. Pollut. *3*, 257–271.

Farrington, J. W. & Quinn, J. G., 1973. Petroleum hydrocarbons in Narragansett Bay I. Survey of hydrocarbons in sediments and clams. – Estuar. coast. mar. Sci. *1*, 71–79.

Farrington, J. W., Goldberg, E. D., Risebrough, R. W., Martin, J. H. & Bowen, V. T., 1983. U.S. "Mussel Watch" 1976-1978. An overview of the trace-metal, DDE, PCB, hydrocarbon and artificial radionuclide data. – Environ. Sci. Technol. *17*, 490–496.

Fisher, W. S. & Newell, R. I. E., 1986. Salinity effects on the activity of granular hemocytes of American oysters, *Crassostrea virginica*. – Biol. Bull. mar. biol. Lab., Woods Hole *170*, 122–134.

Fisher, W. S. & Tamplin, M., 1988. Environmental influence on activities and foreign-particle binding by hemocytes of American oysters, *Crassostrea virginica*. – Can. J. Fish. aquat. Sci. *45*, 1309–1315.

Frazier, J. M., 1975. The dynamics of metals in the American oyster, *Crassostrea virginica*. I. Seasonal effects. – Chesapeake Sci. *16*, 162–171.

Frazier, J. M., 1976. The dynamics of metals in the American oyster, *Crassostrea virginica*. II. Environmental effects. – Chesapeake Sci. *17*, 188–197.

Gabriel, K. R. & Sokal, R. R., 1969. A new statistical approach to geographical variation analysis. – Syst. Zool. *18*, 259–278.

Holsteiner, R. P., 1977. Trends in population levels of the American oyster *Crassostrea virginica* Gmelin on public reefs in Galveston Bay, Texas. – Tech. Ser. Tex. Parks Wildl. Dept. *24*, 1–90.

IBWC, 1985–1989. Flow of the Rio Grande and related data. International Boundary and Water Commission United States and Mexico Water Bulletin, Department of State, El Paso, Texas.

Jumars, P. A., Thistle, D. & Jones, M. L., 1977. Detecting two-dimensional spatial structure in biological data. – Oecologia *28*, 109–123.

Khan, R. A., 1990. Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. – Bull. environ. Contam. Toxicol. *44*, 759–763.

Lytle, T. F. & Lytle, J. S., 1990. Heavy metals in the Eastern oyster, *Crassostrea virginica*, of the Mississippi Sound. – Bull. environ. Contam. Toxicol. *44*, 142–148.

Mackin, J. G. & Ray, S. M., 1955. Studies on the effect of infection by *Dermocystidium marinum* on ciliary action in oyster (*Crassostrea virginica*). – Proc. natn. Shellfish. Ass. *45*, 168–181.

Mackin, J. G., 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. – Publ. Inst. mar. Sci. Univ. Tex. *7*, 132–229.

Marble, D. F., 1967. Some computer programs for geographic research. – Spec. Publ., Dept. Geogr., Northwest. Univ., Evanston, Ill. *1*, 1–201.

Martinčić, D., Nürnberg, H. W., Stoeppler, M. & Branica, M., 1984. Bioaccumulation of heavy metals by bivalves from Lim Fjord (North Adriatic Sea). – Mar. Biol. *81*, 177–188.

Morales-Alamo, R. & Mann, R., 1989. Anatomical features in histological sections of *Crassostrea virginica* (Gmelin, 1791) as an aid in measurement of gonad area for reproductive assessment. – J. Shellfish Res. *8*, 71–82.

NOAA, 1985–1989. Climatological data annual summary (for respectively Texas, Louisiana, Mississippi, Alabama and Florida). NOAA (National Climatic Data Center), Asheville, North Carolina, ■ pp.

NOAA, 1987. National estuarine inventory data atlas, Vol. 2: Land use characteristics. NOAA (Office of Oceanographic and Marine Assessment), Washington, D.C., ■ pp.

Páez-Osuna, F. & Marmolejo-Rivas, C., 1990. Occurrence and seasonal variation of heavy metals in the oyster *Saccostrea iridescens*. – Bull. environ. Contam. Toxicol. *44*, 129–134.

Parizek, J., Kalouskova, J., Babicky, A., Benes, J. & Paulik, L., 1974. Interaction of selenium with mercury, cadmium, and other toxic metals. In: Trace element metabolism in animals. Ed. by W. Hoekstra, J. Suttie, H. Ganther & W. Mertz. Univ. Park Press, Baltimore, *2*, 119–132.

Phelps, H. L., Wright, D. A. & Mihursky, J. A., 1985. Factors affecting trace metal accumulation by estuarine oysters *Crassostrea virginica*. – Mar. Ecol. Prog. Ser. *22*, 187–197.

Philander, G., 1989. El Niño and La Niña. – Am. Scient. *77*, 451–459.

Phillips, D. J. H., 1976a. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. – Mar. Biol. *38*, 59–69.

Phillips, D. J. H., 1976b. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. – Mar. Biol. *38*, 71–80.

Phillips, D. J. H., 1977a. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments – a review. – Environ. Pollut. *13*, 281–317.

Phillips, D. J. H., 1977b. Effects of salinity on the net uptake of zinc by the common mussel *Mytilus edulis*. – Mar. Biol. *41*, 79–88.

Phillips, D. J. H., 1977c. The common mussel *Mytilus edulis* as an indicator of trace metals in Scandinavian waters. I. Zinc and cadmium. – Mar. Biol. *43*, 283–291.

Powell, E. N., Gauthier, H., Stanton Jr., R. J. & Staff, G., 1984. Estimation of the size of molluscan larval settlement using the death assemblage. – Estuar. coast. Shelf Sci. *18*, 367–384.

Powell, E. N., Gauthier, J. D., Wilson, E. A., Nelson, A., Fay, R. R. & Brooks, J. M., 19■. "Dermo" and the El Niño/Southern oscillation. Are yearly changes in *Perkinsus marinus* parasitism in oysters (*Crassostrea virginica*) controlled by climatic cycles in the Gulf of Mexico? – Mar. Ecol. ■ (in press).

Presley, B. J., Taylor, R. J. & Boothe, P. N., 1990. Trace metals in Gulf of Mexico oysters. – Sci. total Environ. *96*, 551–593.

Ratkowsky, D. A., Thrower, S. J., Eustace, I. J. & Olley, J., 1974. A numerical study of the concentration of some heavy metals in Tasmanian oysters. – J. Fish. Res. Bd Can. *31*, 1165–1171.

Ray, S. M., 1966. A review of the culture methods for detecting *Dermocystidium marinum*, with suggested modifications and precautions. – Proc. natn. Shellfish. Ass. *54*, 55–69.

Roesijadi, G. & Klerks, P., 1989. Kinetic analysis of cadmium binding to metallothionein and other intracellular ligands in oyster gills. – J. exp. Zool. *251*, 1–12.

Roesijadi, G., Kielland, S. & Klerks, P., 1989. Purification and properties of novel molluscan metallothioneins. – Archs Biochem. Biophys. *273*, 403–413.

Ropelewski, C. F. & Halpert, M. S., 1986. North American precipitation and temperature patterns associated with the El Niño/Southern Oscillation (ENSO). – Mon. Weath. Rev. U.S. Dep. agric. *114*, 2352–2362.

Sanders, J. G., 1980. Arsenic cycling in marine systems. – Mar. environ. Res. *3*, 257–266.

Sanders, J. G., Osman, R. W. & Riedel, G. F., 1989. Pathways of arsenic uptake and incorporation in estuarine phytoplankton and the filter-feeding invertebrates *Eurytemora affinis*, *Balanus improvisus* and *Crassostrea virginica*. – Mar. Biol. *103*, 319–325.

Scott, G. I. & Lawrence, D. R., 1982. The American oyster as a coastal zone pollution monitor: a pilot study. – Estuaries *5*, 40–46.

Shuster, C. N. & Pringle, B. H., 1969. Trace metal accumulation by the American eastern oyster, *Crassostrea virginica*. – Proc. natn. Shellfish. Ass. *59*, 91–103.

Sindermann, C. J., 1983. An examination of some relationships between pollution and disease. – Rapp. P.-v. Réun. Cons. int. Explor. Mer *182*, 37–43.

Sokal, R. R. & Oden, N. L., 1978a. Spatial autocorrelation in biology 1. Methodology. – Biol. J. Linn. Soc. *10*, 199–228.

Sokal, R. R. & Oden, N. L., 1978b. Spatial autocorrelation in biology 2. Some biological implications and four applications of evolutionary and ecological interest. – Biol. J. Linn. Soc. *10*, 229–249.

Soniat, T. M., 1985. Changes in levels of infection of oysters by *Perkinsus marinus*, with special reference to the interaction of temperature and salinity upon parasitism. – Northwest Gulf Sci. *7*, 171–174.

Soniat, T. M. & Gauthier, J. D., 1989. The prevalence and intensity of *Perkinsus marinus* from the mid northern Gulf of Mexico, with comments on the relationship of the oyster parasite to temperature and salinity. – Tulane Stud. Zool. Bot. *27*, 21–27.

Stegeman, J. J. & Teal, J. M., 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster *Crassostrea virginica*. – Mar. Biol. *22*, 37–44.

Trenberth, K. E., Branstator, G. W. & Arkin, P. A., 1988. Origins of the 1988 North American drought. – Science, N.Y. *242*, 1640–1645.

Ulanowicz, R. E., Caplins, W. C. & Dunnington, E. A., 1980. The forecasting of oyster harvest in central Chesapeake Bay. – Estuar. coast. mar. Sci. *11*, 101–106.

Uncles, R. J., Stephens, J. A. & Woodrow, T. Y., 1988. Seasonal cycling of estuarine sediment and contaminant transport. – Estuaries *11*, 108–116.

USGS, 1985–1989. Water resources data (for respectively Texas [Vol. 1–3], Louisiana, Mississippi, Alabama and Florida [Vol. 1–4] for water years 1985, 1986, 1987, 1988). U.S. geol. Survey Water-Data Rep.

Wade, T. L., Atlas, E. L., Brooks, J. M., Kennicutt II, M. C., Fox, R. G., Sericano, J., García-Romero, B. & Defreitas, D., 1988. NOAA Gulf of Mexico Status and Trends Program: Trace organic contaminant distribution in sediments and oysters. – Estuaries *11*, 171–179.

Wallace, J. M. & Gutzler, D. S., 1981. Teleconnections in the geopotential height field during the northern hemisphere winter. – Mon. Weath. Rev. U.S. Dep. agric. *109*, 784–812.

White, M. E., Powell, E. N., Ray, S. M., Wilson, E. A. & Zastrow, C. E., 1988. Metabolic changes induced in oysters (*Crassostrea virginica*) by the parasitism of *Boonea impressa* (Gastropoda: Pyramidellidae). – Comp. Biochem. Physiol. *90* (A), 279–290.

Wilson, E. A., Powell, E. N. & Ray, S. M., 1988. The effect of the ectoparasitic pyramidellid snail, *Boonea impressa*, on the growth and health of oysters, *Crassostrea virginica*, under field conditions. – Fish. Bull. U.S. *86*, 553–566.

Wilson, E. A., Powell, E. N., Craig, M. A., Wade, T. L. & Brooks, J. M., 1990. The distribution of *Perkinsus marinus* in Gulf coast oysters: its relationship with temperature, reproduction, and pollutant body burden. – Int. Revue ges. Hydrobiol. *75*, 533–550.

Windom, H. L. & Smith, R. G., 1972. Distribution of iron, magnesium, copper, zinc, and silver in oysters along the Georgia coast. – J. Fish. Res. Bd Can. *29*, 450–452.

Wright, D. A. & Zamuda, C. D., 1987. Copper accumulation by two bivalve molluscs: salinity effect is independent of cupric ion activity. – Mar. environ. Res. *23*, 1–14.

Zatoogian, G. E., 1980. *Crassostrea virginica* as an indicator of cadmium pollution. - Mar. Biol. *5* 275–284.

Zatoogian, G. E. & Cheer, S., 1976. Accumulation of cadmium by the American oyster, *Crassostrea virginica*. - Nature, Lond. *261*, 408–409.

Zatoogian, G. E. & Hoffman, G. L., 1982. Arsenic uptake and loss in the American oyster, *Crassostrea virginica*. - Environ. Monit. Assess. *1*, 345–358.

NOAA COASTAL SERVICES CENTER LIBRARY
3 6668 14106 7498