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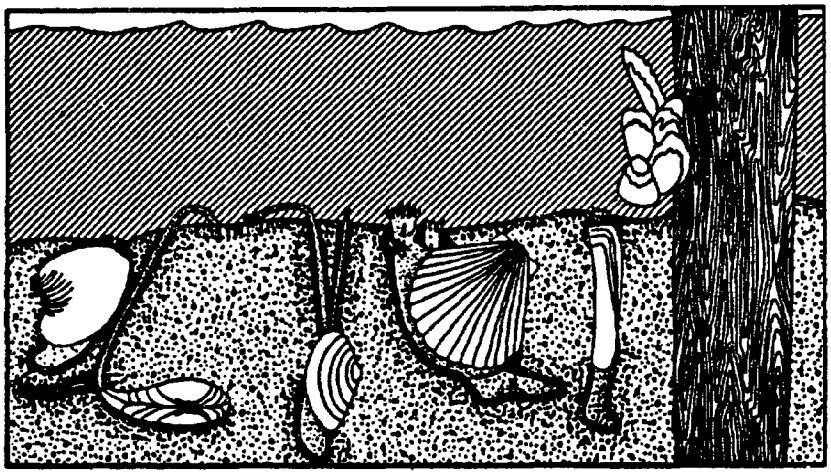
NOAA STATUS AND TRENDS
Mussel Watch Project
Year 7 Technical Report

The Geochemical and Environmental Research Group

Texas A&M Research Foundation



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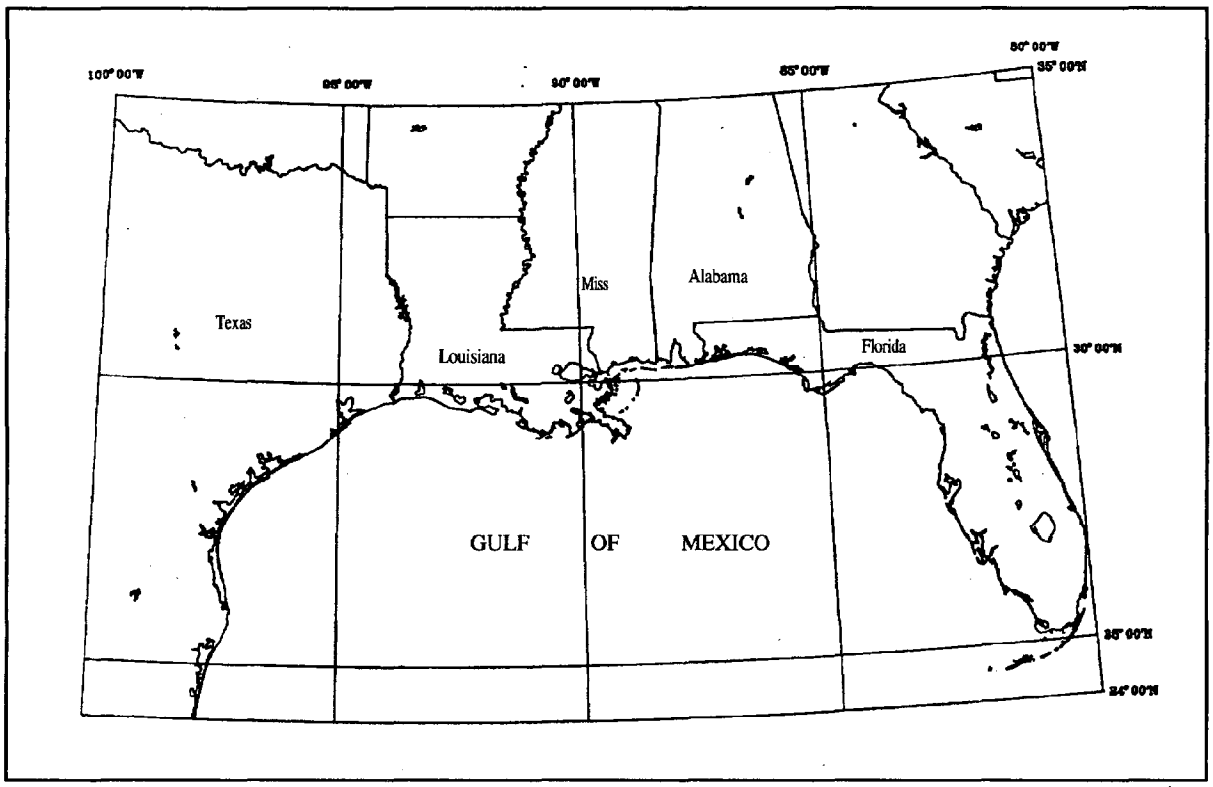

January 1994

NOAA NATIONAL STATUS AND TRENDS

Mussel Watch Project

Year 7 Technical Report

Prepared by

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Table of Contents

Introduction 1-1

Reprint 1: *The Usefulness of Transplanted Oysters in Biomonitoring Studies* 1-11

Reprint 2: *Overview of the First Four Years of the NOAA National Status and Trends Mussel Watch Program* 1-21

Reprint 3: *Trace Organic Contamination in Galveston Bay Oysters: Results from the NOAA National Status and Trends Mussel Watch Program* 1-31

Reprint 4: *Indicators of Trace Metal Pollution in Galveston Bay* 1-37

Reprint 5: *Oysters as Biomonitors of the APEX Barge Oil Spill* 1-45

Reprint 6: *Field Studies Using the Oyster* Crassostrea virginica *to Determine Mercury Accumulation and Depuration Rates* 1-53

Reprint 7: *Trace Metal Chemistry of Galveston Bay: Water, Sediment and Biota* 1-59

Reprint 8: *Mercury Bioaccumulation by Shrimp (*Penaeus aztecus*) Transplanted to Lavaca Bay, Texas* 1-81

Reprint 9: *Polynuclear Aromatic Hydrocarbon Contaminants in Oysters from the Gulf of Mexico (1986-1990)* 1-87

Reprint 10: *Butyltin Concentrations in Oysters from the Gulf of Mexico During 1989-1991* 1-97

Reprint 11: *The American Oyster (*Crassostrea virginica*) as a Bioindicator of Trace Organic Contamination* 1-109

NOAA'S NATIONAL STATUS AND TRENDS (NS&T) MUSSEL WATCH PROGRAM — GULF OF MEXICO

The purpose of the NOAA National Status and Trends (NS&T) 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?
(2) Are these conditions getting better or worse?

This report represents the Year 7 Technical Report from this multi-year project. These questions have been addressed in detail as evidenced by the scientific papers and reports that have resulted from the Geochemical and Environmental Research Group's (GERG) interpretations of the Gulf Coast data (Table 1). Publications not included in GERG's previous Technical Reports are contained in this technical report.

This report is an update on the current condition of the Gulf of Mexico coastal zone, based on results from Years 1 through 7 of the NOAA NS&T Mussel Watch Project. Following is a brief sampling survey of these years:

Year 1 - 49 sites (147 stations) of the original 51 sites were successfully sampled. Sediments and oysters were analyzed at triplicate stations from all sites.

Year 2 - 48 sites (144 stations) of the original 51 sites were successfully sampled. Sediments and oysters were analyzed at triplicate stations from all sites.

Year 3 - Twenty (20) sites were added to the original list of 51 sites for a total of 71 sites. Sixty-four (64) sites (192 stations) of the 71 sites were sampled (only 19 of the new sites were sampled). Oysters were analyzed at triplicate stations from all sites. Sediments were analyzed at only the new sites (three stations analyzed per site).

Year 4 - Seven (7) new sites were added (only six of the new sites were successfully sampled). Sixty-seven (67) sites (201 stations) of the 78 total sites were sampled. Oysters were analyzed at triplicate stations from all sites. Sediments were analyzed at only the new sites (three stations analyzed per site).

Year 5 - Three (3) new sites were added to the sampling project (only two of these sites were successfully sampled; 79:MBDR and 80:PBSP). Sixty-eight (68) sites (204 stations) of the 80 total sites were sampled. Oysters were analyzed at triplicate stations from all sites.

Sediments were analyzed at only the new sites (three stations analyzed per site).

Year 6 - Two (2) new sites were added to the sampling project (81:BHKF in Bahia Honda Key, FL and 63:LPGO in Lake Pontchartrain, LA). Sixty-four (64) sites (192 stations) were sampled. Oysters were analyzed at triplicate stations from all sites. Sediments were analyzed at only the new sites (three stations analyzed per site).

Year 7 - Five new sites were established including three new sites in Puerto Rico (Sites 86 to 88) and two new sites in Choctawhatchee Bay (Sites 84 and 85). Sixty-seven (67) sites were analyzed. Only one oyster analysis was conducted at each of the old sites on a composite from the three stations. Sediments were analyzed at the five new sites and one site in Florida (PBPH) (three stations analyzed per site).

Details of the sample collection and location of field sampling sites are contained in a separate report titled "Field Sampling and Logistics in Year 7".

The oyster and sediment samples were analyzed for contaminant concentrations [trace metals, polynuclear aromatic hydrocarbons (PAH), pesticides and polychlorinated biphenyls (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 were produced from the sample analyses for Year 7 are found in a separate report titled "Analytical Data".

A complete and comprehensive interpretation of the data from the National 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). As part of the data interpretation and dissemination, over 40 presentations of the NOAA NS&T Gulf Coast Mussel Watch Project were given at national and international meetings. With seven years of data, the question of temporal trends of contaminant concentrations has been addressed. A general conclusion found for most contaminants measured is that the concentrations have remained relatively constant over the seven-year sampling period. This general trend, however, is not observed at all sites. Some sites show significant changes (both increases and decreases) among the years. Continued sampling is addressing 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, 20 new sites were added. These sites were chosen to be closer to urban areas, and therefore, to the sources of contaminant inputs. These 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. Over the subsequent years of the project (Years 4 through 7) additional sites have been added to increase the representative coverage of the Gulf of Mexico and U.S. Caribbean territories.

While sampling sites for this project were specifically chosen to avoid known point sources of contamination, 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 through 7 are closer to urban areas and 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.

This document represents one of three report products as part of Year 7 of the NS&T Gulf of Mexico projects. The other two reports are entitled:

- Analytical Data, Year 7
- Field Sampling and Logistics, Year 7

Table 1. **GERG/NOAA NS&T PUBLICATIONS**

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Reprint 1

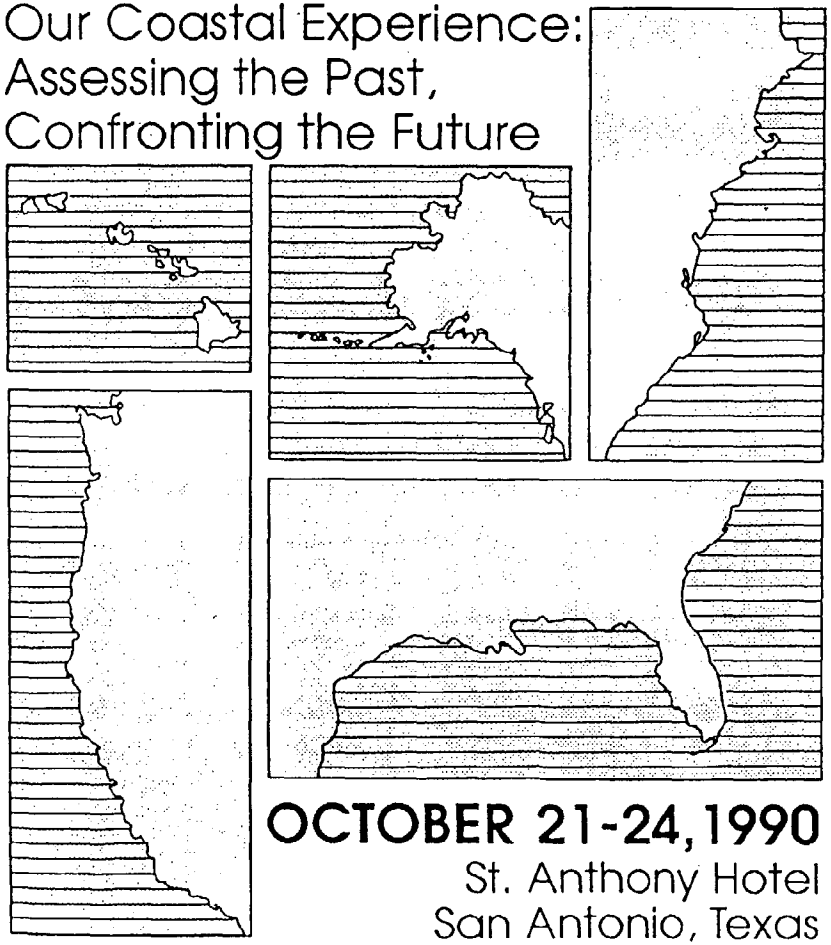
The Usefulness of Transplanted Oysters in Biomonitoring Studies

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

The Coastal Society Twelfth International Conference

CONFERENCE PROCEEDINGS

Our Coastal Experience: Assessing the Past, Confronting the Future



OCTOBER 21-24, 1990

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The Usefulness of Transplanted Oysters in Biomonitoring Studies

José L. Sericano, Terry L. Wade, and James M. Brooks
Texas A&M University

Abstract

This study was designed to examine the uptake and depuration of selected organic contaminants of environmental concern, i.e., polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), by transplanted oysters (Crassostrea' virginica) in Galveston Bay, Texas and to establish the feasibility of using transplanted oysters for biomonitoring the contamination status in areas were no indigenous bivalves are present. Transplanted oysters bioaccumulated individual PAHs and low molecular weight PCBs to concentrations that were not statistically differentiable from the levels encountered in native oysters within 30 to 48 days. In contrast, high molecular weight PCBs did not reach equilibrium in transplanted oysters; whose high molecular weight PCB concentrations were lower than those measured in indigenous oysters during the seven-week uptake period. During the depuration phase of this study, originally uncontaminated oysters depurated PAHs and low molecular wight PCBs at a faster rate than chronically contaminated oysters. Clearance of high molecular weight PCBs was limited in both oyster populations.

Introduction

Contamination of the coastal marine environment 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 has been 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 (Goldberg et al., 1978; Farrington et al., 1980; Phillips, 1980; Risebrough et al., 1983) and its concept has been applied to many monitoring programs during the last decade (Farrington et al., 1983; Martin, 1985; Tavares et al., 1988; Wade et al., 1988; Sericano et al., 1990).

The National Oceanic and Atmospheric Administration's National Status and Trends Program (NOAA's NS&T) is designed to monitor the current status and long-term effects of selected organic and inorganic contaminants of environmental concern, e.g. polynuclear aromatic hydrocarbons (PAHs), chlorinated pesticides, polychlorinated biphenyls (PCBs), and trace metals, along the coasts of the U.S. by measuring their concentrations in bivalves over a number of years. During the first five years of this program (1986-1990), the intent was to sample all the locations

prescribed by NOAA. However, locations depleted or devoid of living oysters caused by virtue of diseases, predators, excessive freshwater runoff, harvesting, or dredge material burying entire reefs compromised this goal. Therefore, in some instances, it was not possible to obtain samples. After the first five years of the NS&T, nearly 20% of the original locations presented some of the above-mentioned sampling problems that left the database 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, i.e. polynuclear aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), in oysters (Crassostrea virginica) through transplantation experiments in two locations in Galveston Bay, Texas.

Materials and Methods

Experimental design

Approximately 250 oysters of similar dimensions 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 in the upper part of the Bay (Figure 1). Composite samples of 20 transplanted and 15 indigenous oysters were collected at 0, 3, 7, 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 Ship Channel site were also transplanted to the Hanna Reef area. Composite samples of 20 oysters from each population were collected at 3, 6, 18, 30, and 50 days after transplantation.

Analytical method

The analytical procedures used during this study are modifications of previously reported methods (MacLeod et al., 1985) and are fully described elsewhere (Wade et al., 1988; Sericano et al., 1990).

Results and Discussion

The concentrations of some of the organic contaminants 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 (Figure 2a). The PAHs accumulated to the highest concentrations by transplanted oysters were: pyrene> fluoranthene> chrysene> benzo(e) pyrene> benzo(b)-anthracene (Figure 2b). Clams and mussels exposed to sediments contaminated with high PAH concentrations accumulated pyrene> benzo(e) pyrene> benzo(b) fluoranthene> benz(a) anthracene (Obana et al., 1983) and chrysene> benzo(b) fluoranthene> fluoranthene> benzo(e)pyrene> benz(a)anthracene (Pruell et al., 1986), respectively.

Hanna Reef and Ship Channel oysters showed statistically significant depuration ($p<0.05$) of four- and five-ring PAHs after relocation to the Hanna Reef area (Figures 2c and 2d). Depuration of these aromatic compounds by both groups of oysters was approximately exponential. This is indicated in Figure 3, where the concentration of selected PAHs plotted on a semi-log plot approximate straight lines.

Kinetics parameters describing uptake and release of PAHs can be calculated assuming the first-order equation

$$(1)\quad dC_t/dt = k_u C_w - k_d C_t$$

where C_t is the PAH concentration in the transplanted oyster at time=t, C_w is the PAH concentration in the seawater, and k_u and k_d are the uptake and depuration rate constant, respectively. If the C_w at Hanna Reef is regarded as zero, i.e., $C_w=0$, which is considerably reasonable because of the very low PAH concentrations measured in indigenous oysters, then equation (1) reduces to

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

or, after integration,

$$(3)\quad \log C_t = \log C_o - (k_d/2.301)t$$

where C_o is the PAH concentration in oysters at the time of their relocation to the Hanna Reef area. Using this equation and the PAH concentrations

corresponding to both oyster populations during the depuration period, values of k_d can be calculated. Statistical analyses, at the $a=0.05$ level, of the regression lines of the logarithm of the concentrations versus sampling time for the depuration period showed significant differences between the slopes, i.e., depuration rates, measured for Hanna Reef and Ship Channel oysters.

The biological half-life, $t_{1/2}$, can be derived from equation (3)

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

The half-lives are reported in Table I. They ranged from 10.4 and 12.4 days for pyrene to 25.6 and 38.5 days for fluoranthene in Hanna Reef and Ship Channel oysters, respectively. Most of the values were, however, between 10 and 16 days.

Recently, Pruell et al. (1986) reported the half-lives for selected PAHs in mussels (Mytilus edulis) exposed to environmentally contaminated sediments. The calculated half-lives compared well with the values measured in this study (Table I).

PCB concentrations in transplanted oysters increased from 30 ng/g to 850 ng/g after the 48-day exposure period. Pentachlorobiphenyls were the compounds accumulated to the highest concentrations in transplanted and native oysters (Figures 4a and 4b). In comparison, practically no octa-, nona-, or decachlorobiphenyls were detected in either oyster group. Contrasting with 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 seems evident that a longer exposure period is needed for the higher molecular weight PCBs to reach an steady state concentration (Figure 5).

Hanna Reef and Ship Channel oysters showed statistically significant depuration ($p<0.05$) of low molecular weight PCBs when relocated to the Hanna Reef area (Figures 4c and 4d). Originally uncontaminated oysters depurated PCBs at a faster rate than chronically contaminated oysters. The depuration rates of high molecular weight PCBs were significantly slower in both oyster populations. This differential PCB depuration can be observed in Figures 4b and 4d showing the concentrations of selected PCBs at the end of the uptake and depuration periods. Biological half-lives for these PCBs in Hanna Reef and Ship Channel oysters ranged from 21 to 129 days and from 20 days to >year, respectively (Table 1). Pruell et al. (1986) reported half-lives for tri- to hexachlorobiphenyls in mussels exposed to resuspended contaminated sediments ranging from 16.3 to 45.6 days. Similar to the present

study, the biological half-lives of PCBs increased with the number of chlorine atoms in the biphenyl rings. Langston (1978) also reported that the less chlorinated PCB congeners were depurated more rapidly by bivalves (Cerastoderma edule and Macoma balthica) with half-lives from 5 to 21 days for di- to tetrachlorobiphenyls. In contrast, hexachlorobiphenyls, and some of the pentachlorobiphenyls, did not decrease in concentration during the 21-day study. Courtney and Denton (1976) reported that environmentally contaminated clams and clams exposed to Aroclor 1254 in the laboratory did not depurated PCBs during three months in control seawater.

In summary, PAHs and low molecular weight PCBs were rapidly accumulated by transplanted oysters. Apparent steady-state concentrations were reached after 30 to 48 days. In contrast, high molecular weight PCBs did not reached an equilibrium plateau within the seven-week exposure to high PCB concentrations. However, the still-increasing concentrations measured for these PCBs by the end of the exposure period seems to indicate that, given enough time, equilibrium concentrations will eventually be reached. When back-transplanted to the Hanna Reef area, originally uncontaminated and chronically exposed oysters depurated PAHs with half-lives ranging from 10.4 to 23.6 days and from 12.4 to 38.5 days, respectively. These rates were similar to those calculated for tri- and tetrachlorobiphenyls but faster than those estimated for heavier molecular weight PCBs. Despite the limitations discussed in the text, transplanted oysters are considered valuable bioindicators of environmental contamination by PAHs and PCBs in areas lacking indigenous bivalves.

Acknowledgements

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TABLE I. Biological half-lives of selected PAHs and PCBs in transplanted and indigenous oysters

COMPOUND	OYSTERS HANNA REEF	OYSTERS SHIP CHANNEL	MUSSELS[1]
Phenanthrene	-	-	-
Fluoranthene	25.6	38.5	29.8
Pyrene	10.4	12.4	-
Benzo(a)anthracene	13.2	15.3	17.8
Chrysene	12.3	15.8	14.2
Benzo(e)pyrene	11.5	16.1	14.4
PCB#26	21	20	-
PCB#52	28	47	-
PCB#110	45	147	-
PCB#118	75	>year	-
PCB#149	129	>year	-
PCB#22	-	-	16.3
PCB#101	-	-	27.9
PCB#128	-	-	36.5
PCB#153	-	-	45.6

1 Pruell *et al.*, 1986

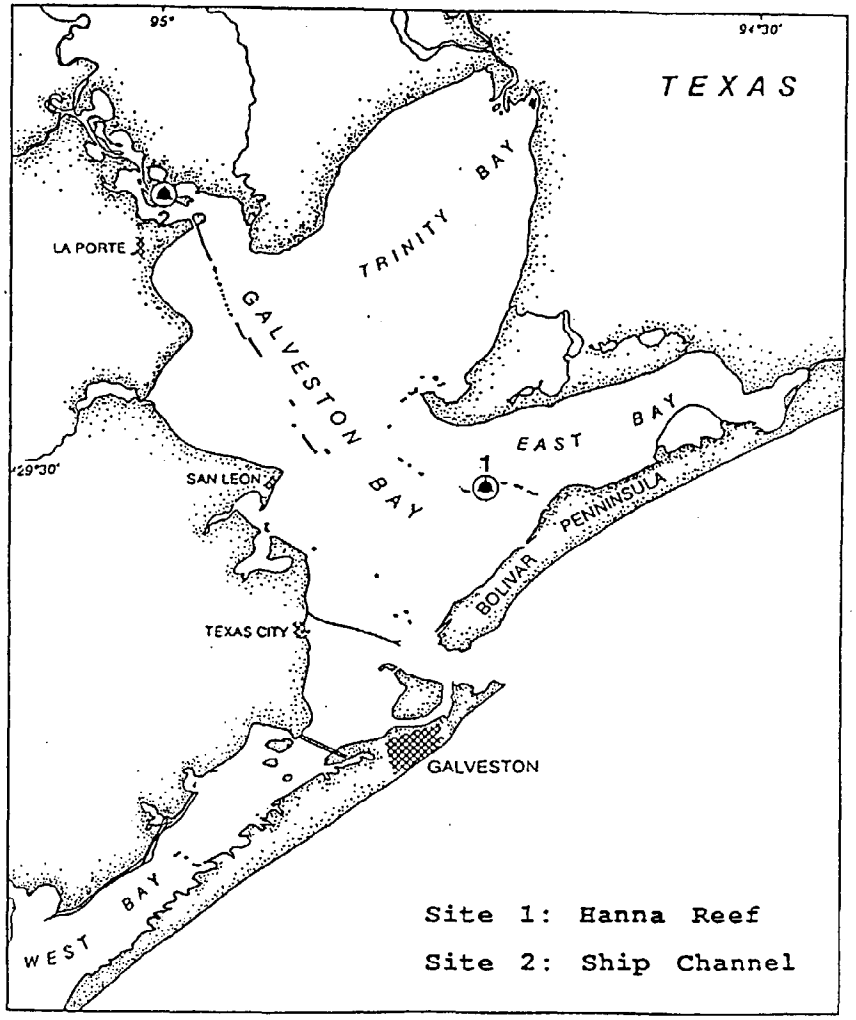


Figure 1: Galveston Bay transplantation sites.

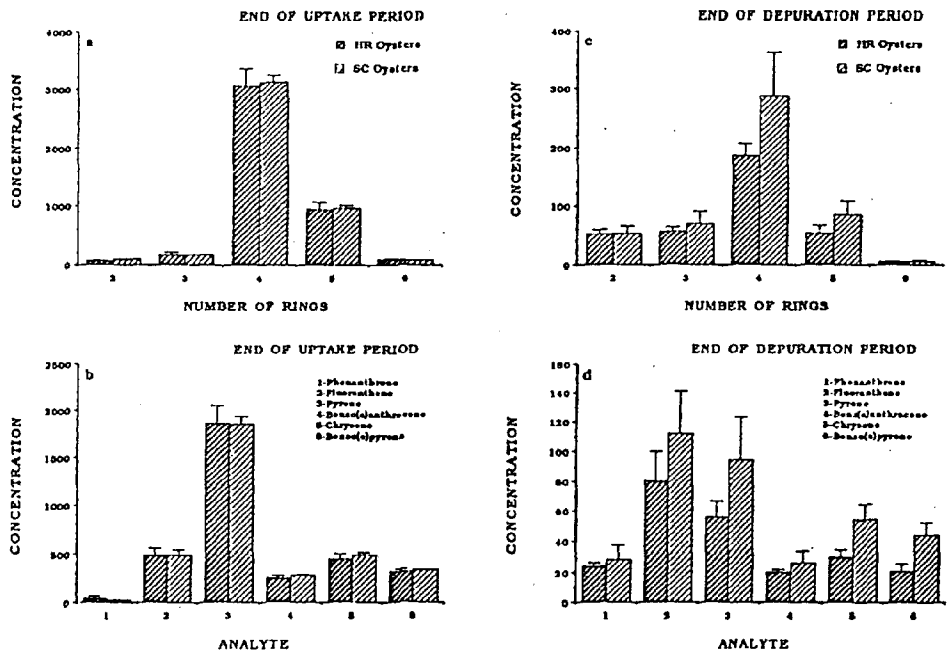


Figure 2: Total and selected individual polynuclear aromatic hydrocarbon concentrations (ng/g, dry weight) in Hanna Reef and Ship Channel oysters after the uptake and depuration periods. The error bars represent one standard deviation from the mean (n=4).

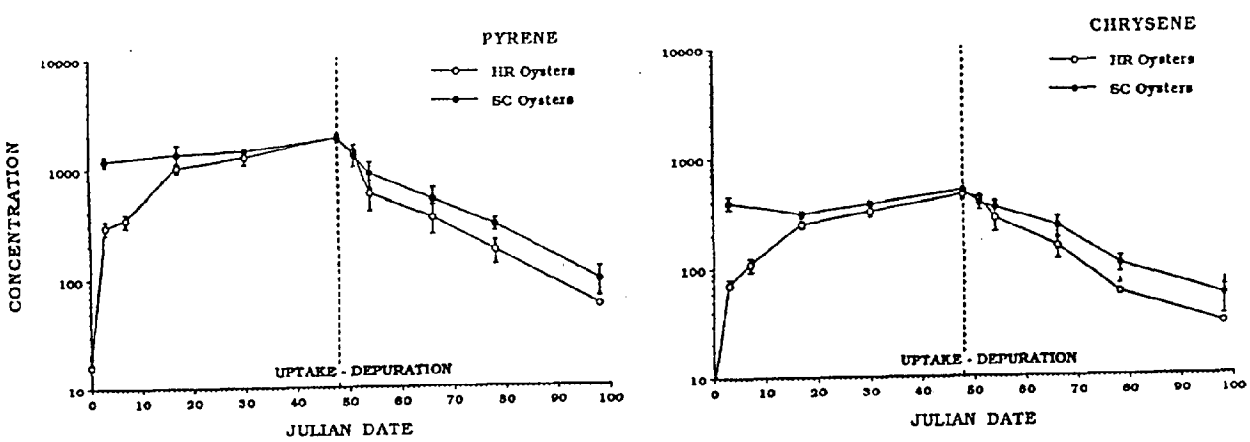


Figure 3: Selected PAH concentrations (ng/g, dry weight) in Hanna Reef and Ship Channel oysters during the uptake and depuration phases of the experiment.

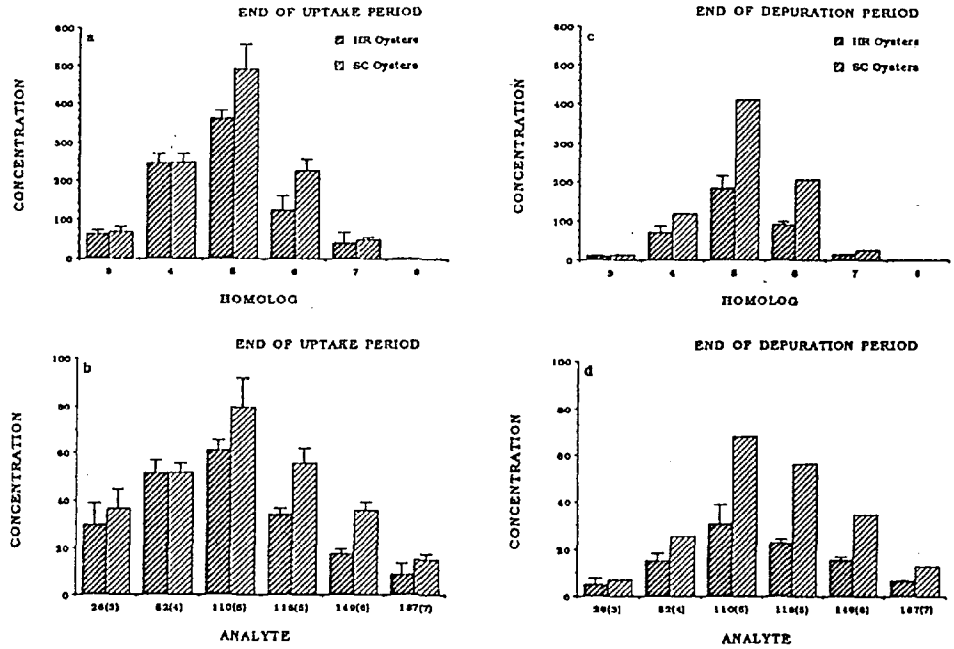


Figure 4: Homolog and selected individual polychlorinated biphenyl concentrations (ng/g, dry weight) in Hanna Reef and Ship Channel oysters after the uptake and depuration periods. The error bars represent one standard deviation from the mean (n=4).

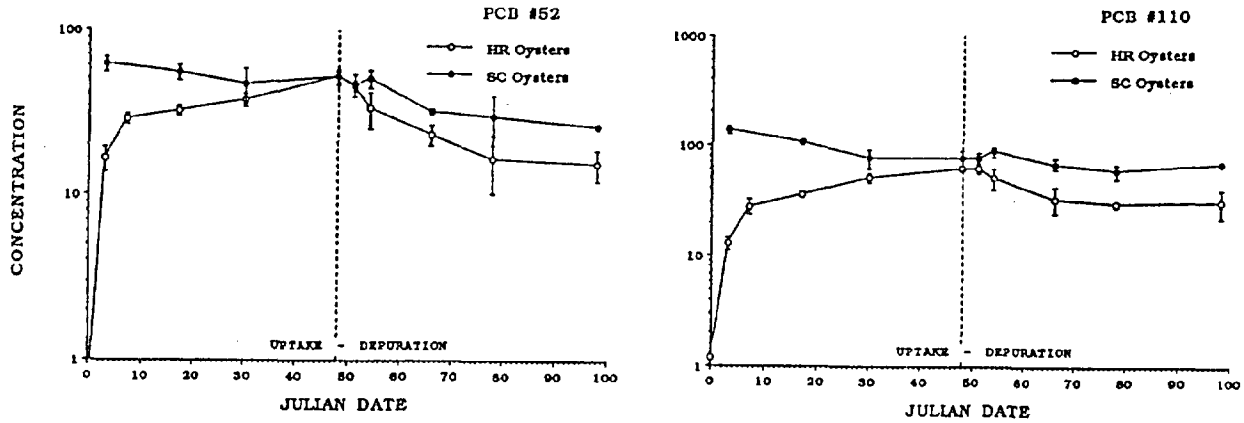


Figure 5: Selected PCB concentrations (ng/g, dry weight)in Hanna Reef and Ship Channel oysters during the uptake and depuration phases of the experiment.

Reprint 2

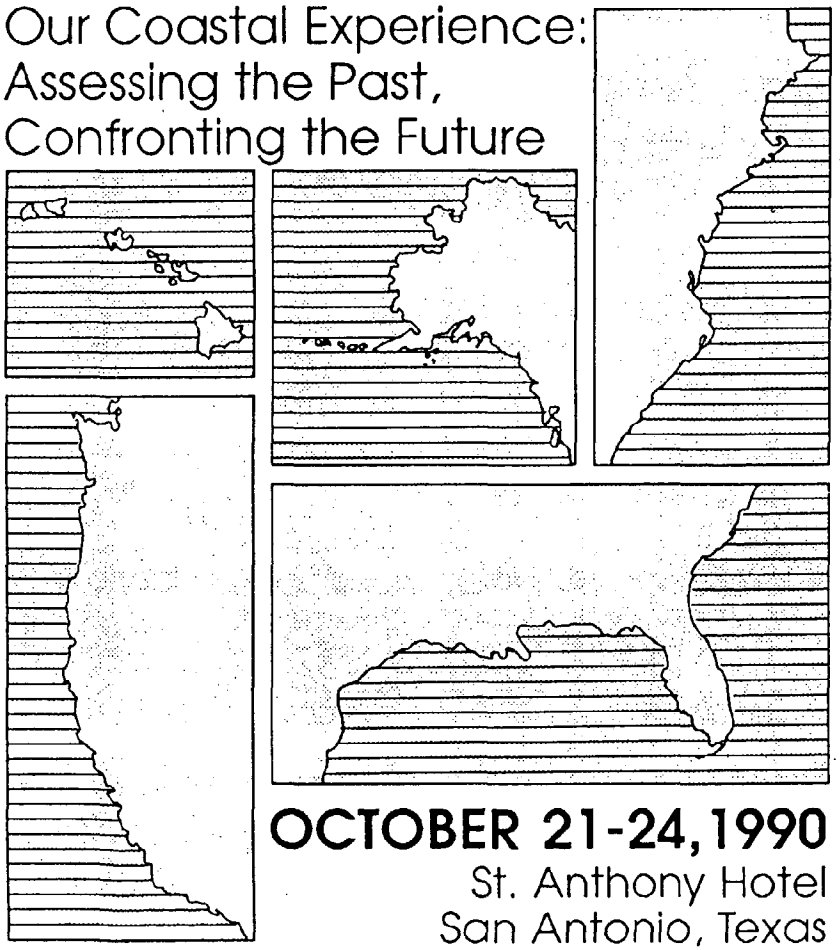
Overview of the First Four Years of the NOAA National Status and Trends Mussel Watch Program

Terry L. Wade, José L. Sericano, James M. Brooks, and Bobby J. Presley

The Coastal Society Twelfth International Conference

CONFERENCE PROCEEDINGS

Our Coastal Experience: Assessing the Past, Confronting the Future



OCTOBER 21-24, 1990

St. Anthony Hotel
San Antonio, Texas

Overview of the First Four Years of the NOAA National Status and Trends Mussel Watch Program

Terry L. Wade, Jose L. Sericano, James M. Brooks, and Bobby J. Presley
Texas A&M University

Abstract

Oysters are utilized as bioindicator organisms to characterize the current status and long-term trends for 13 trace elements and 57 organic contaminants from 75 Gulf of Mexico sampling sites. Sampling sites are distributed throughout the U.S. waters of the Gulf of Mexico, away from known point sources of input, and are sampled yearly in the winter to provide a geographical description of the chronic contaminant loading of the entire U.S. Gulf on a regional basis. Three stations at each site are analyzed individually to assess natural intra-site variability so that significant changes can be detected. Extensive intercomparison exercises assure the comparability of analytical measurements with companion studies on the East and West Coasts. The first four years of data for the Gulf of Mexico represents over 40,000 individual data points. The general trend from this large data set is contaminant concentrations that show no changes during the four-year sampling period. There are, however, certain sites that have experienced significant changes in contaminant concentration over the last four-year sampling period, including monotonic increases and decreases. Generally, the concentrations of the various contaminants do not show any significant relationship to each other. This is probably due to different input sources. Higher concentrations of most contaminants are associated with proximity to large urban areas. Two areas that appear to be exceptions to this generalization, St. Andrew Bay, FL and Choctawhatchee Bay, FL, are discussed in more detail.

Introduction

The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Mussel Watch Program has sampled and analyzed bivalves from U.S. coastal areas since 1986. This report summarizes the first four years of NS&T data for the Gulf Coast of the U.S. Sampling sites give coverage of the Gulf Coast from southernmost Texas to southernmost Florida. Portions of the data have been previously discussed (Wade et al., 1988, 1989, and 1990; Wade and Sericano, 1989; Sericano et al., 1990a and b) and only an overview is presented here.

Methods

The NS&T program utilizes standard operating procedures and a strong quality assurance/quality control program for trace element and trace

organic analyses. Details of these methods are found elsewhere (Brooks et al., 1989; Wade et al., 1988). The accuracy and precision of these methods have been established by several intercalibration exercises conducted by the U.S. National Institute of Standards and Testing and the Canadian National Research Council.

Results and Discussion

Exact sample location and the years in which samples were collected at each site are presented elsewhere (Wade et al., 1990). The geographical distribution for selected contaminants or suite of contaminants is shown in Figures 1 to 7. The sites are listed in geographical sequence starting with the southernmost Texas site and continuing along the coast to the southernmost Florida site. The smaller bars on Figures 1 to 4 represent "plus one standard deviation".

Trace metal concentrations in oysters varied considerably from site to site; in general, these variations were consistent over the four-year period. That is, the same sites showed consistently above or below average concentrations each year. The high concentrations, with very few exceptions, could not be shown to be associated with known activities of man, such as the presence of industry or oil well drilling operations. However, the fact that high values were often found in only one part of a particular bay (e.g., Tampa or Galveston Bay) while at other nearby sites in these same bays the concentrations were average or below for trace metals, suggests localized inputs of these metals.

Regional trends in trace metal concentrations in oysters are more likely to be due to geologic or climatic factors than to activities of man. Regional trends can be seen for only a few of the 13 metals assessed and, even for these, large site-to-site variations are superimposed on rather subtle regional trends. For example, Figure 1 shows the distribution of selenium (Se) for the entire Gulf Coast. A gradual decrease in concentration is apparent when concentrations from Texas and Louisiana are compared to those in south Florida, even though some high values are found in northern Florida.

Arsenic (Figure 2) is usually thought to be chemically similar to Se, but it shows a distribution pattern almost opposite to that of Se (Figure 1). Arsenic (As) is much higher in some of the Florida oysters than elsewhere on the Gulf Coast, yet some Florida oysters, for example those from most sites in Tampa Bay, had very low arsenic concentrations all four years. Only the Tampa Bay site at Navarez Park near the city of St. Petersburg was significantly enriched in As. Even the new site at Knight Airport on the edge of the city of Tampa was low in As. 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 As concentration in oysters and phosphate rock occurrence, shipping, or mining. The As distribution does seem to be controlled by local environmental inputs, as do certain other metal distributions. There seems to be no other explanation for high and low concentrations of trace metals to occur at adjacent sites, often in a given bay, and to have these patterns consistent from year to year.

Mercury (Hg) is generally enriched in Florida sites (Figure 3), where 12 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 where harvesting of oysters has been limited because of the potential threat to human health.

Silver (Ag) distribution (Figure 4) was more similar to that of Se than to As, being somewhat enriched in Texas relative to Florida. The most interesting feature of the Ag distribution is the low values in central Louisiana. This same pattern was seen for cadmium (Cd) and is somewhat surprising because central Louisiana Bays have been extensively disturbed by oil exploration activities and are immediately downstream of the Mississippi River outflow. In this area, then, intense activities by man does not seem to be influencing trace metal concentrations in oysters.

The regional geographical distribution of the concentration of the sum of 18 individual polyaromatic hydrocarbons (PAHs) (Wade et al., 1988) is shown in Figure 5. The concentration of PAHs for regional sites are plotted as the average. For example, for Galveston Bay 6 sites are averaged.

Two PAHs, fluoranthrene and pyrene, generally account for more than 25% of the total amounts detected. The predominance of these compounds would suggest the major source of PAHs is probably combustion and not oil seeps or oil spills. In general, higher PAH concentrations are found at major river mouths where you also generally find large urban areas and associated industrial complexes. This is not surprising, since urban runoff and sewage treatment plants are well known chronic sources of PAHs.

The Panama City and St. Andrew's Bay regions are exceptions to this trend. Their is no major river in these locations, yet they have the highest PAH concentrations. It is possible that these sites were affected by an episodic input of petroleum (i.e., spill). The hydrocarbon distribution at these sites indicates they may be contaminated by used crank case oil.

An extensive interpretation of the chlorinated pesticide and polychlorinated biphenyl (PCB) data has been published elsewhere (Wade and Sericano, 1989; Wade et al., 1988 and 1990; Sericano et.al., 1990a and b). Total DDT (sum of o-p'DDE + p-p'DDE + o-p'DDD + p-p'DDD + o-p'DDT + p-p'DDT) regional distribution for oyster

samples collected along the U.S. Gulf of Mexico coast is shown in Figure 6. Total DDT is the most abundant chlorinated pesticide found in Gulf of Mexico oysters. Most of the DDT is present as the metabolites, DDE and DDD. Less than 10% of the total contaminant load in oysters is the parent compound, DDT.

The regional distribution of total DDT shows that four of the five highest concentrations are associated with major river outfalls including the Brazos, Mississippi, Mobile, and Choctawatchee Rivers. There were also relatively high total DDT concentrations at St. Andrew's Bay and Panama City, although no major rivers are found there. These are the same regions where the PAHs were the highest. DDTs associated with soils may be transported downstream and collect in estuaries. This process provides a plausible explanation of the higher total DDT associated with major river outfalls. The continued use of DDT in Mexico and other Latin American countries and its atmospheric transport and deposition to the sampling areas is another possible source.

The regional distribution of PCBs is shown in Figure 7. PCBs were detected in all NS&T oyster samples analyzed from Gulf of Mexico waters. The highest regional concentration was in St. Andrew's Bay. As mentioned before for PAH and total DDT, this is an anomalous station and at present we do not know the reason for the high concentrations at this site. Possible sources of contaminants at this site may be nearby oil storage tanks and a paper/pulp mill. The PCB concentrations do not show much difference on a regional basis. All the regions have average concentrations within a factor of 5. There are somewhat higher concentrations near areas of higher population density (i.e., Galveston Bay, Mobile Bay, etc.).

Conclusions

Most of the contaminants monitored by the Status and Trends Program have relatively long environmental half-lives. These contaminants, in general, show no change in environmental concentrations over the first four years of this study as seen in the standard deviation for trace metals (Figures 1 to 4). There are specific sites that are exceptions to this general trend and they merit further detailed examination.

Acknowledgements

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_____, 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. Pages 274-280 In: Proceedings, Marine Technology Society '90, Washington, D.C., 1990.

As (ppm)
0
10
20
30
40
Texas
LA-MS-AL
Florida

Se (ppm)
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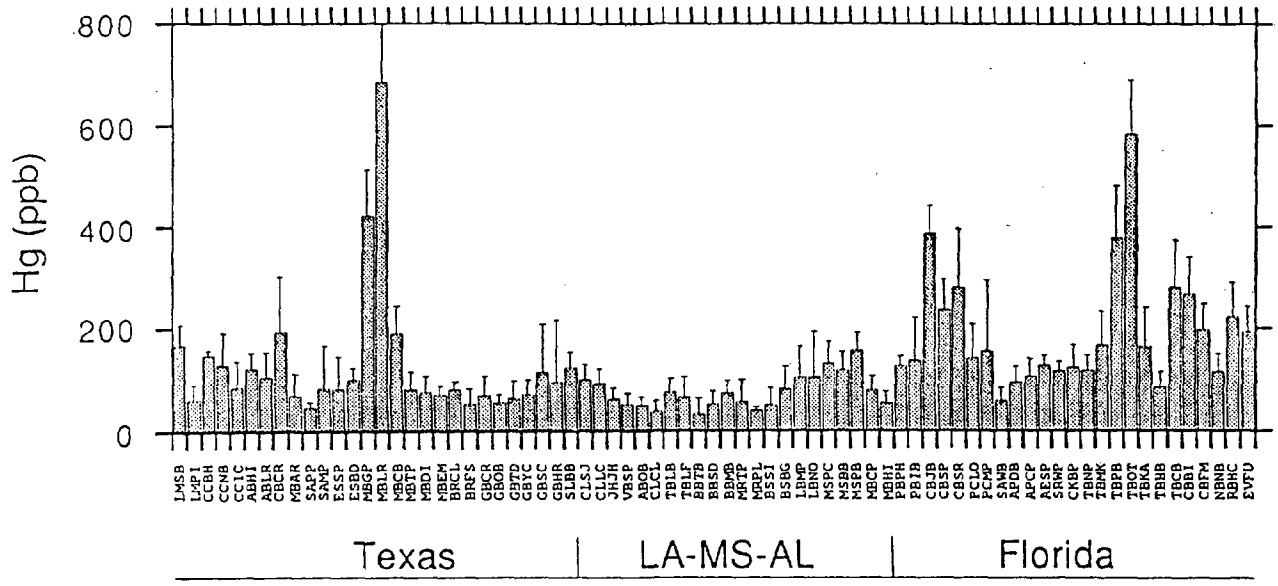
6
8
10
Texas
LA-MS-AL
Florida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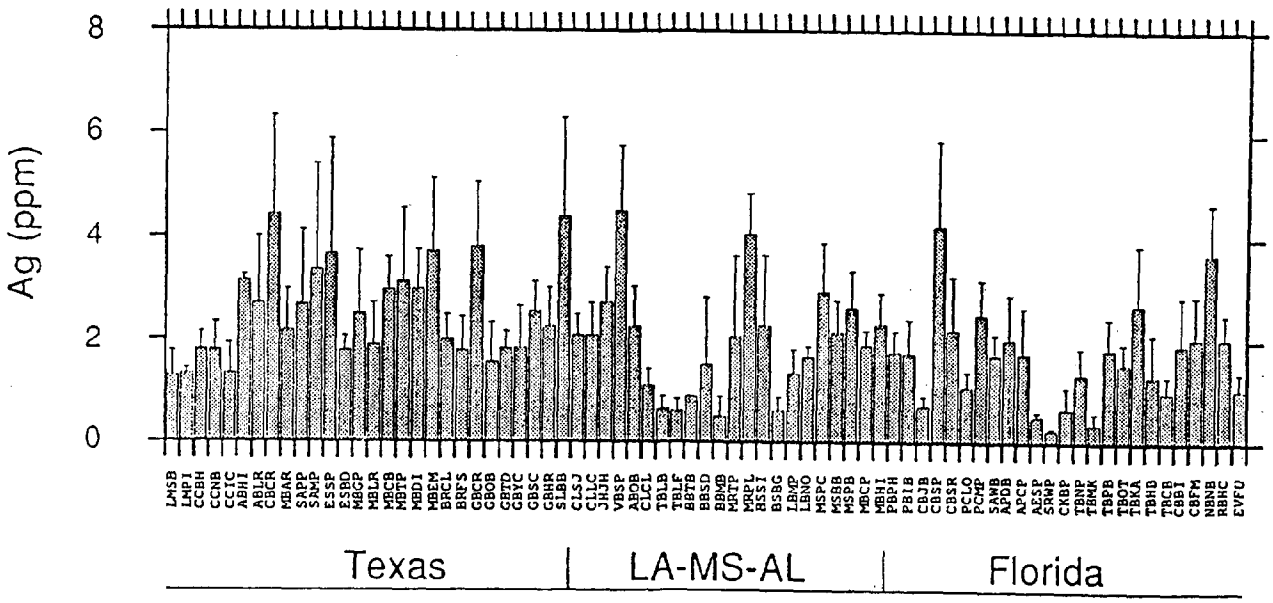
Texas
LA-MS-AL
Florida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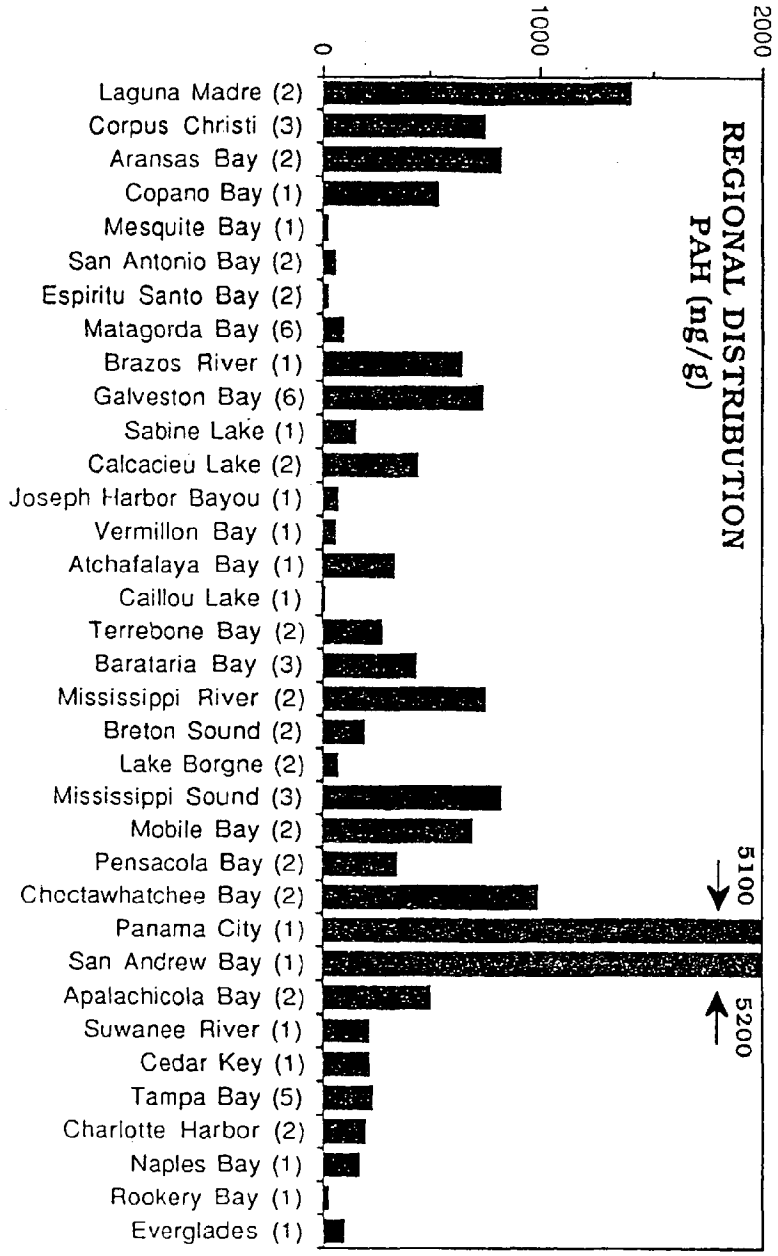
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Mesquite Bay (1)
San Antonio Bay (2)
Espiritu Santo Bay (2)
Matagorda Bay (6)
Brazos River (1)
Galveston Bay (6)
Sabine Lake (1)
Calcacieu Lake (2)
Joseph Harbor Bayou (1)
Vermillon Bay (1)
Atchafalaya Bay (1)
Caillou Lake (1)
Terrebone Bay (2)
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Breton Sound (2)
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Mobile Bay (2)
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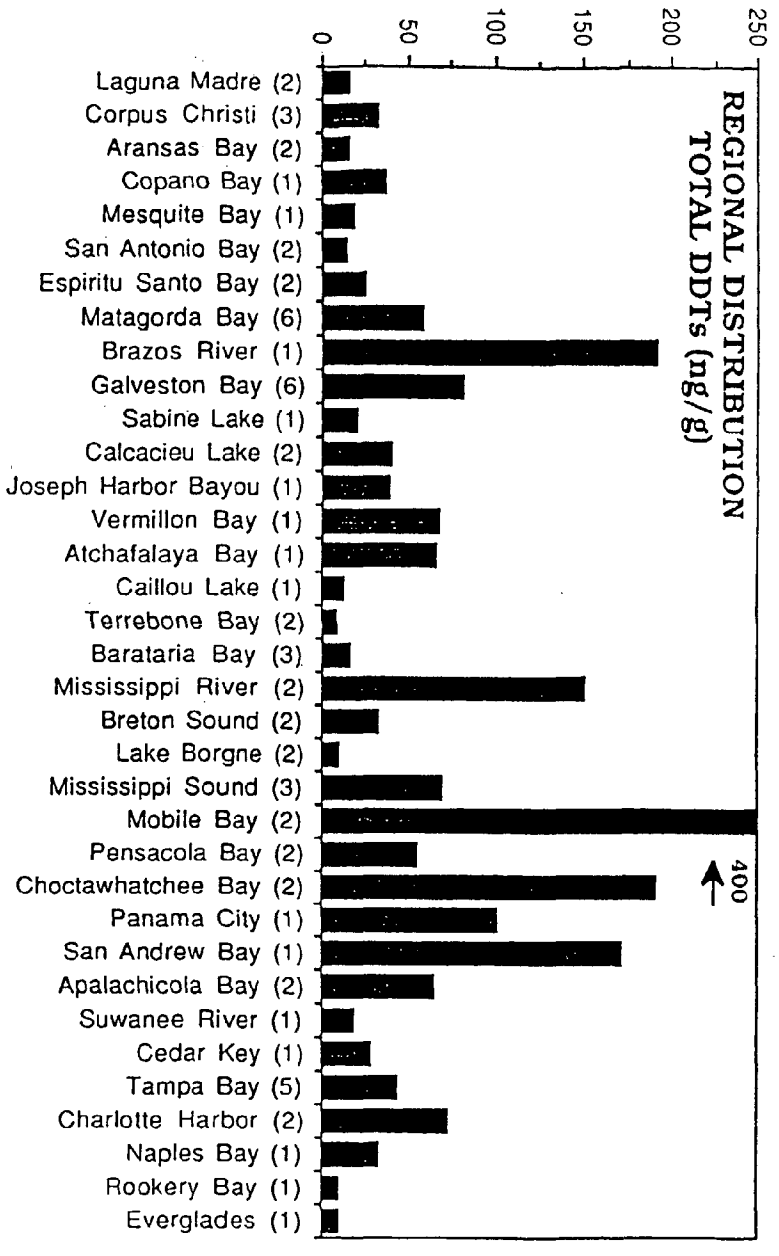
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Brazos River (1)
Galveston Bay (6)
Sabine Lake (1)
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Vermillon Bay (1)
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Caillou Lake (1)
Terrebone Bay (2)
Barataria Bay (3)
Mississippi River (2)
Breton Sound (2)
Lake Borgne (2)
Mississippi Sound (3)
Mobile Bay (2)
Pensacola Bay (2)
Choctawhatchee Bay (2)
Panama City (1)
San Andrew Bay (1)
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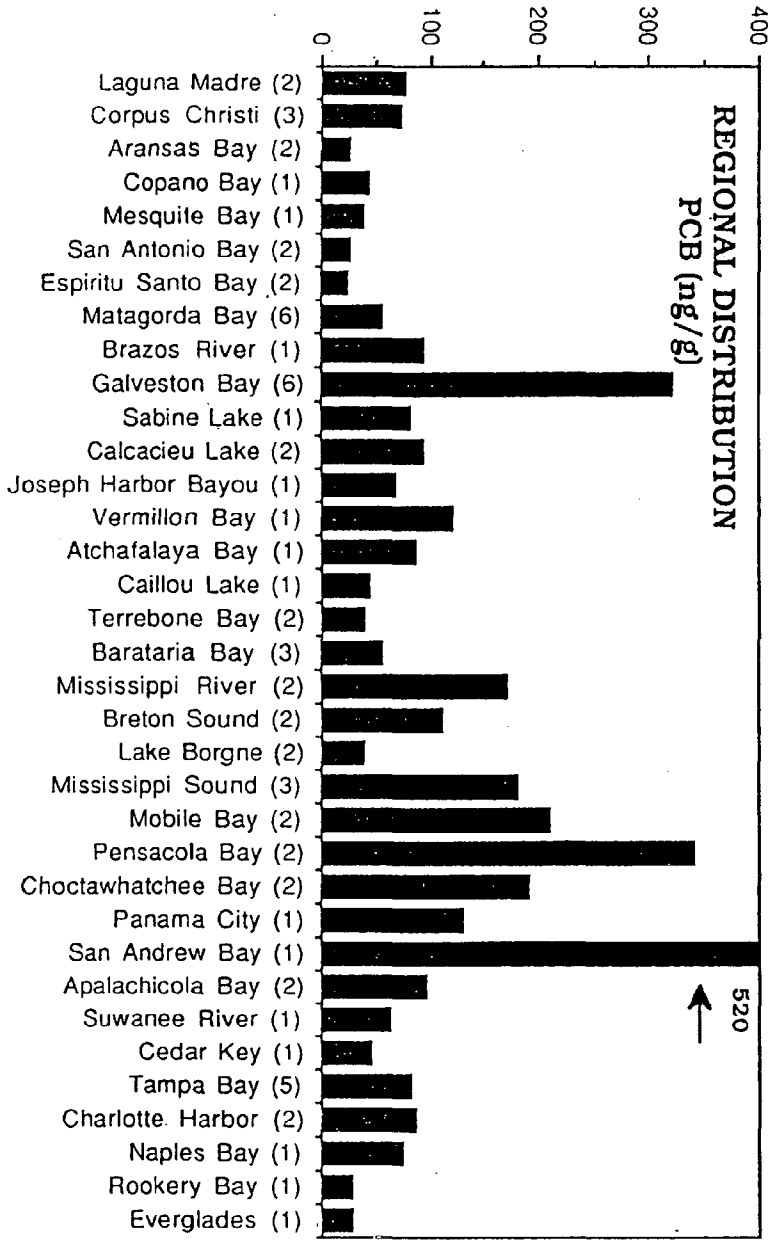
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Aransas Bay (2)
Copano Bay (1)
Mesquite Bay (1)
San Antonio Bay (2)
Espiritu Santo Bay (2)
Matagorda Bay (6)
Brazos River (1)
Galveston Bay (6)
Sabine Lake (1)
Calcacieu Lake (2)
Joseph Harbor Bayou (1)
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Atchafalaya Bay (1)
Caillou Lake (1)
Terrebone Bay (2)
Barataria Bay (3)
Mississippi River (2)
Breton Sound (2)
Lake Borgne (2)
Mississippi Sound (3)
Mobile Bay (2)
Pensacola Bay (2)
Choctawhatchee Bay (2)
Panama City (1)
San Andrew Bay (1)
Apalachicola Bay (2)
Suwanee River (1)
Cedar Key (1)
Tampa Bay (5)
Charlotte Harbor (2)
Naples Bay (1)
Rookery Bay (1)
Everglades (1)

Reprint 3

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

Terry L. Wade, Thomas J. Jackson, James M. Brooks, José L. Sericano, Bernardo Garcia-Romero and Dan L. Wilkinson

Proceedings

The Second State of the Bay Symposium
February 4-6, 1993

Editors

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It is important to determine the current status of contaminant concentrations in order to assess the environmental response to management decisions that reduce or stop the input of selected contaminants. To fill this information gap with high quality data for 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 contaminant inputs, including Galveston Bay. Oysters were employed as sentinel organisms because they are cosmopolitan, sedentary, bioaccumulate, able to provide an assessment of bioavailability, not readily capable of metabolizing contaminants, able to survive pollution loading, 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 U.S. 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, including petrochemical complexes and refineries, bordering the Galveston Bay estuarine system, 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. Galveston Bay NOAA NS&T sampling sites (Figure 1) included the Ship Channel (GBSC), Yacht Club (GBYC), Todd's Dump (GBTD), Hanna Reef (GBHR), Offats Bayou (GBOB) and Confederate Reef (GBCR). Samples were collected in the winter starting in January of 1986 at four sites (GBYC, GBTD, GBHR, GBCR) and in December of 1987 (Year 3) at two additional sites (GBSC, GBOB). Samples were collected at some of these sites at other times 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 (PAH), polychlorinated biphenyls (PCB), chlorinated pesticides (e.g., DDT, chlordane), and

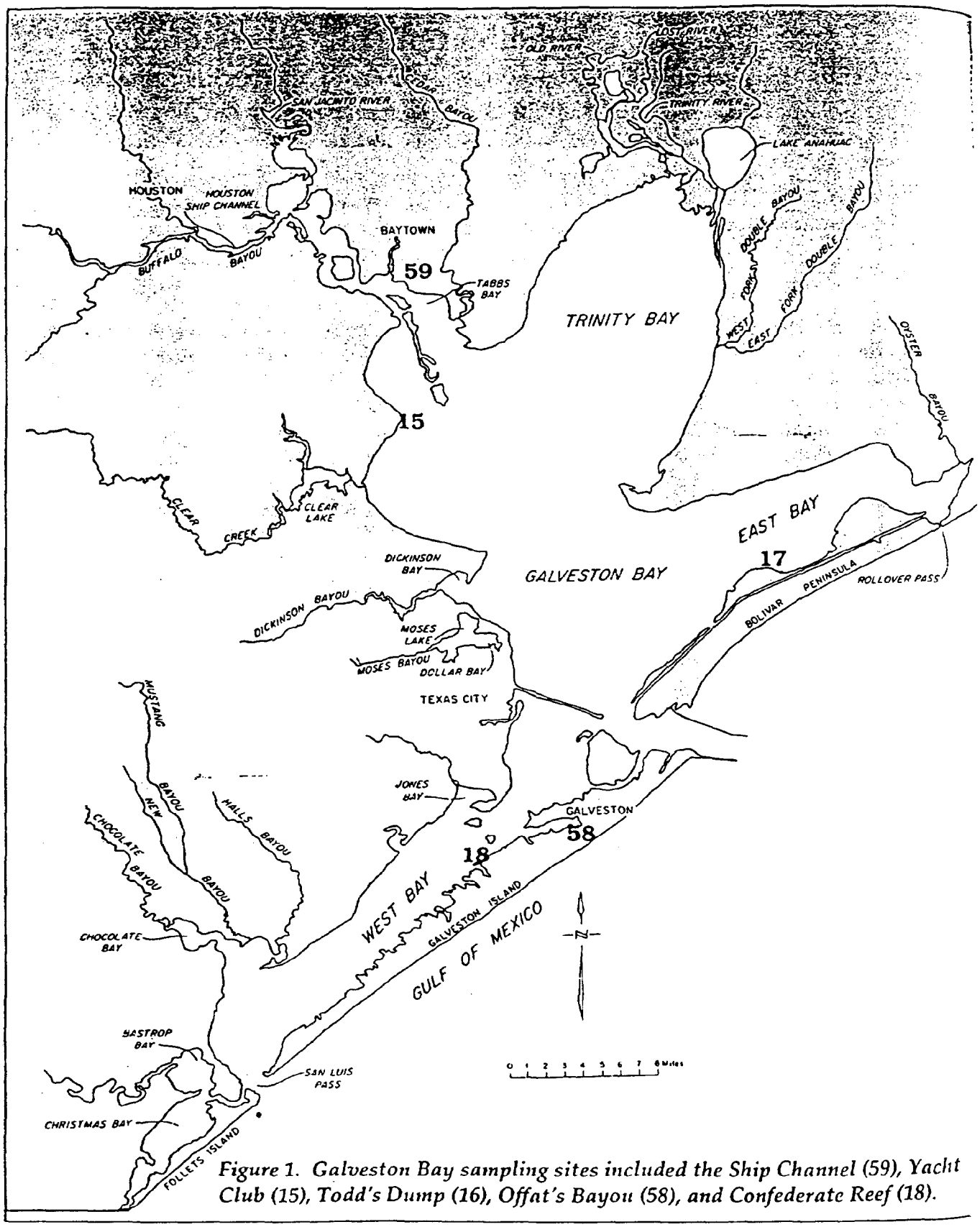

Figure 1. Galveston Bay sampling sites included the Ship Channel (59), Yacht Club (15), Todd's Dump (16), Offat's Bayou (58), and Confederate Reef (18).

tributyltin. Sampling started in the winter of 1985/86 and is continuing with sampling each winter. Seven years of data are currently available and Year 8 sampling has just been completed. All sample analyses were performed using standard operating procedures (SOPs) to provide high quality, precise, accurate, and reproducible data. Data quality was further assured by yearly 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.

Contaminant concentration patterns were similar for most contaminants. The upper bay sites (GBSC, GBYC) had higher concentrations than the mid-bay sites (GBTD, GBHR) for PAH, DDT, PCB, and butyltins. Sites from the lower bay (GBOB, GBCR) 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. For example, total PAH average concentrations ranged from 20 to 15,000 ng/g. The higher concentrations were measured in oysters from the upper portion of Galveston Bay (i.e., GBSC and GBYC) and near the city of Galveston (i.e., GBCR and GBOB). Oyster samples from areas farther away from urban centers (i.e., GBHR and GBTD) 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 suggests that the major source of PAHs is from 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 the GBYC and GBSC were the most contaminated while oysters from GBHR had the lowest residue concentrations.---These concentrations agree with the ranges reported earlier for Galveston Bay bivalves. The median concentrations found in Galveston Bay for PAH, chlordane, dieldrin, PCB, and butyltins are higher than the median concentrations found throughout the Gulf of Mexico for the NS&T Program. The median DDT concentrations found in Galveston Bay are about the same as those found for the entire Gulf of Mexico. Therefore, compared to the rest of the Gulf of Mexico the median concentrations of most organic contaminants are generally higher in Galveston Bay. However, when Galveston Bay sites are compared to all U.S. NS&T sites none of the concentrations, with the exception of chlordanes at GBYC and GBSC, are ranked as high on a national scale.

Sample collections at other times of the year indicate some seasonal variability of contamination concentrations. This may result from the loss of a considerable amount of contaminants by oysters during spawning. Other studies of Galveston Bay oysters indicate that body burdens of contaminants can change due to accumulation and depuration. These preliminary studies indicate that more information regarding the use of oysters as bioindicators would provide for better interpretation of the data from the NS&T program.

Reprint 4

Indicators of Trace Metal Pollution in Galveston Bay

Bobby J. Presley and Kuo-Tung Jiann

Proceedings

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Indicators of Trace Metal Pollution in Galveston Bay

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Sediments and organisms are usually more reliable and more convenient media for trace metal analysis than is water. Even polluted bay and estuarine water is very low in trace metal concentration, making it difficult to analyze reliably. Furthermore, concentrations in water are subject to rapid changes with changing metal inputs. Sediments and organisms have higher metal concentrations and they integrate values over time so less frequent sampling is needed.

Oyster (*Crassostrea virginica*) 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 USEPA 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 pollution levels in the environment, good accumulators of pollutants, widely distributed along coasts, and easy to collect and analyze. Sediments also respond to changes in pollutant trace metal inputs because most pollutant metals are particle reactive; that is, they readily attach to particles which can then sink to the bottom and become part of the sediments.

Oysters have been collected at six different sites in Galveston Bay (GB) since 1986 as part of NS&T. Each site is on an identifiable oyster reef and, for the first 5 years, twenty oysters were taken from each of three stations, the stations being 100 and 500 m apart. Currently only one station is sampled at each site. Each site has been sampled once each year, except two of the sites were not sampled the first two years. The twenty oysters from each station are combined and analyzed as a single sample each year. In most cases, stations are located hundreds of meters to many kilometers away from any obvious point sources of pollutant inputs in an attempt to characterize large areas of GB, rather than to identify specific point sources of pollutant input. Similar NS&T sampling is conducted in all other major bays and estuaries along the U.S. Gulf of Mexico coastline. The program allows different bays to be compared and pollutant concentration changes with time at a given bay to be documented.

Data obtained by atomic adsorption spectrophotometry (AAS) after acid digestion of oysters from the first four years of NS&T have been reported (Presley et al., 1990, 1991). 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. Blanks and reference materials were analyzed with the samples. Precision and accuracy of the data was estimated to be ±10%.

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 (Presley et al., 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.

Presley et al. (1991) reported that the oysters collected in Galveston Bay during the first four years of NS&T were similar in trace metal content to those collected elsewhere along the Gulf coastline, i.e., there was no indication of generalized trace metal pollution in GB. The average Ag, Cd, Cr, Fe, Mn, and Pb in GB oysters differed by 10% or less from the Gulf-wide average. Copper was 13% higher in GB, while Ni was 15% higher, and Se 16% higher. Zinc, however, was 43% higher. Furthermore, the highest Zn levels were found along the industrialized west side of Galveston Bay.

The four year NS&T sampling and analysis of oysters from the Gulf Coast discussed by Presley et al. (1990, 1991) has been continued for three more years with at least an additional three years planned. The basic patterns in concentration variability seen earlier have not changed significantly. With few exceptions, Galveston Bay oysters continue to be about average in trace metal content when compared to oysters from other bays along the Gulf Coast. Furthermore, oysters from near the entrance to the inland part of the Houston Ship Channel and from the industrialized western shoreline have about the same metal content as those from pristine areas of East and West Bays.

In non-funded student research designed to further investigate the relationship between trace metal concentrations in oysters and proximity of industry, samples were taken at twelve sites at the end of June and at the end of September, 1992. At most sites, 10-30 individual oysters were taken. They were collected, handled, and analyzed as described previously (Presley et al., 1990). No oysters were collected in extreme northern Galveston Bay, but shoreline samples were taken near Eagle Point and the highly industrialized areas of Texas City. Samples were also taken in central GB along the open-water part of the Houston Ship Channel and from East and West Bays.

Most trace metal concentrations were lower in oysters collected in September, 1992, than those collected at the same locations in June, 1992. In many cases, the decrease was by a factor of two and was, thus, larger than most site to site differences in the bay. It is very unlikely that this change was caused by human activity because there is little correlation between metal concentrations in oysters and proximity to population or industry, and even Fe concentrations in the oysters changed by up to a factor of two. Rather, the change in trace metal concentration must be related to some physiological change in the oysters. In order to minimize such changes, oysters are always collected in December for NS&T. The September, 1992, data is similar to the six-year average NS&T data, so perhaps oysters change in metal content less during fall and winter.

Silver concentrations are above the Gulf-wide average in several GB samples, but with no clear relationship to proximity to industry. Very high Ag concentrations were found in oysters collected at Confederate Reef in years V and VI (1990-1991) of NS&T, but not in previous years. A site on Deer Island near Confederate Reef was sampled for the 1992 student work. Oysters from it were somewhat higher than average in Ag content, but no more so than those from other sites in Galveston Bay. It is possible that human activity is responsible for the silver and zinc enrichments but no specific causative activity can be identified. In any case, the enrichments are not high enough to harm the oysters or human health.

Based on the discussion above and other data from our laboratory, oysters seem to integrate trace metal concentrations in the surrounding environment for one to two months. For a longer integration period sediments can be analyzed. As part of the unpublished student work reported here, sediments were collected at nineteen locations throughout Galveston Bay, including Morgan's Point and other locations along the industrialized northern and western shoreline, as well as locations far back into East Bay well away from industry. The sediment was sieved to separate the <63 μm grain size fraction, which was analyzed along with an aliquot of the unsieved bulk sample. Analysis was by AAS after both a partial leach of the sample with 0.5 N HCl and complete dissolution using HNO_3-HCl-HF. Results showed the sediment to be generally constant in trace metal concentration from place to place when the <63 μm size fractions were compared and to be similar to sediment from other Texas bays which were analyzed for NS&T. Average concentrations of metals in the <63 μm fraction of Galveston Bay sediments and the percentage of that metal leachable with 0.5 N HCl are shown in Table 1, along with average values for other Texas bays (normalized to 100% <63 μm grain size). Data from Morse et al. (in press) on another set of sediment samples taken from throughout GB confirms the relative constancy of trace metal concentrations. The most notable exception to sediment trace metal constancy found in the present work was a sample taken near the end of the Texas City Dike. It had <0.5% fine material but that fine material was enriched in several metals. Based on other data from this laboratory, it may well be that the fine fraction of very sandy sediment is easily enriched in trace metals from human activity.

Table 1. Average concentrations of trace metals in the <63 μm size fraction of Galveston Bay and other Texas bay sediments.

		Fe (%)	Ag (ppm)	As (ppm)	Cd (ppm)	Cu (ppm)	Ni (ppm)	Pb (ppm)	Zn (ppm)
GB	Avg	2.9	0.164	8.21	0.157	28.7	23.9	24.5	98.8
GB	S.D.	0.7	0.040	1.57	0.106	15.5	4.4	4.6	22.7
GB	Leach (%)	15	61	19	76	56	21	68	33
TX	Avg.	2.12	0.156	7.91	0.253	15.1	17.7	24.5	85.3
TX	S.D.	0.83	0.055	3.27	0.171	3.5	4.2	6.0	25.2

Several species of finfish (flounder, drum, trout, catfish, etc.) as well as blue crabs and oysters were collected from Galveston Bay in May-September, 1990, for the Galveston Bay National Estuary Program (GBNEP) (Brooks, 1992). These were analyzed for trace metals in our laboratory following procedures used for NS&T (Presley et al., 1990). The samples came from near Morgan's point, Eagle point, Hannah Reef and Carancahua Reef; thus, from areas of contrasting proximity to population centers and industry. In spite of the contrasts between the collection sties, no clear differences were found in trace metal concentrations in the organisms. Furthermore, the GB organisms were similar in trace metal content to organisms from non-polluted bays elsewhere.

Oysters are better accumulators of trace metals and, being attached to the sediment, should better characterize a site than the other organisms collected for GBNEP. The GBNEP oysters were generally similar in trace metal content to NS&T oysters from GB, but somewhat lower in Zn concentration. Zinc was also less clearly related to population and industry than in NS&T.

GB fish flesh was much lower in trace metals than oyster flesh and while isolated high values were found, concentrations were generally similar to those found in non-contaminated bays elsewhere. Trace metals in fish showed no relationship to population or industry. Fish livers proved to have much higher concentrations of trace metals (except for Hg) than fish flesh and high variability, but again no clear relationship to population or industry. Blue crabs from GB were generally intermediate in trace metal concentration between fish flesh and oyster flesh with similar high variability and lack of correlation with population or industry. The GBNEP data, therefore, gave no indication of trace metal pollution in Galveston Bay.

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Reprint 5

Oysters as Biomonitors of the APEX Barge Oil Spill

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

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OIL POLLUTION CONTROL. A COOPERATIVE EFFORT

OYSTERS AS BIOMONITORS OF THE APEX BARGE OIL SPILL

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ABSTRACT: *The collision of the Greek tanker ship* Shinoussa *resulted in a spill of an estimated 692,000 gallons of catalytic feed stock oil into Galveston Bay on July 28, 1990. Oysters were collected from Galveston Bay Todds Dump (GBTD) 235 days previous to the spill and 6, 37, 132, and 495 days after the spill. Oysters were also collected from Galveston Bay Redfish Island (GBRI), a site known to be impacted by the spill, 37 and 110 days after the spill. The concentration of the 24 polynuclear aromatic hydrocarbons (PAH) measured for the National Oceanic and Atmospheric Administration's national status and trends program (NS&T) site showed a sharp increase from about 100 ng/g to over 600 ng/g one week after the spill compared to concentrations 235 days previous to the spill. The concentration of the 24 NS&T PAH in oysters from GBRI ranges from 400 to over 1000 ng/g. Soon after the spill the concentration of the 24 NS&T PAH at Todds Dump decreased to levels not statistically different from pre-spill samples. However, analyses of alkylated and sulfur containing aromatic compounds indicate the oysters were still contaminated with Apex barge oil at least 37 and 110 days after the spill at GBTD and GBRI, respectively. Data from NS&T sampling at GBTD more than a year after the spill (495 days) indicates the presence of alkylated aromatic hydrocarbons that may be from Apex barge oil still in the area. It appears that a sink of Apex barge oil (i.e., in sediments) may periodically be released by storms or other events into the ecosystem near GBTD. Therefore, bioavailable Apex barge oil is still present and may adversely affect oysters 495 days after the spill.*

Oysters are analyzed as part of NOAA's national status and trends (NS&T) program[9] to determine the current status and long-term trends of selected contaminant loadings. As part of this program, polynuclear aromatic hydrocarbons (PAH), toxic components of oils, are measured. Coastal waters are continually impacted by chronic inputs of PAH from wastewater treatment plants, storm water runoff, atmospheric deposition, and the like. The NS&T program is designed to determine the extent of this chronic contamination throughout the entire U.S. coastal area including the Gulf Coast. However, sporadic inputs of PAH into the coastal environment also come from small- and large-scale oil spills.[4] The seven years of historical NS&T data along with more recent U. S. EPA environmental monitoring and assessment-near coastal (EMAP-NC) data can be used as the basis for a geochemical and environmental response strategy (GEARS) as described by Brooks et al.[1] This approach utilizes available data to provide historical control sites to determine the extent of a spill and allow for a better estimate of ecosystem exposure.

On July 28, 1990, an estimated 692,000 gallons of catalytic feed stock oil product was spilled into Galveston Bay when a tanker collided with three Apex barges in the Houston Ship Channel. The spill was within a mile of Todds Dump (GBTD), one of the historical NS&T oyster sampling sites. The spill resulted in the closure of recreational and commercial fisheries for several days. A study of fish exposed to the Apex barge oil spill indicated that they efficiently metabolize the PAH after the initial insult.[6,7] This report discusses the use of oysters as biomonitors of the Apex barge oil spill at the historical NS&T Todds Dump site and at Redfish Island, a site reported to be impacted by the oil spill.[3]

Materials and methods

Oysters (*Crassostrea virginica*) were collected for analyses from Galveston Bay Todds Dump and Redfish Island. Individual stations at each site are generally from 100 to 1,000 m apart. An analysis at each GBTD, from routine NS&T sampling program, represents a composite of 20 individual oysters. However, samples from GBTD and GBRI taken 6, 37, and 110 days after the spill represent from 1 to 20 oysters depending on availability.

Tissue extraction followed the method used for NS&T.[9] 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 is extracted three times with dichloromethane using a tissuemizer. The solvent is concentrated to approximately 20 mL in a flat-bottomed flask equipped with a three-ball Snyder column condenser. The tissue extract is then transferred to Kuderna-Danish tubes heated in a water bath (60° C) to concentrate the extracts to a final volume of 2 mL. During concentration, the solvent dichloromethane is exchanged for hexane.

The tissue extracts are fractionated by alumina:silica (80 to 100 mesh) open-column chromatography. The silica gel is activated at 170° C for 12 hours and partially deactivated with 3 percent distilled water (v/w). Twenty grams of silica gel are slurry packed in dichloromethane over 10 grams of alumina. Alumina is activated at 400° C for four hours and partially deactivated with 1 percent distilled water (v/w). The dichloromethane is replaced with pentane by elution. The extract is then applied to the top of the column. The extract is 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 is further purified by high-performance liquid chromatography to remove lipids. The lipids are removed by size exclusion using dichloromethane as an isocratic mobile phase (7 mL/min) and two 22.5 × 250 mm Phenogel 100 columns.[5] The purified aromatic fraction is collected from 1.5 minutes prior to the elution of 4,4'-dibromo-octafluorobiphenyl to 2 minutes after the elution of perylene. The retention times of the two marker peaks are checked prior to the beginning and at the end of a set of ten samples. The purified aromatic fraction is concentrated to 1 mL using Kuderna-Danish tubes heated in a water bath at 60° C.

Quality assurance for each set of 20 samples includes a procedural blank, matrix spike, duplicate, and tissue standard reference material (NIST-SRM 1974), which are carried through the entire analytical

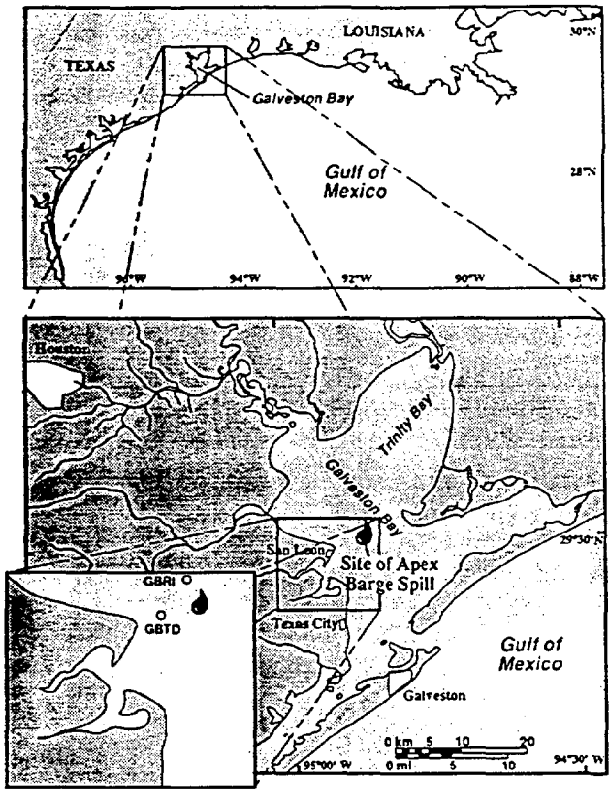

Figure 1. Location of Galveston Bay Todds Dump (GBTD) and Galveston Bay Redfish Island (GBRI) collection sites and the Apex barge oil spill

scheme. Internal standards (surrogates) are added to the samples prior to extraction and are used for quantitation. The surrogates are d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene. Surrogates are 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 are added just prior to GC-MS analysis.

Gas chromatography-mass spectrometry (GC-MS). The PAHs were separated and quantified by GC-MS (HP5890-GC interfaced to a HP5970-MSD). The samples were injected in the splitless mode onto a 0.25 mm × 30 m (0.32 μm 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 multicomponent standard at five concentrations ranging from 0.01 ng/μL to 1 ng/μL. 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 to confirmation ion.

Calibration check samples are run with each set of samples (beginning, middle, and end), with no more than 6 hours between calibration checks. The calibration check must maintain an average response factor within ± 10 percent for all analytes, with no one analyte greater than ± 25 percent of the known concentration. A laboratory reference oil solution is also analyzed with each set of samples to confirm GC-MS system performance and peak identification.

Results and discussion

The location of the Apex barge oil spill is shown in Figure 1. A detailed account of the spill, including cleanup and bioremediation activities has been published.[3] The spilled oil was described as a catalytic feed stock or similar to a No. 5 fuel oil[8] with a density of 0.92 g/mL. The Apex barge oil is not a typical Gulf Coast oil, but resembles a distillate or refined product. This is apparent from the gas chromato-

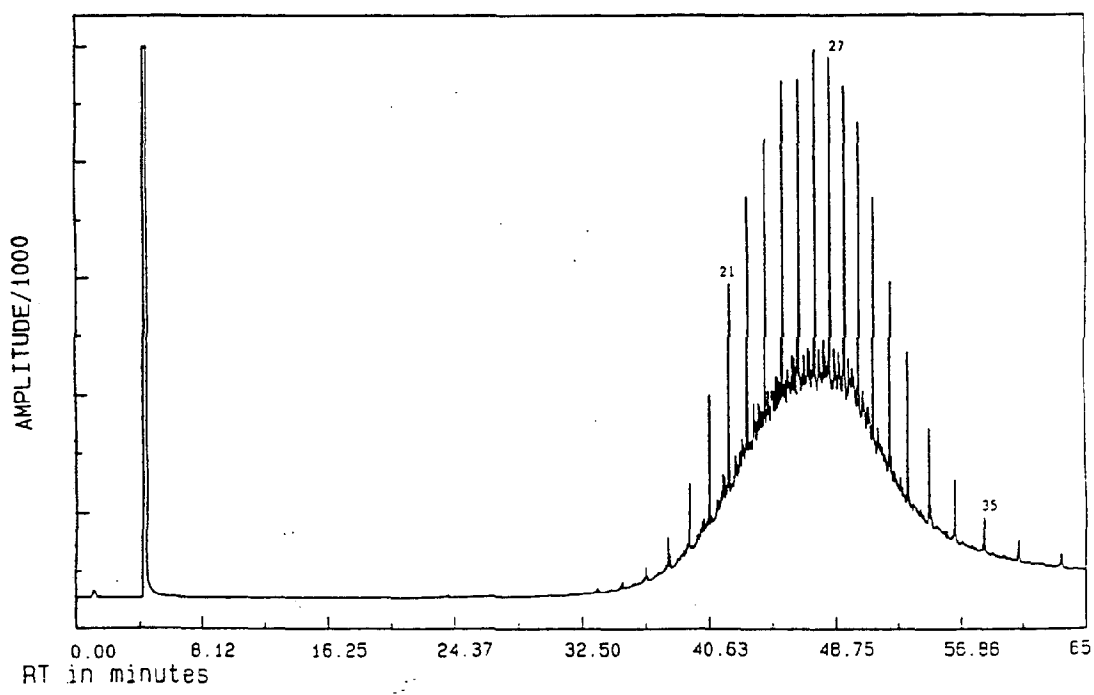

Figure 2. Gas chromatogram of Apex barge oil spilled into Galveston Bay (normal alkanes with 21, 27, and 35)

Table 1. Oyster NS&T PAH, total PAH, and C_3-phenanthrene concentrations before and after the Apex barge oil spill

Site	Collection date	Days before (−) or after spill	NS&T PAH (ng/g)	Total PAH (ng/g)	C_3-phenanthrenes (ng/g)
GBTD3	12/6/89	−235	141	1,057	10
GBTD 2	12/6/89	−235	175	471	10
GBTD 1	12/6/89	−235	323	1,893	10
Apex Spill	7/28/90	0	–[1]	–[1]	–[1]
GBTD	8/3/90	6	705	14,411	2,293
GBTD	9/3/90	37	122	852	77
GBTD 1	12/7/90	132	120	877	122
GBTD 2	12/7/90	132	150	1,204	178
GBTD 3	12/7/90	132	364	4,313	781
GBTD	12/5/91	495	236	1,997	213
GBRI 1	9/3/90	37	790	19,146	2,735
GBRI 2	9/3/90	37	470	12,723	1,636
GBRI	11/15/90	110	1,110	25,213	3,238

1. Not applicable

graph (GC) shown in Figure 2. The GC is a plot of detector response versus increasing temperature and time. The area of peaks and their retention times are used to determine the identity and concentration of the components. The labeled peaks in Figure 2 are normal alkanes with 21, 27, and 35 carbon, respectively. Other peaks represent normal alkanes ranging from 15 to 37 carbons. The presence of only these higher boiling components is consistent with a distillate or refined product and is not typical of a Gulf Coast oil.

The total oyster concentration of the 24 PAH measured as part of NOAA NS&T program, the total of all PAH measured, and the concentration of all the phenanthrenes containing 3 carbon (C_3-phenanthrene) are provided in Table 1. Some of these data are also presented graphically in Figures 3 and 4. The total NS&T PAH ranged from 120 to 1110 ng/g. The concentrations found at GBTD 235 days before the spill ranged from 141 to 323. This GBTD data shows an increase in concentrations as you move from the western onshore station (GBTD-3) to the offshore station (GBTD-1). The 24 NS&T PAH were only diagnostic of the spill at higher concentrations (i.e., greater than 400 ng/g). Total PAH concentrations in oysters from GBTD and GBRI ranged from 471 to 25,213 ng/g. The total oyster PAH concentrations when plotted versus the number of days before or after the spill that the samples were collected (Figure 3) clearly show the influence of the Apex barge spill at concentrations above 10,000 ng/g. Based on total PAH in oysters, the bioavailability of Apex barge spill oil is clearly evident for the sample collected at GBTD 6 days after the spill and samples at GBRI, located closer to the spill, 37 and 110 days after the spill (Table 1 and Figure 3). The oyster total PAH concentration was 852 ng/g at GBTD 37 days after the spill. The total PAH oyster concentration 235 days before the spill at the three GBTD sites was 1057, 471, and 1893, respectively (Table 1). Therefore the concentration at GBTD 37 days after the spill is within the range of concentrations of total PAH found before the spill (Figure 3).

The variability within a site for oyster samples collected on the same date makes evaluation of input from the Apex barge spill more problematic. However, the total PAH in these samples were predominantly lower molecular weight alkylated naphthalenes, alkylated fluorene and C_1- and C_2-phenanthrenes. No C_3-phenanthrenes were detected (Table 1). Based on the fact that the Apex barge oil that was spilled contained predominantly higher molecular weight hydrocarbons (Figure 2), it is not surprising that C_3-phenanthrenes might be better indicators of oyster exposure to the Apex barge oil than NS&T PAH or total PAH. Therefore, the concentration of C_3-phenanthrene was plotted versus the days before or after the spill (Figure 4). This log plot indicates a clear distinction between oysters collected before the spill and those collected after the spill. All samples collected after the spill had detectable concentrations of C_3-phenanthrenes, while none of the samples collected before the spill do.

The data for all the PAH individual compounds or groups of compounds that are present in the Apex barge oil as well as oysters collected from GBTD 235 days before and 132 days after the spill are shown in Figure 5, 6, and 7, respectively. A description of the components that were measured and plotted is listed in Table 2. These plots provide a "fingerprint" of the Apex barge oil and the PAH distribution found in the oysters. Clear differences appear in these fingerprints. The GBTD sample from NS&T Year 5 (235 days before the spill) sampling contains mostly peaks in the left side of the plot (Figure 6) indicating a predominance of lower molecular weight PAH. The GBTD NS&T Year 6 (132 days after the spill) sampling has mostly peaks in the midrange of the plot (Figure 7). The fingerprint from the GBTD oysters after the oil spill is similar to the fingerprint for the Apex barge oil (Figure 5). This indicates that these oysters are still being exposed to Apex barge oil. The NS&T oyster samples collected in Year 7 (495 days after the spill) also appear to contain a PAH fingerprint consistent with bioaccumulation of Apex barge oil.

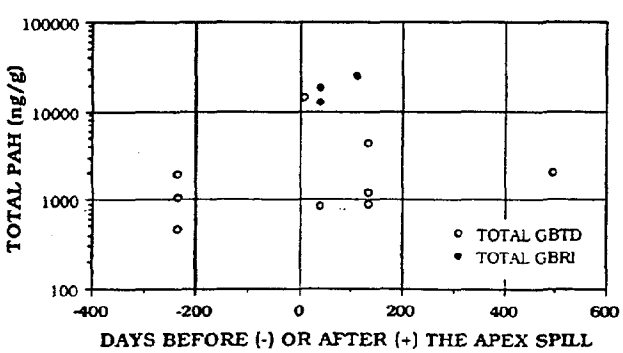

Figure 3. Total PAH concentrations in oysters before and after the Apex barge oil spill

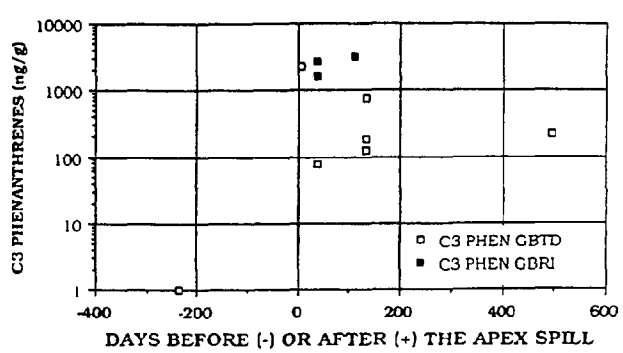

Figure 4. C_3-phenanthrene concentrations in oysters before and after the Apex barge oil spill

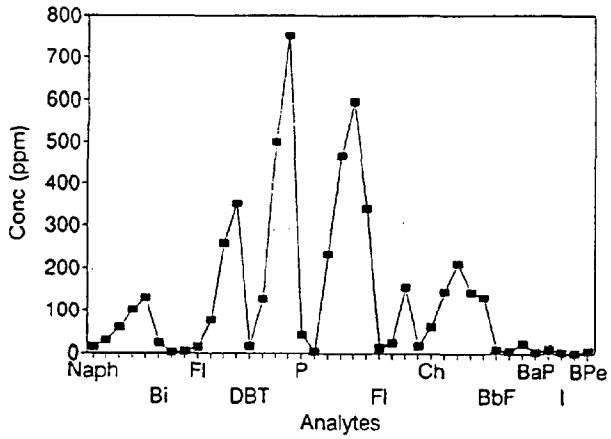

Figure 5. Apex barge oil PAH fingerprint (see Table 2 for abbreviations)

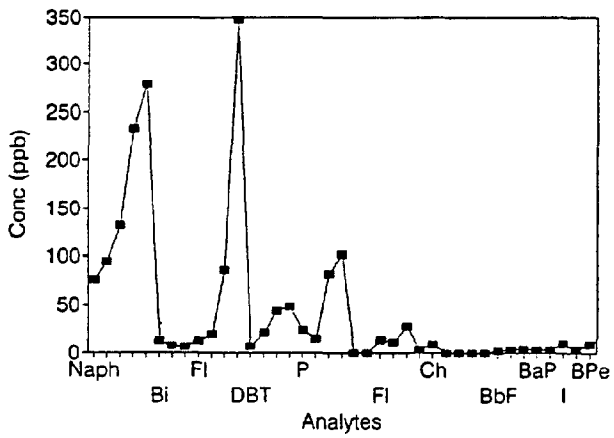

Figure 6. Oysters PAH fingerprint 235 days before the Apex barge oil spill, NS&T Year 5 (see Table 2 for abbreviations)

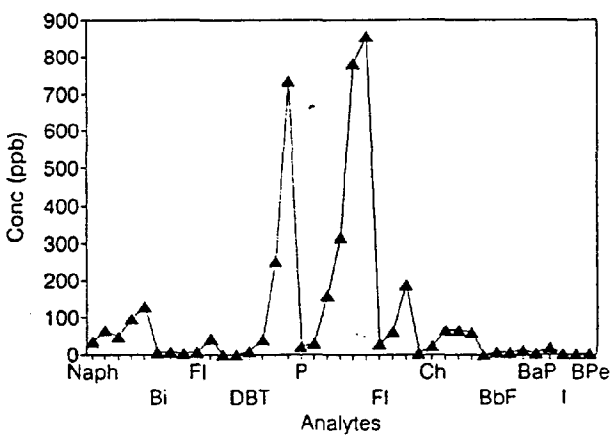

Figure 7. Oyster PAH fingerprint 132 days after the Apex barge oil spill, NS&T Year 6 (see Table 2 for abbreviations)

Table 2. Polynuclear aromatic hydrocarbons (PAH) analyzed

Abbreviation[1]	Analyte
Naph	naphthalene
	C_1-naphthalenes
	C_2-naphthalenes
	C_3-naphthalenes
	C_4-naphthalenes
Bi	biphenyl
	acenaphthylene
	acenaphthene
Fl	fluorene
	C_1-fluorenes
	C_2-fluorenes
	C_3-fluorenes
DBT	dibenzothiophene
	C_1-dibenzothiophenes
	C_2-dibenzothiophenes
	C_3-dibenzothiophenes
P	phenanthrene
	anthracene
	C_1-phenanthrene–anthracenes
	C_2-phenanthrene–anthracenes
	C_3-phenanthrene–anthracenes
	C_4-phenanthrene–anthracenes
Fl	fluoranthene
	pyrene
	C_1-fluoranthene–pyrenes
	benz (a) anthracene
Ch	chrysene
	C_1-chrysenes
	C_2-chrysenes
	C_3-chrysenes
	C_4-chrysenes
	benz (b) fluoranthene
BbF	benz (k) fluoranthene
	benzo [e] pyrene
BaP	benzo [a] pyrene
	perylene
I	indeno [1,2,3-cd] pyrene
	dibenz [a,h] anthracene
BPe	benzo [g,h,i] perylene

1. Used in Figures 5 through 7

Conclusions

The analyses of oyster samples before and after the Apex barge oil spill indicate that oysters do act as biomonitors and that the spilled oil is bioavailable. Measurement of total PAH was diagnostic of exposure for months after the spill; however the complication of other chronic sources of input make diagnosis of exposure specific to Apex barge oil more difficult. The use of fingerprinting, using all of the available PAH data coupled with the historical NS&T data, suggests that Apex barge oil or a similar oil is still present in the vicinity of the GBTD NS&T sampling site. The Apex barge oil could be trapped in the sediments in the shallow surrounding areas. There is then the potential for periodic releases during storms or other events that disturb the sediments. A more extensive data set might have provided enough information to quantitate this possibility better. However, since the decision was made to do a Type A assessment of the damage,[2] which is based on a natural resource damage assessment model for coastal and marine environments (NRDAM/CME) and not on environmental monitoring, no extensive data set exists. The damage assessment model does not consider the fact that bioavailable PAH from the Apex barge oil spill may still be present 495 days after the spill. While the use of computer models to assess damage is a politically expedient measure, in the case of the Apex barge spill there is insufficient data to determine if it adequately considers long-term damage to the environment. More research is needed to address this possible limitation of the model.

Acknowledgments

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Reprint 6

Field Studies Using the Oyster *Crassostrea virginica* to Determine Mercury accumulation and Depuration Rates

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Environmental Contamination and Toxicology

Field Studies Using the Oyster *Crassostrea virginica* To Determine Mercury Accumulation and Depuration Rates

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Mercury as an environmental hazard, especially with regard to human health, has been of concern since the Minamata disaster (Huddle et al. 1975). From 1966 to 1970 a chlor-alkali plant in Point Comfort, Texas released mercury-enriched wastewater (up to 29.9 kgHg/day) into Lavaca Bay (TWQB 1977). Since 1970 the Texas Department of Health (TDH) has periodically closed and then re-opened portions of Lavaca Bay to the harvesting of crabs and finfish based on their levels (< >0.5 ppm Hg wet weight) of mercury. A 1988 closure remains in effect as of this writing (Wiles, 1993). Mercury contamination in Lavaca Bay organisms thus continues to be a problem 22 years after the chlor-alkali plant ceased releasing mercury into the bay. The goal of the following research was to better understand the behavior of mercury in Lavaca Bay.

Oysters have been widely used as indicator species in metal pollution studies (Goldberg et al. 1983). Most such programs have focused on the concentrations of metals in oysters from different geographic areas. This study, however, investigated the rate and amount of mercury a "clean" oyster would accumulate when transplanted to a contaminated estuary and the rate of mercury depuration by contaminated oysters placed in a clean environment. The oysters were additionally analyzed for Ba, Cu, Fe, P, and Zn to test for the possible involvement of these metals in mercury accumulation and depuration.

MATERIALS AND METHODS

In August 1991, mature Crassostrea virginica were collected from an uncontaminated area, Carancahua Reef, in Carancahua Bay, Texas for use in the accumulation study. The oysters were placed in nylon bags with a mesh size of 0.5 cm. Each bag contained about 50 oysters. The bags were taken about 16 km away to Lavaca Bay and a control site, Keller Bay (see Fig. 1) and placed on plastic grates, which prevented them from sinking into the soft sediment at the 1 m deep sites. Nine transplanted oysters from each site were collected on days 0, 7, 14, 21, 36, and 51, placed in plastic bags, and frozen for later analysis.

The depuration study, also conducted in August 1991, used C. virginica from

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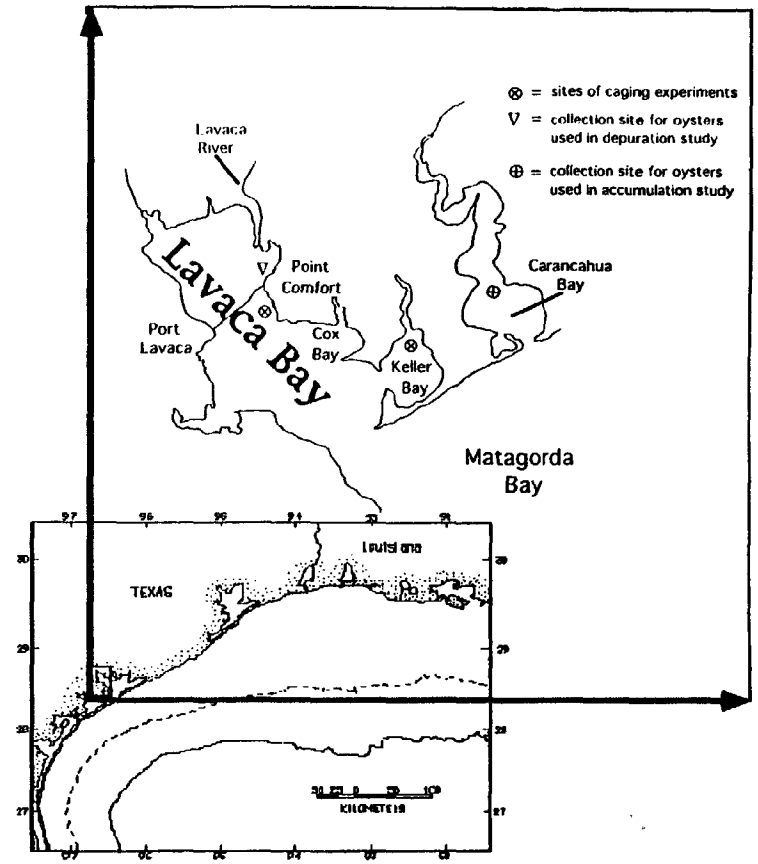

Figure 1. Map of Lavaca Bay showing caging sites and oyster collection locations for the accumulation and depuration experiments.

an area of North Lavaca Bay known to be contaminated with Hg. These oysters were placed in a contaminated area of lower Lavaca Bay, as a control site, and in uncontaminated Keller Bay (see Fig. 1). Deployment techniques and collection times were the same as those used in the accumulation study.

On each sampling date oysters were removed from the experimental bags in both the accumulation and depuration experiments and nine individuals from the natural population of C. virginica at the respective sites were collected.

In the lab, oysters were thawed and opened with a stainless steel knife. The soft tissue was removed with a teflon spatula and plastic forceps, rinsed in

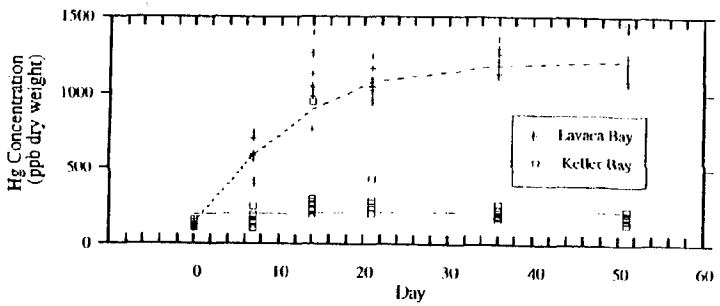

Figure 2. Mercury concentrations in Carancahua Reef oysters transplanted to Lavaca and Keller Bays for the 51-d accumulation experiment.

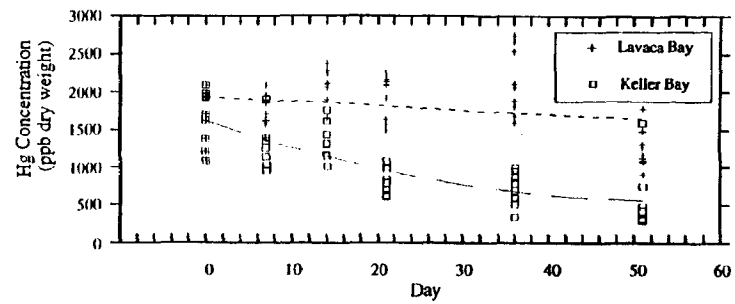

Figure 3. Mercury concentrations in oysters collected from North Lavaca Bay when transplanted to lower Lavaca and Keller Bays for the 51 day depuration experiment.

Table 1. Certified and experimental data for National Bureau of Standards reference material 1566a Oyster Tissue and the elemental detection limits obtained in this study.

Element	Hg (ppb)	Ba (ppm)	Cu (ppm)	Zn (ppm)	P (ppm)	Fe (ppm)
NBS Certified 1566a Oyster Tissue	64.2 ± 6.7	NC	66.3 ± 4.3	830 ± 57	6230 ± 180	539 ± 15
Experimental 1566a Oyster Tissue (n=15)	55.7 ± 2.0	1.49 ± 4	73.8 ± 4.7	935 ± 36	6203 ± 260	549 ± 43
Detection Limit	6	0.4	10	90	680	110

NC = not certified

distilled-deionized water, weighed, freeze dried, and homogenized before analysis. All oysters were individually digested according to a modification of the USEPA 245.1 (USEPA 1990) method and analyzed for mercury by cold vapor atomic absorption spectrophotometry (Hatch and Ott 1968). Samples were additionally analyzed for Ba, Cu, Fe, P, and Zn using a modified Applied Research Laboratories, Inc., (ARL) SpectraSpan® VI Direct Current Argon Plasma (DCP) Emission Spectrophotometer following ARL's instructions (ARL 1991). Every digest (about 30 samples) included two aliquots of the reference material, 1566a Oyster Tissue, certified by the National Bureau of Standards. The certified and experimental values from the 1566a Oyster Tissue and the detection limits for each element are listed in Table 1.

Statistical analyses using SAS Institute, Inc. software (SAS INSTITUTE INC. 1985) were performed on the data from the three oyster groups, accumulation (AA), depuration (DD), and the natural population (NP) to investigate possible relationships among the variables analyzed. The Spearman correlation test and the general linear model (GLM) were used to find correlations and linear relationships among variables. The dry weight of the oysters was used as a covariant in the GLM. The Least Square Means test, LSMEANS, was used to verify changes in Hg with time during the caging experiments.

RESULTS AND DISCUSSION

Oysters removed from Carancahua Bay readily accumulated mercury when placed in Lavaca Bay. Mercury accumulation was rapid through the first 14 days of exposure and leveled off with time (Fig. 2). The rate of Hg accumulation between days 0 and 14 averaged 70 ppb Hg per day. From day 15 to 51 mean daily Hg uptake ranged from 0 to 10 ppb. The Hg levels in the control oysters from Carancahua Bay did not significantly change over the 51-d experiment when caged in Keller Bay. The GLM procedure showed a significant (p <0.05) difference in Hg levels between sites and days collected.

The LSMEANS test showed that Hg concentrations in oysters caged in Lavaca Bay on days 7, 14, 21, 36, and 51 were all significantly higher than day 0 at p <0.05, while the Hg levels on days 14, 21, 36, and 51 were not significantly different from one another.

The results of the depuration study showed that contaminated oysters released Hg when placed in an uncontaminated environment, Keller Bay (Fig. 3). The average Hg level dropped from 1660 ± 363 ppb on day 0 to 550 ± 416 ppb on day 51. The rate of Hg depuration varied throughout the experiment. The rate was highest on days 14 to 21, averaging a loss of 72 ppb Hg per day. According to the LSMEANS test, concentrations in oysters caged in Keller Bay on days 21, 36, and 51 were significantly lower than Hg levels in oysters from North Lavaca Bay transplanted to Keller Bay on day 0 at p <0.05.

Table 2. Correlations among variables measured in the oyster accumulation and depuration experiments and the natural populations in Keller (KB) and Lavaca Bay (LB). Upper, Spearmans rho; lower, P value

Variables	Accumulation		Natural Population		Depuration	
	KB	LB	KB	LB	KB	LB
Cu and Zn	0.83100	0.81849	0.91104	0.87307	0.89350	0.82946
	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)
Zn and P	0.40244	0.42855	0.28371	0.40736	0.32464	0.37663
	(0.003)	(0.001)	(0.04)	(0.002)	(0.02)	(0.005)
Ba and Fe	0.64172	0.62662	0.60290	0.68725	0.51649	0.49675
	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)
Cu and P	0.37744	0.41719	NS	0.36245	0.36627	0.49388
	(0.005)	(0.002)		(0.002)	(0.007)	(<0.0001)
P and Fe	0.35252	NS	0.28380	NS	0.41407	0.33303
	(0.01)		(0.04)		(0.002)	(0.01)
Dry Wt. and Hg	NS	-0.40646	-0.48637	NS	-0.46336	-0.38954
		(0.002)	(0.0003)		(0.0004)	(0.004)
Hg and Cu	NS	NS	NS	0.55418	0.66808	0.29423
				(<0.0001)	(<0.0001)	(<0.0001)
Hg and Zn	NS	NS	NS	0.56905	0.70215	0.43221
				(<0.0001)	(<0.0001)	(<0.0001)
Cu and Fe	NS	NS	NS	0.26899	0.49198	0.37663
				(0.05)	(0.0002)	(0.005)

NS = Not significant

The average oyster living in the transplant sites in Lavaca and Keller Bays contained 2068 ± 676 ppb Hg and 354 ± 124 ppb Hg, respectively. The Carancahua Bay oysters caged in Lavaca Bay for the accumulation experiment increased dramatically in Hg concentration but did not reach the high average Hg levels in the natural population of oysters in Lavaca Bay. Similarly, oysters caged in Keller Bay for the depuration experiment decreased in Hg from 1660 ± 363 to 550 ± 416 but did not acquire the low Hg concentrations found in the natural population of oysters in Keller Bay.

The transplant experiments clearly show that C. virginica rapidly accumulated mercury when placed in a contaminated environment, Lavaca Bay. Furthermore, mercury-contaminated C. virginica from Lavaca Bay were found to quickly depurate Hg when placed in an uncontaminated environment, Keller Bay. Although the initial rate of Hg uptake was much faster than was the release, the oysters in the accumulation and depuration experiments both changed average Hg levels by about 1000 ppb over the 51-d experiment. The results of this study confirm earlier work (Riegal 1990)

showing that Hg contamination is a continuing problem in Lavaca Bay and that the Hg is readily available for bioaccumulation by oysters.

Positive correlations between Cu and Zn, Zn and P, and Ba and Fe were found in every experiment (Table 2). Copper and phosphorus were positively correlated in every oyster group except the natural population from Keller Bay. A positive relationship between P and Fe and a negative correlation between Hg and oyster dry weight were found in several experiments. Correlations were also seen between Hg and Cu, Hg and Zn, and Cu and Zn in oysters from Lavaca Bay, i.e., those collected for the depuration experiment and the natural population of oysters from Lavaca Bay.

Positive relationships between Cu and Zn in bivalves such as those found here have been noted in previous studies (Páez-Osuna and Marmolejo-Rivas 1990a, Marcus and Thompson 1986, Wright et al. 1985, and Páez-Osuna and Marmolejo-Rivas 1990b). Copper and zinc are both biologically active elements, but the reason for their strong correlation is not understood. Previous work (George and Pirie 1980) found that Zn transferred in the plasma of Mytilus edulis was mostly associated with granules that also contained Fe, S, P, K, and Ca. Others have indicated that M. edulis sequesters Zn and Fe in lysosomes in various cell types (Lowe and Moore 1979). George et al. (1978) found Zn and Cu were immobilized in individual granular amoebocytes; granular cells which contained Cu and Zn were present in Ostrea edulis, O. angasi, and C. gigas. It is believed that oysters concentrate Cu, Zn, P and other metals in granules to detoxify and change them to an excretable form (George and Pirie 1980, George et al. 1978). The correlations between Cu and Zn, Cu and P, and Zn and P found in this study could be related to granular formation in C. virginica, but no documentation of this was obtained.

The relationship among the elements Hg, Zn, and Cu may be a result of the high Hg concentrations in the water and sediment in Lavaca Bay. Perhaps Cu and Zn function in protective mechanisms in the detoxification of Hg in C. virginica. Another possible explanation is that Lavaca Bay oysters contain more metal-binding granules or low molecular weight binding proteins, metallothioneins, which could detoxify the metals (Lobel and Payne 1984). The strongest correlation between the three elements was found in oysters in the depuration study, where Cu and Zn closely followed the trend of decreasing Hg with time.

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Reprint 7

Trace Metal Chemistry of Galveston Bay: Water, Sediment and Biota

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Trace Metal Chemistry of Galveston Bay: Water, Sediments and Biota

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ABSTRACT

*Galveston Bay is the second largest estuary in Texas. It receives major urban runoff from the Houston area, its major river drains the Dallas–Ft Worth Metroplex, and the area surrounding the Bay is intensely industrialized, with chemical and petroleum production being especially prominent. Consequently, there are serious concerns about the possible contamination of the Bay and previous studies have indicated toxic metals at elevated concentrations (*e.g. *NOAA, 1989*a*).*

We have conducted an extensive investigation of Galveston Bay trace metals, in which their distribution in the water column, oysters and sediments were determined. Results of the water column and oyster analyses indicate that metal levels in open areas of Galveston Bay are currently similar to those in more pristine bays elsewhere. Industrial metal inputs to the Bay have not led to greatly increased concentrations in water, sediments and biota. However, the sediment analyses indicated that such inputs may have been significant in the past. Total Cu, Zn, Pb, and Ag concentrations in the waters, determined by state-of-the-art clean

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techniques, are 1, 2·7, 0·3, and 0·006 μg liter⁻¹, respectively, and are mostly regulated by the dynamics of sediment suspension and settling. This leads to a correlation of particulate trace metal concentration with the suspended particulate matter (SPM) concentrations, and trace metal enrichment in particles at low SPM concentrations. Forty-four percent of the individual sediment sampling sites exhibited an 'anomalous' concentration with respect to at least one of the metals studied and about half of these sites were directly associated with dredge spoils. The study also indicated that many of the metals are significantly converted to a coprecipitate with pyrite in the top 10 cm of sediment.

INTRODUCTION

Galveston Bay, with a surface area of 1600 km^2, is one of the largest embayments on the US coastline. The Bay water is, however, very shallow, averaging only about 2 m in depth and is largely cut off from the Gulf of Mexico by the Bolivar Peninsula and Galveston Island. Tides, which average about 40 cm in height, thus exchange water primarily through a channel between these two land barriers. It is generally accepted that winds are often more important than tides in Bay circulation and water exchange (NOAA, 1989*a*), but exact current patterns in the Bay and the residence time of water in the Bay with respect to exchange with the Gulf of Mexico are not well known. A mean residence time for waters in the Bay of about 40 days has been estimated from its average salinity of 15 and an average river flow of 12 km^3 $year^{-1}$ (Armstrong, 1982).

The Trinity River supplies about 70% of the freshwater that enters the Bay, with the remainder coming from the San Jacinto River and from ungauged flows (NOAA, 1989*a*). The Dallas Ft Worth Metroplex with its 4 million people is located 400 miles up the Trinity River from Galveston Bay and is separated from the Bay by a large freshwater lake. The Houston metropolitan area with its 3 million people is immediately adjacent to upper Galveston Bay and drains directly into it through the San Jacinto River and the Houston Ship Channel. The Houston–Texas City–Galveston area is intensively industrialized, especially by the petroleum, petrochemical and chemical industries. For example, 30–50% of the US chemical production and oil refineries are situated around Galveston Bay. This industrialization and the large population results in Houston being the third largest seaport in the US in terms of total shipping tonnage. Galveston Bay receives more than half of the total permitted wastewater discharges for the state of Texas and a total of about 5 km^3 $year^{-1}$ of wastewater input.

The combination of large population, high industrialization, shallow depth and restricted water exchange gives Galveston Bay the potential for serious trace metal contamination problems. Such problems have, however, not been well documented. Hann & Slowey (1972) showed sediments in the upper, confined parts of the Houston Ship Channel to be highly enriched in several trace metals and this has been confirmed by yearly sampling by the Texas Water Quality Board and Texas Water Commission (TWC) since 1974 (Texas Water Commission, 1987). However, sediment from the ship channel where it crosses the open Galveston Bay did not appear to be contaminated in these early studies. TWC analyses for trace metals in Houston Ship Channel water show a decline in all metals between 1974 and 1986 but the quality of the data is in question, so no firm conclusions can be reached. The only reliable previously published data for dissolved trace metals in open Galveston Bay waters are thought to be those of Tripp (1988) who determined only As and Sb concentrations. These were both near normal seawater values in the open Bay but As was enriched by up to a factor of five in the Houston Ship Channel and Sb was depleted there.

One of the feared effects of contaminant inputs into coastal embayments is this buildup in water and sediments leading to accumulation and negative effects in biota. For this reason, oysters and sedentary organisms have been advocated as pollution biomonitors (Goldberg, 1975; Goldberg *et al.*, 1983). Bioaccumulation in benthic fauna can occur from both dissolved and particulate forms. Generally higher trace metal concentration in either water or sediments lead to elevated concentrations in the biota, depending on the organism type, trace metal speciation and solid carrier phases. Bioavailability of trace metals can be controlled by many factors, for example, by the amount and speciation of iron in sediments. An inverse correlation between bioaccumulation of Pb and bound Fe concentration in sediments has been found (Luoma & Bryan, 1978). Therefore, a general overview of trace metal chemistry has to include all three reservoirs (i.e. water, sediments, and biota) and has to pay special attention to the chemical forms of Fe and other geochemical indicator elements or key physicochemical variables. Very few such comparisons which used state-of-the-art techniques for the analysis of all three reservoirs are available in the open literature.

In order to obtain such an overview indicating possible heavy metal contamination in Galveston Bay, we have combined the results of three studies. These studies were of trace metal concentrations in both dissolved and particulate form in the water column (studied by Benoit and Santschi), concentrations in oysters (studied by Presley and Taylor), and their form and distribution in sediments (studied by Morse).

METHODS

Water column-associated metals

Water samples for trace metal analysis were collected in summer and fall 1989, along a transect from the Trinity River near the town of Anahuac, through Trinity and Galveston Bays, and out to the Gulf of Mexico (Fig. 1). Samples were not collected in the Houston Ship Channel/San Jacinto arm of the Bay, but water from this source would have been included as an admixture, especially in higher salinity samples. The data do not reflect a truly synoptic picture of the Bay system for either collection for two reasons. First, the size of the Bay made it impossible to collect across the entire salinity gradient in a single day. Second, for the summer collection, additional samples were collected during a trial run two weeks before the other samples.

Salinity was monitored using a refractometer, and samples were collected in salinity intervals of approximately 5‰. The refractometer had a precision of about ±1 and reported salinities were measured in the laboratory by argentometric titration (Amer. Public Health Assoc., 1985). Salinity sometimes changes rapidly and unpredictably over short distances, at times decreasing locally in the seaward direction. Likewise, turbidity is very patchy. To help verify that samples collected at a given station were drawn from a discrete water mass, salinity was checked periodically during sample collection.

For measurements of trace metals in water column samples, ultraclean techniques were used during all stages of sample collection, transport, handling, processing, and analysis (Patterson & Settle, 1976). A separate sample was collected by peristaltic pumping through a 0·4 μm Nuclepore filter for measurement of salinity, suspended particulate matter, alkalinity, and DOC.

Immediately after return to shore, water samples were preserved by acidification with 2 ml ultrapure HNO_3 per liter of seawater within a portable laminar-flow clean bench. Filters were unloaded from their Teflon assemblies and transferred to acid-cleaned 15 ml screw-cap vials. Filtered water samples were digested in their original bottles using the preservation acid and ultrasonification for 60 min at 60°C. Filters were digested in their original vials using 10 ml of 0·5% HNO_3 and the same heat and sonification.

Extraction columns consisted of a 2 cm bed of silica-immobilized 8-hydroxyquinoline (Sturgeon *et al.*, 1981; Marshall & Mottola, 1983) contained in a 1·5 cm diameter glass chromatography column connected to a 500 ml glass reservoir. The column was precleaned by passage of

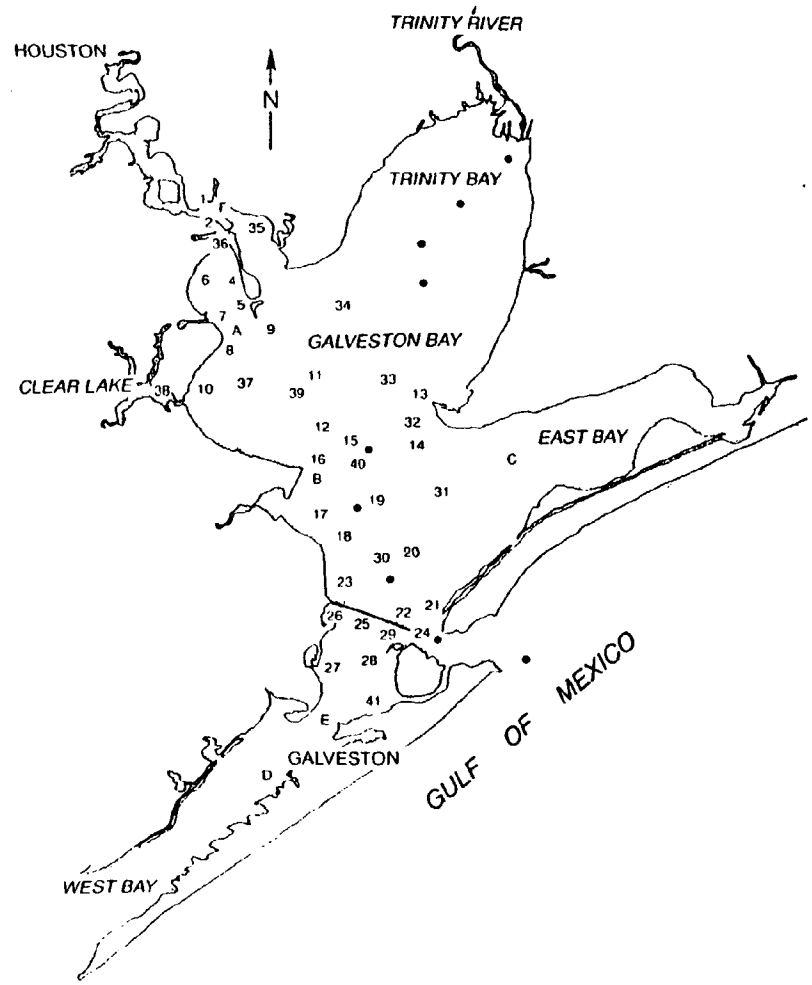

Fig. 1. Locations of sampling sites in Galveston Bay. Solid circles are water column sites. Numbers are sediment sites. Letters are oyster sites. A = Galveston Bay Yacht Club (GBYC); B = Todd's Dump (GBTD); C = Hanna Reef (GBHR); D = Confederate Reef (GBCR); E = Offats Bayou (GBOB); F = Ship Channel (GBSC).

approximately 300 ml of a solution 1 M in HCl and 0·1 M in HNO_3 (Seastar brand). The concentration of zinc in the effluent was monitored until it nearly matched the added acid. A sample of the final acid rinse was collected and measured for a column blank for all metals, though this was usually negligible. The column was rinsed with 10 ml of 18 Mohm water to remove traces of acid. Immediately before preconcentration, aliquots were decanted in a clean bench and titrated to determine the amount of ultrapure NH_4OH required to adjust the pH to 8·0±0·5. Approximately 200 ml of seawater sample (the entire 10 ml of digestion solution was used for filters) was passed through the column, which was then rinsed with 10 ml ultrapure water to remove sea salts. Metals were then eluted with 15 ml of 1 M HCl/0·1 M HNO_3 and collected in a 15 ml acid-cleaned plastic vial that was checked for contamination by rinsing with the same acid and testing for Zn.

Lead, Cu, and Ag were measured in duplicate in acid-washed Teflon autosampler cups in the graphite furnace of a Perkin Elmer Zeeman 5100 atomic absorption spectrophotometer equipped with Zeeman background correction, pyrolized furnace tubes and L'vov platforms. Injection volumes were 40 μl for Cu and 150 μl (3 × 50 μl) for Ag and Pb. Zinc was measured by manual injection of 10 μl since the autosampler introduced unacceptably high blanks of unknown source. Manual injections were repeated until reproducible absorbance readings were obtained and verified (typically 3 to 5 injections).

Column yields were monitored by parallel measurement of certified reference seawaters (CASS-2, S = 29·2‰, or SLEW-1, S = 11·6‰, Research Council of Canada) for one out of every five columns. Silver column yields were determined on spiked seawaters, since the seawater reference materials are not certified for silver. Concentrations were calculated by correcting for metals in the elution acid, the column blank, and column yield (typically near 90%). Bottle blanks, determined on distilled water collected in the field using the same filtration system, were negligible. Column yields had a standard deviation close to 20%, and introduced most of the uncertainty in the final calculated concentrations. Duplicate samples (20% of collected samples) and replicate laboratory analyses (10% of measurements) agreed within the calculated uncertainties.

We believe that our measurements are reliable for several reasons: (1) state-of-the-art clean techniques were used throughout; (2) the method was checked frequently against certified reference seawaters; (3) the concentration ranges measured are similar to values found by careful investigators working on other estuaries; and (4) the trends in the data are geochemically reasonable, i.e. they correlate in a simple manner with ancillary key physicochemical variables.

Alkalinity and salinity were measured by titration, DOC by the wet digestion method (persulfate–phosphoric acid), and suspended particulate matter (SPM) by gravimetry. Wet digestion may not quantitatively measure dissolved organic carbon (DOC) in open ocean waters (Sugimura & Suzuki, 1988), but we believe it gives reliable results for qualitatively different DOC found in estuaries. Virtually all SPM analyses were measured in duplicate. Here, we only report selected results as they relate to sediment and oyster data. A full account of these data will be given elsewhere (Benoit *et al.*, 1992).

Oyster-associated metals

Oysters (*Crassostrea virginica*) were collected at six different sites in Galveston Bay during 1986–90 as part of the National Status and Trends Program (GERG, 1990). Each site was on an identifiable oyster reef (Fig. 1) and at each, twenty oysters were taken from each of three stations, the stations being 100–500 m apart. Each site was sampled once each year, except two of the sites (GBOB and GBSC) were not sampled during the first two years. The twenty oysters from each station were combined and analyzed as a single sample each year.

Oysters were usually handpicked from exposed reefs, but in deeper water they were taken by dredge or tongs. In most cases stations were located hundreds of metres away from obvious point sources of contaminant inputs such as industrial discharges in an attempt to characterize large areas of Galveston Bay, rather than to identify specific point sources. The new sites added in year three were, however, selected to be closer to industrial areas or population centers than were the original four sites. Stations were reoccupied as closely as possible each year, both in time and space.

Frozen oysters were returned to the laboratory where they were brushed to remove mud from the shells and allowed to thaw. They were then opened under clean room conditions using a stainless steel oyster knife. The tissue was washed sparingly with distilled-deionized water to remove any adhering mud and was put into a 500 ml Teflon jar. When the jar was approximately one-third full, or when all twenty oysters from a given station had been added, three solid Teflon balls of 3·5 cm diameter were added. The jars were tightly closed and were put into plastic Ziplock bags. The jars were then loaded into an industrial paint shaker and were shaken vigorously for 15–20 min 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.

The digestion vessels were 60 ml capacity screw top 'bombs' of PF Teflon (Savillex Corp., Minnetonka, MN model 561). Digestion of tl approximately 200 mg dry weight samples of oyster tissue used 3 ml of 4 to 1 mixture of ultra-pure nitric and perchloric acids. The samples we first allowed to pre-digest for 2–3 h in the mixture on a warm hotpla while the bombs were covered with Teflon watch covers. The bomt were then tightly closed to a constant torque (2·5 kg-m) with matchir screw caps and were placed in an oven at 130°C for 8 h. After remov. from the oven and cooling, 20 ml of distilled-deionized water was adde The bombs were weighed and from the known empty bomb weight an final solution density (1·04 g ml^{-1}) an exact final solution volume wa calculated. This and the sample weight were used to calculate a dilutio factor for each sample.

Two blanks and two reference materials were digested with every s of 20–40 samples. Reference material used included National Institu of Standards and Technology (NIST, formerly NBS) 1566 oyst tissue, National Institute for Environmental Studies, Japan (NIES) muss tissue, EPA trace metals in fish standard, National Research Counc of Canada (NRCC) DOLT-1 dogfish liver tissue and a Texas A&M University (TAMU) house standard oyster tissue. Repeated analysis c these reference materials and participation in several intercalibratio exercises organized by Dr Shier Berman of the NRCC give an estimat of 10% or better for both the precision and accuracy of the data reporte here.

All data reported here were obtained by atomic absorption spectro metry (AAS). Flame AAS was used for Cu, Fe, and Zn which exhib high concentrations in oysters, cold vapor AAS for mercury and graphit furnace AAS (Perkin-Elmer Corp. model 3030 with Zeeman backgroun correction) for the remaining elements. Some samples of freeze-drie oyster tissue were also analyzed for some elements by neutron activatio analysis (NAA) which required no sample pre- or post-treatment. Agree ment between AAS and NAA was good (±10%) for elements analyzed b both techniques.

The samples were analyzed for Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, N Pb, Se, Si, Sn, and Zn. In addition, temperature, salinity and related en vironmental parameters were measured as were size, sex, parasite pres ence and indicators of health of the oysters (see GERG, 1990).

Sediment-associated metals

The sediments of Galveston Bay are highly variable, with major varia tions in grain size occurring on a small scale. Also, oyster reefs and spoi

banks are widespread. Because of the great heterogeneity of this environment, anything short of a monumental sampling effort will not yield a detailed picture of distributions in the Bay. Whether such an effort is justifiable is open to question because major storms, including hurricanes, which are common in this area, cause substantial redistribution of the sediments. With this in mind, sites were chosen throughout Galveston Bay that were deemed reasonably representative. Sites associated with dredged areas and that have a potential for abnormal metal concentrations were given special emphasis. Sampling locations are shown in Fig. 1.

Samples were collected from the RV *Roman Empire* either by hand coring with a precleaned plastic core tube or with an epoxy-coated grab sampler in deeper waters. Only the top ~10 cm of sediment was used and it was homogenized upon collection in sealed bags from which air was excluded to prevent oxidation. This sampling depth was used because it represents 'near surface' sediments and the sandy nature of many of the sediments often precluded sampling much deeper. The homogenized bag sample was immediately divided between two precleaned plastic bottles which were filled to the top to exclude air, capped and sealed with electrical tape. One bottle was quick-frozen on dry ice for sulfide and metal analyses.

A portion of sediment was weighed and freeze-dried to determine water content. Porosity was calculated by using 2·5 as the solid density and correcting for the weight loss of pore water. Sediment grain-size distributions were determined by wet sieving following the method of Folk (1968).

Organic carbon and carbonate carbon were determined with a LECO Carbon analyzer (CR12) in which a finely ground sample is combusted at 1350°C. A portion of the sediment was acidified with 2 ml of 6 N HCl to remove carbonate carbon and dried on a hotplate for 12 h at ~70°C. This sample was then combusted in the LECO carbon analyzer to yield organic carbon content. The carbonate carbon was then determined from the difference between the total and organic carbon. The precision was 1% (1 SD). A LECO carbon standard of 0·98 wt% C was used for standardization.

Sedimentary iron sulfide minerals were operationally defined as acid volatile sulfide (AVS) and pyrite. AVS was extracted from sediments using a cold HCl (6 N) + $SnCl_2$ (2%) method (Cornwell & Morse, 1987). Pyrite was determined from the difference between AVS and the total reduced sulfur (TRS) concentrations. TRS was extracted using a boiling Cr(II)+acid method (Zhabina & Volkov, 1978, as modified by Canfield *et al.*, 1987). The Cr(II) + acid method has been demonstrated to extract pyrite sulfur effectively and specifically after removal of

metastable iron sulfide minerals by AVS extraction (Canfield *et al.* 1987). Also, this method has been demonstrated to extract none of the organic sulfur from sulfur-containing compounds or natural organic matter, thus, eliminating the interference from organic sulfur (see Canfield *et al.*, 1987). The concentration of hydrogen sulfide evolved by these methods was determined by potentiometric titration with $Pb(ClO_4)_2$. The detection limit of potentiometric titration was ~1 μmol g^{-1}, and the precision was 5% (1 SD).

Metals were extracted from the sediments using leaching techniques. Freeze-dried portions of the frozen samples were subjected to a series of leaching procedures in order to separate sedimentary pyrite from the bulk sediment. Briefly, the sequential extraction procedure involves digestion of the sediment sample with 1 M HCl (reactive fraction), 10 M HF (silicate fraction) and concentrated HNO_3 (pyrite fraction). A more complete explanation of the sequential extraction procedures and the development of the separation method is given in Huerta-Diaz & Morse (1990). Additionally, frozen samples were extracted using citrate dithionite to determine the reactive fraction for comparison with the 1 M HCl results.

Trace metals (As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb and Zn) were determined by flame atomic absorption (FAA) using a Perkin-Elmer model 2380 spectrophotometer. Metals below the detection limit of this instrument were analyzed by direct injection into a Hitachi model 170-70 graphite furnace atomic absorption (GFAA) spectrophotometer with Zeeman background correction. The analytical precision (± relative standard deviation) was normally between 5 and 10% for flame atomic absorption analyses and between 10 and 15% for graphite furnace atomic absorption analyses. Salt matrix effects for As determination by GFAA were partially overcome by using a Ni Pd ascorbic acid matrix modifier (Robert Taylor, pers. comm.). Determination of Pb by GFAA in samples containing high Fe/Pb ratios (>250) was carried out following the procedure developed by Shao & Winefordner (1989). Mercury was determined with a Laboratory Data Control UV monitor equipped with a 30 cm path length cell, using the well-known cold vapor technique. For samples suspected of having high dissolved organic matter (DOM) concentrations, Hg was measured after destruction of the DOM with bromine monochloride. Detection limits were calculated as 2·5 times the standard deviation of the reagent blank (e.g. Kaiser, 1970; Bruland *et al.*, 1979; Kremling, 1983). All reagents used were ACS reagent grade or better. Milli-Q water was used for the preparation of all aqueous solutions. Acidic working standard solutions were always freshly prepared. All materials were carefully cleaned using established acid leaching procedures. The use of the sequential extraction procedures precluded

comparisons with standard reference materials for which only total metal concentrations are generally available.

RESULTS

Because of the large number (1000s) of analyses done as part of this study, original analytical data are generally not given in this paper. These data are available from the authors upon request.

Trace metals in the water column

Samples were collected over salinities ranging from 0·1‰ to 27·4‰, and particulate matter levels from 2·4 to 48·4 mg $liter^{-1}$ (Fig. 2). Particulate matter did not show the same trend with salinity on the two sampling dates. In August, there was a mid-salinity SPM maximum, while in October, there was an SPM minimum at intermediate salinities. In fact, the two curves are almost inverses of each other. The variations over time at a given location probably reflect different levels of wind-driven mixing and turbulence, and subsequent sediment entrainment in the water column of this shallow estuary.

DOC levels in the fresh water samples were 5·5 ± 1·4 and 5·3 ± 0·2 mg C $liter^{-1}$, while in the Gulf end member they were 0·1 ± 0·3 and 0·29 ±

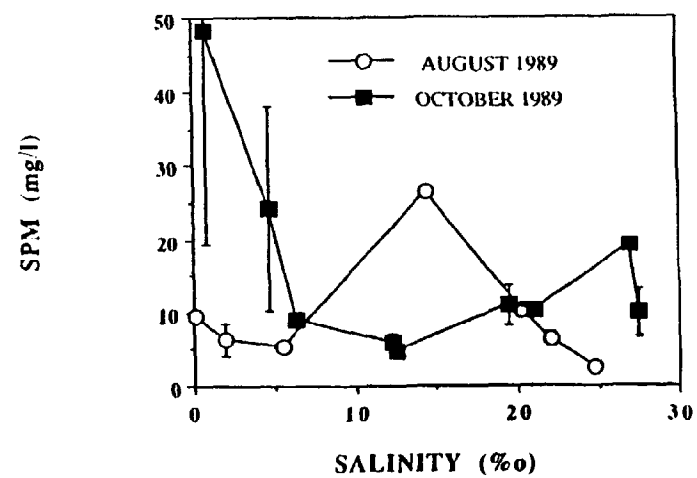

Fig. 2. Suspended particulate matter concentration as a function of salinity. SPM probably reflects local wind stress induced resuspension of bottom sediments.

0·14 mg litre $^{-1}$. DOC showed very consistent behaviour on the two dates, decreasing non-conservatively (curve concave upwards) with salinity in both cases. Therefore, a sink for DOC in the intermediate salinity range is indicated for Galveston Bay. This is consistent with previously observed removal of DOC via flocculation with increasing ionic strength in other estuaries (Boyle *et al.*, 1977). Alkalinity was nearly constant at about 2 meq liter $^{-1}$ at all salinities in this estuary.

Results of trace metal measurements are summarized in Table 1 and illustrated in Fig. 3 as a function of SPM. In general, trace metal concentrations were 5 to 10 times higher than open ocean values (Boyle & Huested, 1983; Bruland & Frank, 1983; Martin *et al.*, 1983; Schaule & Patterson, 1983), and are similar to measurements from other estuaries, conducted by careful analysts (e.g. Bewers & Yeats, 1978; Windom *et al.*, 1983, 1985; Keeney-Kennicutt & Presley, 1985; Mart *et al.*, 1985; Shiller & Boyle, 1985; Valenta *et al.*, 1986). Dissolved metals often did not show systematic variations with salinity, while particulate metal concentrations in the water column (μg liter $^{-1}$ showed trends that were broadly similar to those of suspended particulate matter (mg liter $^{-1}$).

Zinc, Pb, and Cu each had similar concentration ranges on the two dates while dissolved Ag levels were significantly lower at the later date. In August dissolved Ag averaged 5·6 ± 2·2 ng liter $^{-1}$, while in October it was 1·3 ± 0·8 ng liter $^{-1}$. Particulate Ag dropped from 3·6 ± 1·8 to 2·2 ± 0·8 ng liter $^{-1}$, but this is not a statistically significant change (Bevington, 1969). The change in dissolved Ag could result from high freshwater inputs in spring and summer with Ag dilution in freshwater sources, or from a decrease in the organically complexed form of the metal. If the

TABLE 1
Summary of Trace Metal Data in the Water Column of Galveston Bay

	Zn		*Pb*		*Cu*		*Ag*	
	Diss	*Part*	*Diss*	*Part*	*Diss*	*Part*	*Diss*	*Part*
Maximum	4·50	2·56[a]	0·133	0·530	1·41	0·39[a]	8·9	5·9[a]
Minimum	0·30	0·44	0·022	0·026	0·13	0·03	0·2	0·7
Average	1·68	1·04	0·071	0·209	0·86	0·15	3·2	2·8
SD	1·14	0·66	0·029	0·150	0·33	0·10	2·7	1·5
n	17	20	19	19	21	19	20	15

[a]A single particulate sample gave the following values in μg liter $^{-1}$: Zn 6·85, Pb 0·36, Cu 0·80, and Ag 21 ng liter $^{-1}$.
This sample was assumed contaminated and not included in this compilation.
Diss = dissolved, Part = particulate, concentrations are in μg liter $^{-1}$ except Ag which is in ng liter $^{-1}$.

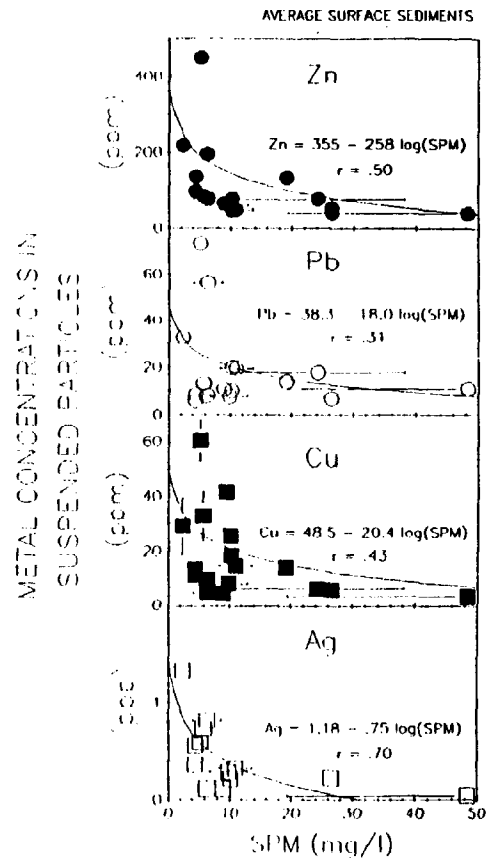

Fig. 3. Metal concentrations in suspended particulate matter as a function of SPM concentrations in the water column. Note that metals have similar concentrations in SPM and in average surficial sediments (dotted lines). Metal concentrations are higher at low SPM levels probably because more fine-grained sediments are suspended at such times.

latter were true, it seems likely that Cu would change in a similar manner. Systematic contamination with Ag alone seems improbable. Further study of the seasonal concentration of dissolved Ag could resolve this question.

Trace metals in oysters

Oysters and other bivalves have been used as 'sentinel' organisms for assessing the contamination of coastal marine water bodies for almost

twenty years. For example, Goldberg *et al.* (1983) report data for a USEPA-funded 'Mussel Watch' program conducted in 1976–78 and the current NOAA funded 'National Status and Trends' (NS&T) program (NOAA, 1985, 1989*b*) is an outgrowth and extension of the 'Mussel Watch' concept. Bivalves are widely recognized as being responsive to changes in contaminant levels in the environment, good accumulators of metals, widely distributed along coasts and easy to collect and analyze. They integrate contaminant levels in the environment over weeks to months and therefore allow areas to be compared even when sampling is limited to a frequency of once or twice per year.

Trace metal concentrations found in oysters collected along the Gulf of Mexico coastline during the first five years of NS&T were generally similar to those reported in oysters taken from non-contaminated water in other parts of the world (Presley *et al.*, 1990). Only a few sites showed obvious trace metal contamination 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 influences. Sites giving higher than average values for most metals were just as likely to be far from population or industrial centres as to be near such areas.

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. the oysters give no indication of generalized trace metal contamination in Galveston Bay. This can be seen by comparing the overall averages (Table 2) for all years and all sites in Galveston Bay with those for the entire Gulf. The Galveston data include five sites sampled all four years and two sites sampled for two years. Three stations were sampled at each site resulting in almost 1500 Galveston oysters being analyzed over the five year period. The Gulf data set includes more than 18 000 oysters, as fifty sites were sampled all five years and an additional twenty sites for three years. Thus, the averaged data are unlikely to be biased by a few abnormal individuals.

The average Cd, Cr, Cu, Mn, and Pb in NS&T oysters from Galveston Bay differs by 10% or less from the Gulf-wide average. This difference is about the same as our analytical precision and is not significant at the 99% confidence level based on a '*t*-test'. Silver is 23% higher in Galveston Bay, Ni is 16% higher and Se is 14% higher. A '*t*-test' of the significance of those figures shows that the Ni averages are not significantly different at the 95% confidence level. Although, the Se average for Galveston Bay oysters is significantly higher than the Gulf-wide average, it is not significantly different from the Texas–Louisiana average. It differs from the Gulf-wide average only because of low Se oysters in Mississippi and Florida.

TABLE 2

Summary Statistics for Trace Metal Concentrations in Galveston Bay and US Gulf of Mexico Oysters Collected for the NOAA NS&T Program in 1986–90 and for the EPA Mussel Watch in 1976–78. All Values in ppm dry weight

	Ag	*As*	*Cd*	*Cr*	*Cu*	*Fe*	*Hg*	*Mn*	*Ni*	*Pb*	*Se*	*Sn*	*Zn*
Galveston Bay													
1986–90[a]	2·77	4·50	4·33	0·53	165	275	0·078	15·9	1·89	0·71	3·42	0·29	3263
SD	2·42	1·08	1·58	0·44	61	142	0·066	8·1	0·64	0·45	0·88	0·22	1648
US Gulf of Mexico													
1986–90[a]	2·24	9·69	4·20	0·55	156	320	0·142	14·8	1·64	0·69	2·99	0·23	2417
SD	1·59	7·00	2·46	0·42	107	243	0·156	8·8	1·29	0·93	1·33	0·16	1753
GB:GOM	1·23	0·46	1·03	0·96	1·06	0·86	0·55	1·08	1·16	1·03	1·14	1·24	1·35
Significance of *t*-test of means	<0·01	<0·01	0·64	0·63	0·44	0·11	<0·01	0·29	0·08	0·86	<0·01	<0·01	<0·01
US Gulf of Mexico													
1976–78[b]	1·8	—	4·6	—	162	—	—	—	2·7	0·9	—	—	1940
SD	1·5	—	2·6	—	138	—	—	—	1·4	0·9	—	—	1480

[a] *Crassostrea virginica*: for Galveston Bay, $n = 78$ pooled samples of 20 oysters each; for GOM, $n = 874$ pooled samples of 20 oysters each.
[b] *Crassostrea virginica*: mean ± 1 SD; EPA Gulf Mussel Watch; Goldberg *et al.* (1983).

The high average Ag in Galveston Bay oysters is caused by a 300% enrichment found at a site on Confederate Reef in 1990. The enrichment appears to be real because it was in all three of the twenty oyster pooled samples collected at that site. No cause for the enrichment can be suggested, and if these samples are neglected NS&T Galveston Bay oysters would have average silver content.

Arsenic and mercury in Galveston Bay oysters are less than one-half the Gulf-wide average but the Gulf-wide averages are greatly influenced by several sites in southern Florida that produce oysters greatly enriched in arsenic and mercury and by a site in Lavaca Bay, Texas which is enriched in mercury. Oysters from other Texas and Louisiana bays are similar in As and Hg content to those in Galveston Bay.

Tin seems to be about 24% higher than Gulf averages in Galveston Bay, but Sn values are near the detection limit of the method used and a 24% difference may not be meaningful. Finally, Zn is 35% higher in Galveston Bay oysters collected for NS&T than in Gulf-wide average oysters. This difference is highly significant as the high Zn found in all oysters leads to precise analytical determination and few analytical artifacts.

Trace metals in sediments

Sediment characteristics vary widely at the different study sites (Table 3). This reflects the highly heterogeneous environment in Galveston Bay and the impact of man's activities, such as dredging and trawling for shrimp. The sediments are generally poorly sorted mixtures of sand, silt and clay, and exhibit major variations in mean grain size. Porosity averages 62 (±10)%.

The organic carbon content of these sediments is highly variable averaging 0·42 wt% and ranging from 0·05 to 1·18 wt%. It is positively correlated (r^2 = 0·69) with the <62 μm size fraction of the sediments. The $CaCO_3$ content of the sediments is also highly variable averaging 3·9 wt% and ranging from 0·33 to 24·4 wt%. It is not correlated with the <62 μm size fraction (r^2 = 0·00) and is dominantly present as shell fragments.

Average trace metal concentrations are given in Table 4. Also included in this table are the range of trace metal concentrations, trace metal concentrations normalized to the <62 μm grain size fraction, and the percent of the metal in the total reactive fraction (here defined as 1 N HCl extractable + pyrite-associated metal). The average concentrations are similar to values observed in other estuaries (e.g. Naragansett Bay, Santschi *et al.*, 1984; Baffin Basy, Huerta-Diaz & Morse, 1992) especially when grain-size normalized values are used. This is further elaborated in the Discussion section.

TABLE 3
Sediment characteristics C = clay; S = sand; s = silt; C = clay.

Sample	*Sand (wt%)*	*Silt (wt%)*	*Clay (wt%)*	*<62 μm (wt%)*	*Class*	*Porosity (%)*	*Organic-C (wt%)*	*$CaCO_3$ (wt%)*
GB1-1	25	32	43	75	SsC	79	1·16	0·33
GB1-2	66	17	17	34	S	64	0·41	0·76
GB1-3	65	11	24	35	S	62	0·35	1·37
GB1-4	43	48	19	57	SsC	63	0·29	0·85
GB1-5	45	36	19	55	Ss	51	0·24	4·05
GB1-6	44	40	16	56	SsC	61	0·30	1·95
GB1-7	78	14	8	22	S	51	0·18	2·68
GB1-8	75	17	8	24	S	52	0·19	1·08
GB1-9	36	36	28	65	SsC	60	0·43	3·70
GB1-10	65	30	5	35	S	54	0·22	2·01
GB1-11	93	5	2	7	S	45	0·07	0·70
GB1-12	93	4	3	6	S	26	—	—
GB1-13	34	47	19	66	SsC	74	0·52	24·38
GB1-14	34	29	37	66	SsC	69	0·44	1·91
GB1-15	87	9	4	13	S	54	—	—
GB1-16	93	4	3	8	S	49	0·15	11·86
GB1-17	87	7	6	12	S	54	0·18	3·95
GB1-18	98	2	0	2	S	47	0·1	4·25
GB1-19	95	2	3	5	S	47	0·05	0·60
GB1-20	36	36	28	64	SsC	51	0·11	7·87
GB1-21	57	12	32	43	CS	53	0·11	13·46
GB1-22	63	22	15	37	CS	50	0·14	2·46
GB1-23	87	5	8	13	S	48	0·10	6·94
GB1-24	75	8	17	25	S	57	0·22	1·37
GB1-25	49	20	31	51	SsC	67	0·41	1·26
GB1-26	53	20	27	47	SsC	58	0·34	2·92
GB1-27	60	13	27	40	CS	60	0·35	17·48
GB1-28	59	14	27	41	CS	58	0·36	0·95
GB1-29	20	37	43	79	SsC	73	0·67	3·16
GB1-30	88	10	2	11	S	54	0·18	1·77
GB1-31	56	16	28	44	SsC	65	0·46	2·07
GB1-32	8	22	70	92	sC	73	1·11	2·14
GB1-33	27	30	43	272	SsC	76	0·77	2·58
GB1-34	29	25	46	71	SsC	76	0·75	2·13
GB1-35	42	18	40	58	SsC	69	0·61	1·42
GB1-36	5	27	68	95	sC	78	1·18	2·55
GB1-37	36	25	39	64	SsC	80	0·79	6·67
GB1-38	65	22	13	35	sS	57	0·36	1·38
GB1-39	51	27	22	49	SsC	70	0·62	2·48
GB1-40	84	8	8	16	S	69	0·26	1·89
GB1-41	9	28	63	90	sC	77	1·07	2·64

These are combined for other classes of sediment (e.g. Cs = Clayey silt) according to Folk (1968).

TABLE 4
Summary of Metal Concentrations in Galveston Bay Sediments

Metal	*Average concentration*	*Range in concentration*	*Average concentration**	*% Reactive*
As	56	23–98	125	42
Fe	15 900	1 570–40 200	35 200	30
Cr	37	4–102	82	37
Cu	8	2–15	18	51
Hg	0·08	0·01–0·28	0·19	98
Mn	605	165–2 365	1 320	42
Mo	41	25–79	95	29
Ni	26	4–45	58	28
Pb	25	12–46	59	99
Zn	55	6–116	123	44

Concentrations are total in $\mu g\ g^{-1}$. Concentration* concentration normalized to the average fraction of sediment (0·45) in the less than 63 μm grain-size range. Reactive-metal fraction = 1 N HCl extractable-metal + pyrite-metal.

All sediments were observed to be anoxic with the active sulphide mineral formation occurring. Data on the concentrations of AVS and TRS, and the degree of pyritization (DOP) of iron using both citrate dithionite and 1 N HCl extraction techniques to remove reactive-Fe are presented in Table 5. DOP is defined as (Berner, 1970):

$$\mathrm{DOP} = \frac{\text{Pyrite Fe}}{\text{Pyrite Fe} + \text{Reactive Fe}}$$

where pyrite-Fe is assumed equal to 0·5 (TRS–AVS), based on the 1:2 stoichiometry of FE:S in pyrite. The AVS concentration averages 3·6 μmole g^{-1} and ranges from 0·1 to 16·3 μmole g^{-1} TRS concentrations average 58·6 μmole g^{-1} and range from 5·2 to 314·2 μmole g^{-1}. AVS generally represents a small fraction of TRS averaging 7·6% and never exceeding 24% of TRS. Neither TRS nor the fraction of TRS as AVS are well correlated with the <62 μm fraction of the sediment (r^2 = 0·25 and 0·08, respectively).

The DOP values determined by the citrate dithionite and 1 N HCl methods are in good general agreement averaging, respectively, 0·35 and 0·42. This is consistent with the findings of previous studies (e.g. Huerta-Diaz & Morse, 1990). Because of difficulties in applying the citrate dithionite method when determining some trace metals of interest (e.g. Zn), results using the 1 N HCl extraction method for characterizing the non-pyritized reactive fraction were used. The DOP (1 N HCl) of

TABLE 5
Data on Reduced Sulphur and Extent of Pyritization

Sample	*TRS* ($\mu mol\ g^{-1}$)	*AVS* ($\mu mol\ g^{-1}$)	*%AVS* ($\mu mol\ g^{-1}$)	*Pyrite* ($\mu mol\ g^{-1}$)	*DOP-FCD*	*DOP—1 N*
GB1-1	64·5	5·2	8·0	29·7	0·43	0·55
GB1-2	33·2	0·6	1·7	16·3	0·27	0·45
GB1-3	42·4	8·5	20·0	16·9	0·23	0·35
GB1-4	41·1	4·6	11·1	18·3	0·28	0·43
GB1-5	45·6	2·1	4·6	21·8	0·41	0·52
GB1-6	71·1	1·4	1·9	34·9	0·44	0·68
GB1-7	22·7	1·4	6·1	10·6	0·25	0·53
GB1-8	28·9	4·5	15·7	12·2	0·29	0·52
GB1-9	83·3	3·8	4·5	39·8	0·44	0·61
GB1-10	45·7	2·7	6·0	21·5	0·55	0·73
GB1-11	12·3	0·3	2·5	6·0	0·32	0·76
GB1-13	147·3	4·0	2·7	71·6	0·76	0·79
GB1-14	199·7	0·0	0·0	99·9	0·77	0·77
GB1-16	24·8	2·1	8·4	11·4	0·35	0·50
GB1-17	18·4	1·9	10·5	8·3	0·29	0·35
GB1-18	7·9	0·4	4·7	3·8	0·16	0·24
GB1-19	5·2	0·1	1·8	2·6	0·12	0·09
GB1-20	23·0	1·4	6·1	10·8	0·23	0·50
GB1-21	17·2	2·3	13·6	7·4	0·10	0·24
GB1-22	41·4	7·1	17·2	17·2	0·34	0·38
GB1-23	7·0	0·6	8·7	3·2	0·17	0·28
GB1-24	8·5	0·3	4·0	4·1	0·27	0·21
GB1-25	28·8	0·4	1·5	14·2	0·21	0·27
GB1-26	64·6	4·8	7·4	29·9	0·47	0·62
GB1-27	21·2	1·2	5·8	10·0	0·14	0·17
GB1-28	43·3	0·4	0·9	21·5	0·28	0·28
GB1-29	49·7	9·8	19·8	20·0	0·17	0·16
GB1-30	23·6	0·8	3·3	11·4	0·27	0·12
GB1-31	88·7	1·9	2·2	43·4	0·66	0·48
GB1-32	58·9	9·8	16·7	24·5	0·42	0·28
GB1-33	95·8	5·8	6·1	45·0	0·62	0·52
GB1-34	175·2	3·9	2·2	85·6	0·50	0·41
GB1-35	65·5	10·4	15·8	27·6	0·48	0·41
GB1-36	56·9	7·7	13·5	24·6	0·29	0·22
GB1-37	314·2	4·0	1·3	155·1	0·62	0·58
GB1-38	38·8	4·0	10·3	17·4	0·61	0·54
GB1-39	62·6	1·6	2·6	30·5	0·37	0·32
GB1-40	38·5	0·9	2·4	18·8	0·22	0·18
GB1-41	68·8	16·3	23·7	26·2	0·30	0·24
Average	58·6	3·6	7·6	27·5	0·35	0·42
SD	60·1	3·6	6·2	29·8	0·18	0·19

sediments is highly variable, ranging from 0·09 to 0·79. The upper range of values is indicative that Fe may be approaching being the limiting factor for pyrite formation in some of the sediments.

In areas where sediment grain size varies substantially, such as Galveston Bay, total metal concentrations (Table 4) by themselves often are not particularly informative. This is because the metals are often dominantly associated with the fine-grained material and, consequently, variations in metal concentrations given in Table 4 may largely reflect grain size differences (e.g. Trefry & Presley, 1976). The correlation coefficients (r^2) for the total reactive fraction of metals studied in Galveston Bay with the <62 μm grain size fractions are: Fe = 0·80, Mn = 0·52, Ni = 0·66, Cu = 0·57, Zn = 0·73, Cd = 0·16, Pb = 0·47, Cr = 0·69, Mo = 0·18, As = 0·00, and Hg = 0·08. These relationships indicate that most metals, with the exceptions of Cd, Mo, As, and Hg, are dominantly associated with the fine-grained fraction of the sediment. The metals not well associated with this fraction may be incorporated into the sediment by processes other than simple sedimentation (e.g. diffusion of dissolved arsenate and molybdate into the sediment followed by reduction and precipitation) or be associated with fractions such as large carbonate particles.

It is common in the study of trace metal geochemistry to attempt to 'normalize' concentrations by ratioing the trace metals to some other more abundant chemical components such as Fe or Al. Because Al was not determined in this study, the correlations (r^2) of total trace metal concentrations with total Fe concentrations were calculated. The results are: Mn = 0·60, Ni = 0·79, Cu = 0·56, Zn = 0·88, Cd = 0·42, Pb = 0·46, Cr = 0·90, Mo = 0·42, As = 0·01, and Hg = 0·06. Mn, Ni, Zn, Cd, Cr, Mo all exhibit significantly better correlations with Fe than with the fine-grained fraction, whereas Cu and Pb exhibit about the same correlation by both approaches. Arsenic and Hg were the only metals exhibiting a poor correlation with both grain size and Fe.

The primary concern in this study is not with total metal concentrations, but rather with the total reactive fraction which is operationally defined here as reactive metal (1 M HCl extractable) + pyrite-metal. The citrate dithionite extractable fraction was not used since the reagent is substantially contaminated with several of the metals of interest and in some cases this fraction is not well analyzed by graphite furnace atomic absorption spectrometry (see Huerta-Diaz & Morse, 1990, for discussion). The concentration ratios of the total reactive fraction of trace metals relative to total reactive-Fe (Me*) are presented in Table 6. Normalization to total reactive-Fe concentrations was done in order to observe if anomalously high total reactive trace metal concentrations are present in any of the sediments. Such anomalies may be indicative of contamination.

TABLE 6A

Ratios (Me*) of Total Reactive-Metal Concentrations to Total Reactive-Iron Concentrations

Sample	*Mn**	*Ni**	*Cu**	*Zn**	*Cd**	*Pb**	*Cr**	*Mo**	*As**	*Hg**
Galveston Bay										
GB1-1	52·0	1·03	1·40	6·50	0·110	1·42	1·91	1·11	2·14	0·0024
GB1-2	45·3	0·80	1·63	7·17	0·222	3·74	3·73	3·16	12·48	*0·0371*
GB1-3	86·2	0·86	1·31	4·57	0·228	2·11	2·16	2·55	8·76	0·0019
GB1-4	60·6	0·87	1·59	6·75	0·238	2·88	2·82	3·96	*16·08*	0·0089
GB1-5	75·0	1·42	1·10	3·38	0·235	1·58	2·29	1·76	3·57	0·0023
GB1-6	42·6	1·13	1·62	5·36	0·381	2·49	2·62	2·05	7·97	0·0018
GB1-7	97·6	2·91	1·77	*17·44*	*1·067*	*4·81*	4·69	4·50	10·28	0·0026
GB1-8	56·1	2·27	1·82	7·12	0·577	3·96	7·25	4·24	7·91	0·0026
GB1-9	58·1	1·68	1·52	3·49	0·143	1·96	2·41	1·62	3·95	*0·0418*
GB1-10	47·1	2·13	2·39	5·76	0·287	4·04	6·24	2·57	7·40	0·0035
GB1-11	87·5	2·43	1·96	6·49	0·240	*8·88*	*9·13*	*8·78*	*33·30*	0·0061
GB1-13	47·4	2·73	1·09	3·15	0·382	1·24	1·27	0·96	1·24	0·0022
GB1-14	27·0	1·08	0·69	2·29	0·064	0·67	1·06	0·91	1·30	0·0009
GB1-16	*182·1*	*18·28*	*3·64*	*15·64*	*3·330*	*8·36*	5·87	*6·21*	*26·11*	*0·0331*
GB1-17	126·0	5·69	1·69	6·82	*0·981*	3·36	2·28	*5·07*	7·32	0·0032
GB1-18	126·2	*6·40*	1·45	6·70	*0·868*	5·69	4·56	*8·08*	*145·34*	0·0063
GB1-19	47·3	0·80	0·17	6·75	0·000	2·27	2·22	3·94	12·30	0·0018
GB1-20	*226·0*	3·26	1·42	3·72	0·461	2·37	1·38	2·21	5·15	0·0031
GB1-21	*183·3*	*6·04*	1·67	4·75	*0·963*	3·44	3·12	3·48	5·73	0·0067
GB1-22	69·7	1·17	0·66	4·11	0·039	1·28	1·32	1·48	3·12	*0·0111*
GB1-23	84·6	*6·37*	1·45	5·40	*0·889*	4·42	2·99	*4·98*	6·63	0·0062
GB1-24	57·9	1·01	0·93	5·32	0·105	1·75	1·96	2·01	4·11	0·0020
GB1-25	46·7	1·01	1·52	5·44	0·042	1·61	2·54	1·04	3·47	0·0068
GB1-26	45·9	1·47	*3·91*	7·33	0·131	1·53	5·65	1·18	7·20	0·0035
GB1-27	96·9	3·97	2·21	7·47	0·569	2·60	4·80	1·61	3·71	0·0069
GB1-28	31·5	0·89	1·00	5·38	0·059	1·08	2·20	0·63	1·36	0·0021
GB1-29	49·2	1·21	1·15	4·17	0·061	0·90	2·82	0·75	2·84	0·0017
GB1-30	45·0	1·02	0·33	3·85	0·038	1·01	1·98	1·17	4·32	0·0037
GB1-31	26·6	0·85	0·56	2·61	0·019	0·57	1·29	0·74	2·42	0·0023
GB1-32	39·1	0·91	0·83	3·07	0·028	0·75	1·57	0·52	2·12	0·0027
GB1-33	29·4	0·97	0·78	2·89	0·027	0·65	1·71	0·58	2·69	0·0056
GB1-34	31·5	0·96	0·96	3·05	0·000	0·80	1·88	0·92	2·23	0·0028
GB1-35	29·6	0·84	0·89	5·67	0·000	0·79	1·83	0·97	1·95	0·0106
GB1-36	96·9	1·02	1·07	3·64	0·072	0·84	2·04	0·78	1·65	0·0032
GB1-37	24·9	0·99	1·07	2·02	0·089	0·79	1·98	0·91	2·36	0·0023
GB1-38	24·8	1·05	5·97	6·05	0·094	1·83	2·05	3·78	7·97	0·0049
GB1-39	50·0	0·89	0·73	3·40	0·094	0·82	1·66	0·95	2·09	0·0022
GB1-40	55·0	1·02	0·52	3·85	0·065	1·16	2·01	1·12	3·67	0·0027
GB1-41	40·0	0·91	1·48	4·87	0·097	0·92	2·11	0·51	1·53	0·0020
Average	67·9	2·32	1·48	5·47	0·341	2·34	3·73	2·40	6·56	0·0065
SD	46·3	3·09	1·05	3·05	0·580	1·98	1·81	2·07	6·73	0·0094
Baffin Bay										
86 BB2	102·9	2·97	1·93	3·59	0·128	1·32	2·62		-	-
88 BB1	112·0	1·85	1·69	3·53	0·102	5·71	1·97	0·04	0·41	0·00244
88 BB2	94·1	1·61	1·64	3·36	0·120	1·83	1·24	0·21	0·83	0·00461
Average	102·7	2·14	1·75	3·48	0·12	2·85	1·93	0·13	0·63	0·00362
GB/BB	0·66	1·08	0·85	1·57	2·93	0·82	1·93	18·76	10·45	1·80

Values exceeding twice the average are in italics. Baffin Bay sediment data from Huerta-Diaz & Morse (1992). GB/BB = Galveston Bay results divided by Baffin Bay results.
Ratios are molar × 1000.

TABLE 6B

Samples in Which the Value Exceeds Two Times the Standard Deviation of the Mean. Numerical Values Represent (Sample Average)/Standard Deviation

Sample	*Mn**	*Ni**	*Cu**	*Zn**	*Cd**	*Pb**	*Cr**	*Mo**	*As**	*Hg**
GB1-2	—	—	—		—	—	—	—	—	3·25
GB1-7	—	—	—	3·93	—	—	—		—	—
GB1-9	—	—	—				—	—	—	3·76
GB1-11	—	—	—	—	—	3·30	2·98	3·08	3·97	—
GB1-16	2·47	5·17	2·06	3·34	5·15	3·04	1·18	—	2·91	2·83
GB1-18	—	—						2·74	—	—
GB1-20	3·41	—	—	—	—	—	—	—	—	—
GB1-21	2·49	—	—	—	—	—	—	—	—	—
GB1-26	—	—	2·31	—	—	—	—	—	—	—
GB1-38	—	—	4·27	—	—	—	—		—	—

However, if contaminant sources introduce large quantities of iron, this procedure might not be appropriate as an indicator of excess metals. Observed anomalies and the relationship of metal concentrations in Galveston Bay to other sediments will be dealt with in the Discussion section.

Huerta-Diaz & Morse (1990) introduced the concept of degree of trace metal pyritization (DTMP) which is equivalent to DOP for iron. By comparing DTMP with DOP it is possible to relate the pyritization of a given trace metal to that of Fe, which is the dominant metal that is pyritized. The metals can be arbitrarily divided into three major groups: those generally exhibiting considerably less, about the same, and greater pyritization than Fe. Results are shown graphically in Fig. 4. Mn, Zn, Ni and Pb generally fall into the first category, not being as extensively pyritized as Fe. Cu, Cr, an Mo exhibit a large scatter, but most samples fall into the second category, being pyritized about the same as Fe. Arsenic and Hg are often much more extensively pyritized than Fe. The concentrations of Cd were often close to or below detection limits and such a relationship is not reliable for this metal.

DISCUSSION

To our knowledge these are the first reliable, systematic measurements of trace metal concentrations other than As and Sb in the water column of Galveston Bay. Data bases of the Texas Water Commission (TWC), Army Corps of Engineers, and US Geological Survey contain many

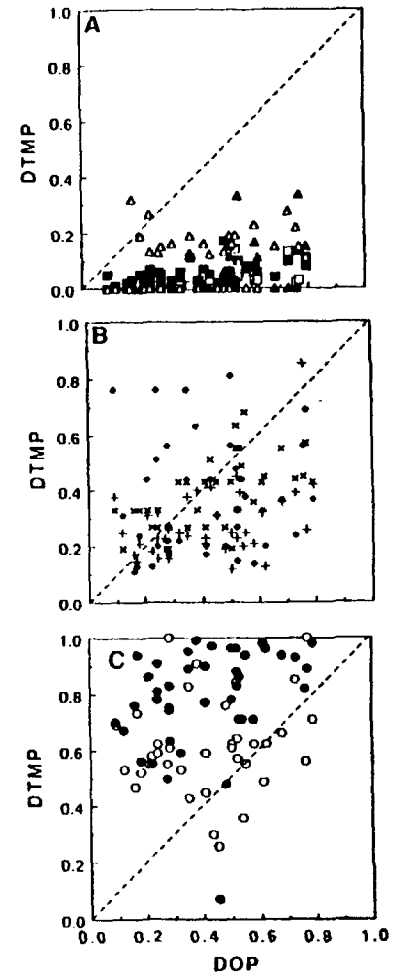

Fig. 4. The relationship between trace metal (DTMP) and iron (DOP) pyritization. (A) Metals generally undergoing less pyritization than Fe (□ = Mn; ■ = Sn; Δ = Pb; ▲ = Ni). (B) Metals generally having a similar degree of pyritization to that of Fe (+ = Cu; × = Mo; ♦ = Cr). (C) Metals generally undergoing greater pyritization than Fe (○ = As; ● = Hg). Dashed lined is for 1 to 1 ratio of DTMP to DOP.

years of trace metal data from surface waters, but these measurements are invalid due to sample contamination and/or insufficient analytical sensitivity. Our values are typically 100 to 1000 times lower than the earlier analyses. The difference most probably reflects our more careful measurements rather than any real change in metal concentrations over time.

Galveston Bay is very shallow, so SPM levels, and their trace metal burdens, should be dominated by resuspension and settling of bottom sediments. Because of this close coupling between the water and sediment columns, it seems likely that particulate metals in the water column will reflect levels in surficial bottom sediments. Supporting this expectation, particulate metal concentrations broadly mirror SPM levels, with Zn and Pb showing mid-salinity maxima in August, and corresponding minima in October. Figure 5 shows the good correlation between particulate Zn and Pb concentrations with SPM concentrations for October samples. The linear correlation is remarkable, since it implies a nearly constant concentration of these two metals in bottom sediments across the entire estuary. The slopes of the two lines give average concentrations (Zn 41 $\mu g\ kg^{-1}$, Pb—12 $\mu g\ kg^{-1}$). The other data show weaker correlations, perhaps because metal concentrations in surficial bottom sediments ordinarily exhibit a wide range, rather than a uniform value.

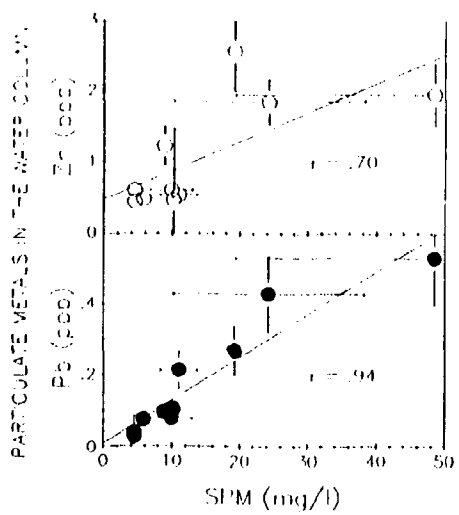

Fig. 5. Filter-retained Pb and Zn concentration in the water column as a function of SPM. The good correlations support the hypothesis that particulate metals in the water column are derived from resuspended bottom sediments. The linear relationship implies that the suspended sediments have nearly a constant metal concentration ($\mu g\ g^{-1}$) at all locations sampled.

In Fig. 3, the concentrations of metal on suspended particles vary over a range that brackets the average values in surface sediments. This is exactly what would be expected if metals on the suspended particles were derived directly from resuspended bottom sediments. The range in concentrations in the water column can be explained by the range in concentration on bottom sediments, but this is probably modulated by the preference of metals for fine sediments and differential resuspension and settling of different sediment size classes. In particular, under conditions of low wind stress and turbulence, SPM concentrations are lower and are probably composed largely of finer sediments, which are enriched in trace metals. Conversely, stronger winds resuspend a greater number of larger particles, which are comparatively depleted in metals. Figure 3 shows that trace metals concentrations on suspended particles do, in fact, decrease with increasing SPM concentration for all four metals. This is consistent with previous observations by Duinker (1983) who associated this inverse correlation to the fact that high levels of SPM include a substantial fraction of heavier, larger-sized particles and aggregates, originating from resuspension of surface sediments. Larger particles are often mainly composed of quartz-grains which have a lower trace metal concentration thus 'diluting' the pool of fine suspended particles enriched in organic carbon and metal oxides, the most efficient carrier phases for many trace metals. Alternatively, this behavior can also be produced by the 'particle-concentration' effect, which has as a cause the presence of trace metal-containing colloidal particles in the filter-passing fraction (Honeyman & Santschi, 1989; Baskaran & Santschi, 1992; Baskaran *et al.*, 1992). One way to test this hypothesis would be to measure particulate trace metal levels as a function of suspended particle size, including colloidal particles.

Dissolved trace metals do not show a simple pattern relative to salinity or SPM concentration (not shown). Based on our researach using naturally occurring radionuclides (Baskaran & Santschi, 1992; Baskaran *et al.*, 1992), we believe that trace metals are scavenged and released very rapidly in Galveston Bay waters. This means that dissolved trace metal levels are the result of rapid particle uptake (adsorption and colloid aggregation) and release from particles (desorption and colloid disaggregation), as well as rapid cycling of particles derived by resuspension of bottom sediments through the water column. Thus, dissolved trace metal levels are controlled mainly by the combination of three processes: (1) resuspension of bottom sediments to yield particulate and colloidal metals, (2) steady-state equilibrium partitioning of metals between solid and liquid phases, (3) steady-state partitioning between particles of different sizes including those in the colloidal size range. As a result, dissolved

trace metal levels should depend on both the quantity and size spectrum of suspended particles, and on their partitioning characteristics. Further research is needed to elucidate the details of such a relationship.

Although the behaviour of the dissolved metals was complex, certain simple trends are worth noting. In general, metal concentrations were lowest near the Gulf salinity end member. This is to be expected, since rivers act as a source of metals to the ocean. Also, at all salinities, dissolved Pb was much lower than particulate Pb, while the opposite was true for Cu. Zinc and Ag showed approximately equal partitioning between dissolved and particulate fractions. This trend matches our expectations since Pb is highly particle reactive (e.g. Balistrieri & Murray, 1984; Santschi *et al.*, 1984), while Cu tends to form soluble organic com-

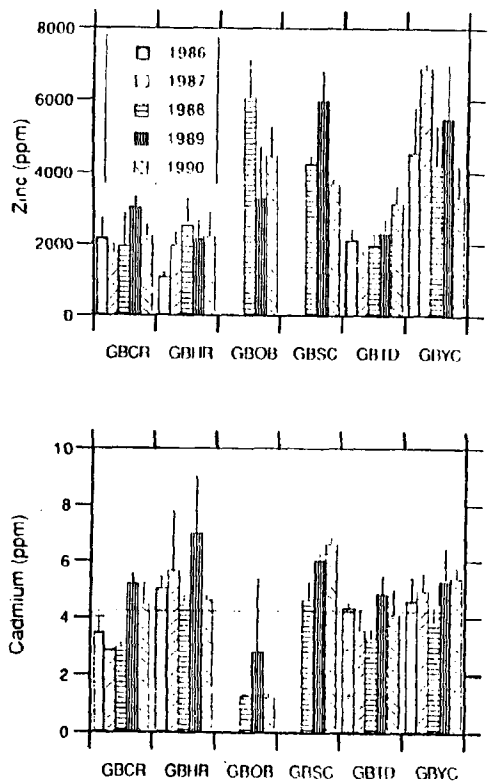

Fig. 6. Annual mean and standard deviation of metal concentrations in oysters collected at six sites in Galveston Bay from 1986–90. Solid horizontal line represents mean of 874 oyster samples collected along US Gulf of Mexico coast from 1986 to 1990. (A) Zn, (B) Cd.

plexes (e.g. Sunda & Hanson, 1987). Dissolved Cu showed the simplest pattern against salinity, decreasing from the fresh to the saline end member via a mid-salinity maximum. This pattern would be consistent with release of dissolved Cu from particles or sediments at mid-salinities. Another possibility is addition of dissolved Cu in water originating from the San Jacinto River or Clear Lake area.

Trace metal concentrations in various estuaries exhibit a wide range, reflecting large local differences in inputs and removal processes. For that reason, it is difficult to compare Galveston Bay to other estuaries, except to say that concentrations are in the same range as measurements elsewhere. Since, unfortunately, these are the first reliable trace metal data for this estuary it is impossible to draw conclusions about historical trends. Because metals in Galveston Bay waters are dominated by exchange with sediments, it seems reasonable that water column metal concentrations are as variable as sediment metal concentrations (see below).

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, it can be seen (Fig. 6) that 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, two of which were not sampled in the first two years of the program, had much higher Zn concentrations. Two of the sites with high Zn concentrations, Ship Channel and Yacht Club, are in northwestern Galveston Bay near industrial waste water inputs and boat basins where Zn contamination might be expected.

The other site with high Zn concentrations was in Offatts Bayou on Galveston Island and is surrounded by residential development and private boat moorings. This site was moved a few hundred metres between year three and year four, and the Zn concentration in oysters was lower by about 50%. This shows the extremely local influence on Zn content of oysters. Local control can be seen even more dramatically in the variations between stations at a given site for a given year (data not given here). Local control on Zn, and on other metals is seen not only in Galveston Bay but also throughout the Gulf of Mexico. For example, oysters from one site in Tampa Bay averaged 200–300 $\mu g\ g^{-1}$ Zn over the first five years of NS&T while a nearby site averaged 6000–8000 $\mu g\ g^{-1}$ (GERG, 1990). On a temporal scale, particularly large changes in Zn were found in Sabine Lake, Texas, another industrialized site. It seems likely that the big changes in Zn from time to time and place to place are caused by human activities, but the exact activity responsible for such a pattern has not been identified. Alternatively, it could be caused by

natural variations in concentrations and chemical form of sedimentary Fe, as suggested by Luoma & Bryan (1978).

Cadmium, Pb, Ag and Hg are often added to the environment in industrial areas by human activities in amounts rivalling those added by natural processes (e.g. Bowen, 1979; Fergusson, 1990) but there is little evidence of anthropogenic inputs of these metals in the Galveston Bay oyster data, except possibly for Pb. Confederate Reef and especially Hanna Reef are in open areas of the Bay, well away from industrial activity, yet oysters from these reefs are similar in Cd, Ag and Hg content to those from reefs along the highly industrialized northwestern shore of the Bay and are only slightly lower in Pb. The Cd pattern (Fig. 6) is thus representative of the distribution of these four toxic materials. Note that the highest Hg value found was at Hanna Reef, apparently the most pristine site sampled. Furthermore, the most anomalous Ag value was found at pristine Confederate Reef where all three stations sampled in 1990 were extremely enriched in Ag. We have no explanation for these results.

Attempting to assess whether sediments in a region such as Galveston Bay are contaminated with respect to a given metal is difficult. Much of this difficulty arises from the heterogenous nature of the sediments. For many of the metals under consideration the variation in grain-size distribution can easily lead to variations of a factor of two or more in absolute metal concentrations (Table 4). Consequently, simply giving average total concentrations can be quite misleading, if it is not normalized to grain size (Table 4).

Secondly, in order for contamination to cause a major change in average concentrations, over the entire estuarine system, large amounts of the metal from anthropogenic sources would have to be added (e.g. for Cu which is of intermediate concentration for the trace metals studied, about 60 mg m^{-2} $year^{-1}$ of Cu, equivalent to 80 tons $year^{-1}$ for all of Galveston Bay, would have to be added to the sediments to double their average Cu concentration. This estimate assumes 13 μg g^{-1} Cu interface sediments, and a sediment delivery rate to the Bay of 6×10^6 tons $year^{-1}$ (GURC, 1965). Thus, unless truly massive contamination with a given metal occurs it is generally not possible to detect the enrichment against the background of natural variability using regional averages.

However, while it is difficult to asses whether or not an entire area may be contaminated with a given metal, it is frequently possible to identify local sub-areas that have anomalously high concentrations of a given metal relative to the region under study as a whole. Such areas have often been observed to be close to the source of anthropogenic metal inputs. It must be kept in mind that formation of such 'pockets of contamination' is strongly dependent on the degree of dispersion of the introduced metal, and a variety of other processes such as biological uptake and sedimentation patterns.

As discussed in the Results section, in this study the search for sediments with anomalous metal concentrations was undertaken by ratioing total reactive-metal concentrations to total reactive-Fe concentrations. Total reactive-metal normalized to total reactive-Fe is designated Me*. Average values of this parameter in Galveston Bay are compared to equivalent values in Baffin Bay, Texas (Huerta-Diaz & Morse, 1992) in Table 6. Baffin Bay was chosen for comparison because it is remote from major population centres and similar data exist for sediments from this Bay. (It should be noted that Baffin Bay is generally hypersaline whereas Galveston Bay has a salinity less than that of seawater.) Mn* is less in Galveston Bay, and Ni*, Cu*, and Pb* are similar in both bays. Zn*, Cd*, Cr*, and Hg* are at least 1·5 times higher in Galveston Bay. Mo* and As* are at least an order of magnitude higher in Galveston Bay than Baffin bay, but total As and other metals in the sediments of Galveston Bay are similar to concentrations in other Texas and Louisiana bays (GERG, 1990).

It is not possible to unambiguously ascertain whether the trace metals that are higher in Galveston Bay are so as a result of anthropogenic inputs or differing natural sources and processes in the two bays. For example, Mo and As, which are highly elevated in Galveston Bay sediments relative to Baffin Bay, exist in the water column dominantly as molybdate and arsenate, and As has been observed to be at close to normal concentrations in the open water column (Tripp, 1988). In the sediments they are extensively reduced along with sulphate and incorporated into iron sulphide minerals. The salinity in Baffin Bay is typically about four times higher than in Galveston Bay. Consequently, even if Mo and As were in similar concentrations in the water column, their ratio relative to sulphate would be about four times higher in Galveston Bay. This difference in ratios could then well be reflected in their incorporation into sediments.

Three approaches have been made to try to identify sites in Galveston Bay which have 'anomalous' metal concentrations using Me* values. The first two are given in Table 6A, where samples having twice the average value are given in italics, and Table 6B where samples having a difference of over two standard deviations from the average value are given. The third approach is non-statistical and consists of observing Me* values in histograms (Fig. 7). The choice of values above which Me* is considered 'anomalous' is arbitrary. For some metals, such as Zn, it is relatively obvious, for others, such as Cr it is difficult. The histograms in Fig. 7 are

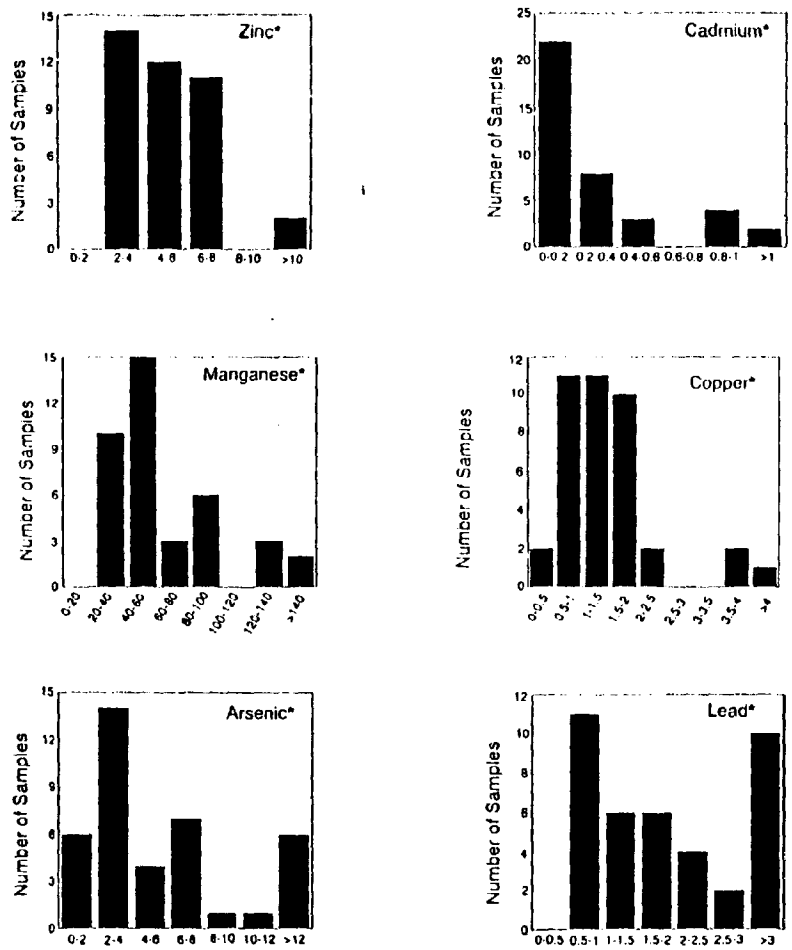

Fig. 7. Histograms of Me* (= reactive-metal to reactive-iron concentration ratio) values for different metals in Galveston Bay sediments.

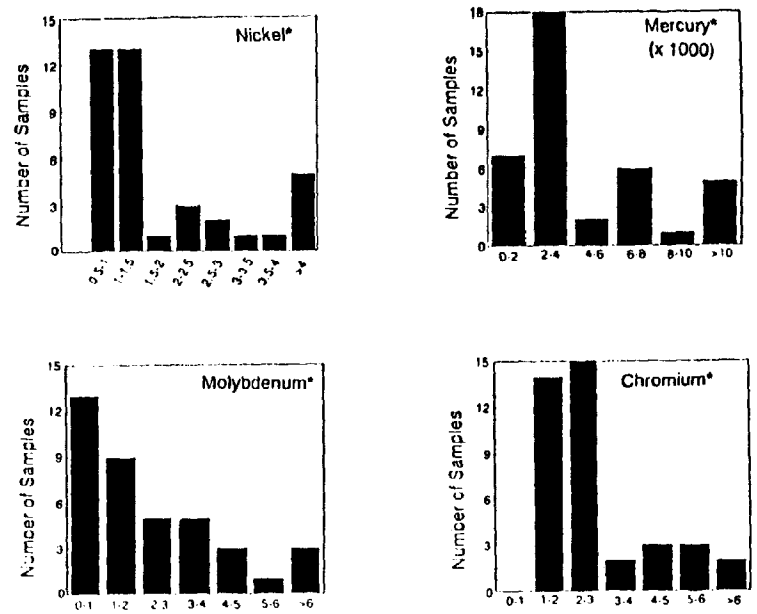

Fig. 7. – *contd.*

arranged in increasing order of difficulty in making such a judgement. The values chosen are Zn* > 10, Cd* > 0·8, Mn* > 120, Cu* > 3·5, As* > 12, Pb* > 3, Ni* > 3, Hg* > 0·01, Mo* > 6, and Cr* > 6.

Almost half (17 out of 39) sites exhibited an anomalous value for at least one metal by at least one of the above criteria (Table 6B). Site GB1-16 off Eagle Point had anomalous values for all metals. The number of sites having anomalous values for each metal is Pb = 9, Ni = 7, Cd and As = 6, Mn and Mo = 5, Cr and Hg = 4, Cu = 3, and Zn = 2. Exceptionally higher values (defined as ~4 times higher the standard deviation above the average) were encountered for Ni* and Cd* at site GB1-16, Cu* at GB1-38, Zn* at site GB1-7, and As* at site GB1-11. While the high Cu* (Clear Lake site) may possibly be explained by leaching of Cu from anti-fouling paints on the many boats moored in this small area, the possible reasons for the other high values are not obvious. It is interesting to note that few of the exceptionally high values occurred near Texas City or directly in the Houston Ship Channel, where higher contaminant metal concentrations might be expected, but that about 60% of the dredge spoil sites had anomalous metal concentrations.

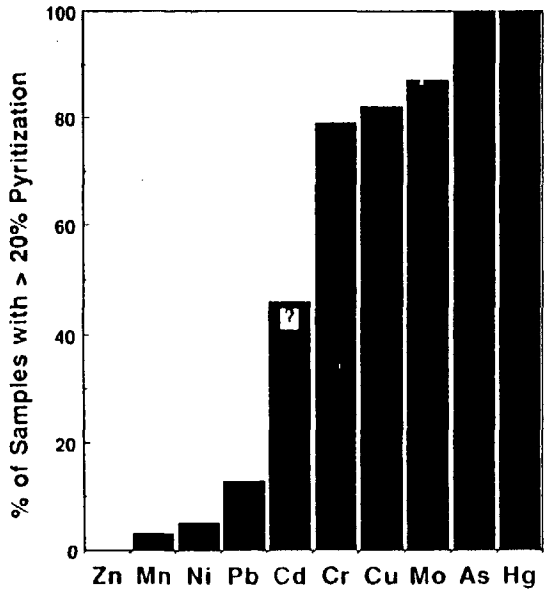

Fig. 8. Histogram of percentage of samples for different metals having greater than 20% of the total reactive fraction pyritized in the top 10 cm of sediment.

A major objective of this investigation of trace metal levels in sed ments was to investigate if extensive pyritization of reactive trace meta takes place near the sediment water interface (approximately top 10 cm In the Results section data were presented indicating that this does occu for several of the metals of interest. Figure 8 is a histogram giving th percentage of each metal that was significantly (here defined as > 20% pyritized. Metals in which less than 15% of the samples fell in this cate gory include Zn, Mn, Ni, and Pb. As previously discussed, the data fc Cd are uncertain due to its low concentration. Over 75% of the Cr, Cu Mo, As and Hg samples were significantly pyritized. It should be note that previous studies (e.g. Huerta-Diaz & Morse, 1992) indicate tha pyritization of metals dominantly occurs in the upper 10 cm of sedimer in fine-grained sediments associated with coastal environments. Conse quently, these observations are of likely general validity for Galvesto Bay. It is also interesting to note that the most extensively pyritize metals, As and Mo, both have total reactive concentrations normalize to Fe over an order of magnitude higher than in Baffin Bay sediments.

SUMMARY AND CONCLUSIONS

Based on extensive investigations into concentrations and chemical forms of selected trace metals in water, sediments and biota (e.g. oysters), we come to the following conclusions:

(1) Trace metal concentrations in the open water column of Galveston Bay are similar to those in apparently more pristine bays and estuaries. Cu, Zn, Pb, and Ag concentrations in the water column of Galveston Bay are low, and are mostly regulated by sediment dynamics (wind and tide generated sediment suspension and settling), leading to significant association with SPM and correlations of their particulate concentrations in the water with suspended matter concentrations. Concentrations of these metals in suspended particles ≥ 0·4 μm, diameter resemble those in the sediments and are higher at low particle concentrations, indicating enrichment in the finer, slower settling fraction.

(2) Except in Zn, trace metal concentrations in oysters from Galveston Bay are similar to those in oysters from pristine areas elsewhere and do not reflect the relative differences in proximity to population and industrialization centres of the different sampling sites in the Bay.

(3) Average trace metal concentrations in the sediments are similar to those from other estuaries. However, due to the large range of concentrations observed for many trace metals, meaningful comparisons require normalization to grain size and reactive-Fe. In Galveston Bay sediments, total reactive Zn, Cd, Cr, and Hg are at least 1·5 times higher, and As and Mo over an order of magnitude higher when normalized to reactive-Fe, than in sediments from Baffin Bay (Huerta-Diaz & Morse, 1992). Since trace metals in the water column closely reflect those in sediments of this estuary, it is likely that the same metals may have been historically elevated in the water column as well. Forty four percent of the individual sites in Galveston Bay exhibit an 'anomalous; concentration with respect to at least one of the metals studied. About half of these sites were directly associated with dredge spoils. It is not possible to unequivocally determine if this is the result of contamination from anthropogenic sources, however, it is probable that these elevated concentrations of metals in the sediment reflect past conditions during which anthropogenic metal inputs were higher.

(4) A major fraction of reactive Cr, Cu, Mo, As, and Hg are immobilized by incorporation into authigenic pyrite in the top 10 cm of sediment. These metals may be transformed via pyrite oxidation (Morse, 1991) to more bioavailable species if the sediments are resuspended in the oxic water column by storms or activities such as dredging and bottom trawling.

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Reprint 8

Mercury Bioaccumulation by Shrimp (*Penaeus aztecus*) Transplanted to Lavaca Bay, Texas

Sally J. Palmer and Bobby J. Presley

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Mercury Bioaccumulation by Shrimp (*Penaeus aztecus*) Transplanted to Lavaca Bay, Texas

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A field study was conducted to determine mercury accumulation rates by brown shrimp, *Penaeus aztecus*, transferred to a mercury contaminated estuary, Lavaca Bay, Texas. Mercury levels in the caged shrimp rose from an average baseline value of 347 ± 163 ppb to 1170 ± 107 ppb in 36 days, resulting in an average rate of mercury uptake of 22 ppb per day. Our results show that shrimp rapidly accumulate Hg when confined to a contaminated area, even though the natural population of shrimp in Lavaca Bay is not contaminated.

As much as 29.9 kg day^{-1} of mercury was released into Lavaca Bay, Texas from 1966 to 1970 by waste water from a chlor-alkali plant (Fig. 1). Since 1970 the Texas Department of Health (TDH) has issued periodic health warnings and bay closures due to elevated (> 0.5 pm wet wt) Hg levels in Lavaca Bay organisms, only to reopen the bay to fishing when the Hg levels decreased. Portions of the bay closed to commercial and sport fishing of finfish and crabs in 1988 have not been reopened as of this writing. Unlike natural population of oysters (*Crassostrea virginica*) and blue crabs (*Callenectes sapidus*) in Lavaca Bay, no high (> 0.5 ppm wet wt) mercury levels in shrimp have been reported by the TDH (Trebatoski & Gooris, 1990) or other researchers who worked in the area (Blanton & Blanton, 1972; Palmer, 1992). Therefore all of Lavaca Bay remains open to shrimping.

Numerous laboratory metal accumulation studies have been conducted using a variety of invertebrates over the years (e.g. King & Davis, 1987; Riisgård & Famme, 1986; Zanders & Rojas, 1992). In the work reported here, instead of a laboratory study, field caging experiments were used to determine the uptake rate of mercury by shrimp confined to a contaminated area of Lavaca Bay. To our knowledge, this is the first time that transplanted shrimp have been used to determine mercury accumulation rates in the field. Although it is impossible to control variables such as temperature, salinity, food supply and turbidity in a field study, the authors felt that a field caging study would better reflect natural conditions than a laboratory study using Lavaca Bay sediment of mercury contaminated food.

Materials and Methods

In July 1991 similarly sized (3.2 ± 0.31 cm rostral length; 3.16 ± 0.31 g wet wt) adult brown shrimp (*Penaeus aztecus*) were collected from Matagorda Bay in a relatively uncontaminated area about 10 km from

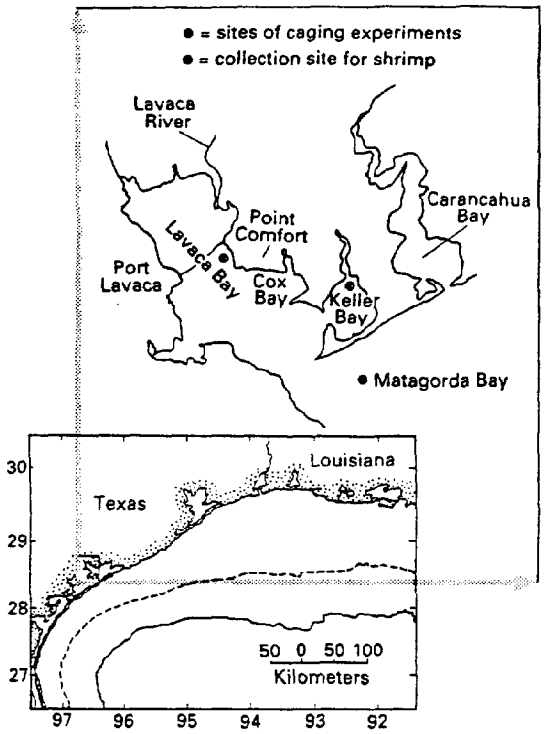

Fig. 1 Map of Lavaca Bay area showing the Matagorda Bay shrimp collection site and the caging sites in Lavaca and Keller Bays.

the most heavily Hg contaminated part of Lavaca Bay. These were transferred to the caging experiment sites in Lavaca Bay and the control site, Keller Bay (Fig. 1). The cages, 20×20×20 cm plastic storage crates, were lined with 3.3 mm plastic mesh and held two shrimp each. To facilitate handling, groups of eight crates were attached to 0.5×1.0 m plastic grates. Individual cages were spaced approximately 7 cm apart on the grates to minimize restriction of water flow around them. At each site the three grates holding the cages were tied together, weighted and pushed at least 1 cm into the sediment. Therefore the caged shrimp could derive food from organic detritus in the bottom sediment (Britton & Morton 1989) as well as plankton and demersal fauna that entered the cage through the mesh (Gleason & Wellington, 1988).

The caged shrimp were sampled six times over a period of 36 days. At each sampling as many as nine individuals were collected from each of the two locations. Immediately after collection the animals were placed in plastic bags and frozen until analysed. The cages that remained in the field after each sampling episode were shifted slightly within the site in hopes of renewing the food supply, but it is nevertheless possible that the shrimp suffered food depravation. Although the average dry weight of the whole shrimp decreased over the experimental period from 1.25 ± 0.39 g (n = 9) to 0.97 ± 0.09 g (n = 6) in Lavaca Bay and 0.71 ± 0.11 g (n = 7) in Keller Bay, the shrimp were vigorous and appeared to be healthy when sampled.

Food chain relationships, especially potential routes of Hg transfer to large commercially important finfish

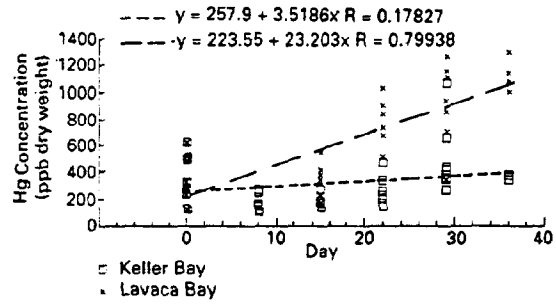

Fig. 2 Mercury concentrations in shrimp from Matagorda Bay confined to Lavaca and Keller Bays. Each symbol represents an individual shrimp sacrificed on that day. Lines are best fit through all data from each bay.

which feed on shrimp, were a prime interest in this study, therefore, the shrimp were analysed whole. Once thawed, the shrimp were rinsed, weighed, freeze dried, and digested according to a modification of USEPA method 245.1 (USEPA, 1990). All samples were analysed in replicate for total mercury using cold vapour atomic absorption spectrophotometry (Hatch & Ott, 1968).

Data quality control

Included with each set of samples analysed were blanks and a dogfish muscle reference material, DORM-1, certified for Hg by the National Research Council of Canada. Analyses of DORM-1 run with each set of shrimp samples were within the certified value for Hg 95% of the time.

Statistical analysis

To determine relationships between total Hg, caging sites and time, statistical analyses using SAS Institute Inc. software (SAS Institute Inc. 1985) were performed. The general linear model (GLM) was used to test for significant differences in Hg levels between Lavaca and Keller Bays and day of the caging experiment. The Least Square Means test, LSMEANS, was used to verify changes in shrimp Hg levels over the duration of the experiment.

Results

Slightly Hg contaminated Matagorda Bay shrimp caged in the highly contaminated portion of Lavaca Bay readily accumulated additional Hg, while shrimp caged in uncontaminated Keller Bay did not significantly change in Hg concentrations during the 36 day experiment (Fig. 2). Average Hg concentrations in shrimp caged in Lavaca Bay climbed from 347 ± 163 ppb dry wt (n = 9) on day 0 to 1170 ± 107 ppb (n = 3) on day 36. Using the shrimp baseline and final mercury concentrations, the average daily rate of Hg uptake was 22 ppb over the 36 day experiment.

The LSMEANS test showed that Hg levels in shrimp caged in Lavaca Bay on day 22 were significantly higher ($p < 0.05$) than baseline concentrations. The GLM procedure indicated a significant difference in Hg concentrations between Lavaca and Keller Bays at every sampling period after day 0 at the $p < 0.05$ level.

Discussion and Conclusions

The accumulation of mercury by shrimp confined to a contaminated area of Lavaca Bay shows that shrimp can become contaminated with Hg if forced to remain in a contaminated area for three weeks or more. The fact that the natural population of shrimp collected in the contaminated area are not contaminated implies that they spend less than three weeks at a time in this area. Additionally, the caging experiment in Keller Bay suggests that slightly contaminated shrimp are slow to depurate Hg. This contrasts with results from a similar experiment where contaminated oysters rapidly depurated Hg when placed in Keller Bay (Palmer *et al.*, 1993). The slow depuration of Hg by shrimp and the low Hg in the natural population of shrimp in contaminated Lavaca Bay implies that the shrimp do not move into and out of the contaminated area on a time cycle that would result in their spending more than a total of three weeks in the contaminated area during their lifetime.

The difference in Hg loss rates between oysters and shrimp may be due to differences in Hg speciation within the organisms, but we have no data to document this. Riisgård & Famme (1986) for example found the retention efficiency, defined as the amount of accumulated mercury divided by the amount of ingested mercury, in shrimp, *Crangon crangon*, to be 4% for inorganic and 75% for organic mercury during their 28 day experiment. It is well known that methyl mercury is more efficiently accumulated and retained than inorganic mercury (Riisgård *et al.*, 1985). Shrimp, unlike oysters consume sediment dwelling organisms. These may contain a higher proportion of methyl mercury than plankton and organic detritus found in the water column, even though our data of total Hg shows these two food sources to be similarly contaminated (Palmer, 1991).

Since an aliquot of a whole homogenized shrimp was used for analysis in this study, concentrations in muscle tissue cannot be obtained directly from this data. However, in a separate study (Palmer, 1992), 18 Matagorda Bay shrimp collected with those used in the caging accumulation study were dissected into muscle (abdominal tissue), exoskelton, and head. The results showed that average Hg levels in abdominal tissue were 2.25 times greater than in head and exoskeleton. Therefore it is likely that the edible muscle tissue of the shrimp caged in Lavaca Bay became more contaminated during the 36 day exposure period than did the whole organism.

The observation that the natural population of shrimp caught from Lavaca Bay are not contaminated with mercury suggests that shrimp spend much of their time and obtain much of their food in non-contaminated areas of the bay or coastal ocean. The relative importance of water, sediment, and food in the accumulation of Hg by shrimp is still poorly understood and could not be resolved in this study because all three media are known to be enriched in Hg at the caging site near the old chlor-alkali plant (Palmer, 1992).

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

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POLYNUCLEAR AROMATIC HYDROCARBON CONTAMINANTS IN OYSTERS FROM THE GULF OF MEXICO (1986–1990)

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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 Oceanographic and Atmospheric Administration's (NOAA's) National Status and Trends (NS&T) Mussel Watch Program exhibit a log-normal 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 & 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 & 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 (Fig. 1). Individual stations at each site are generally from 100 to 1000 m 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

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

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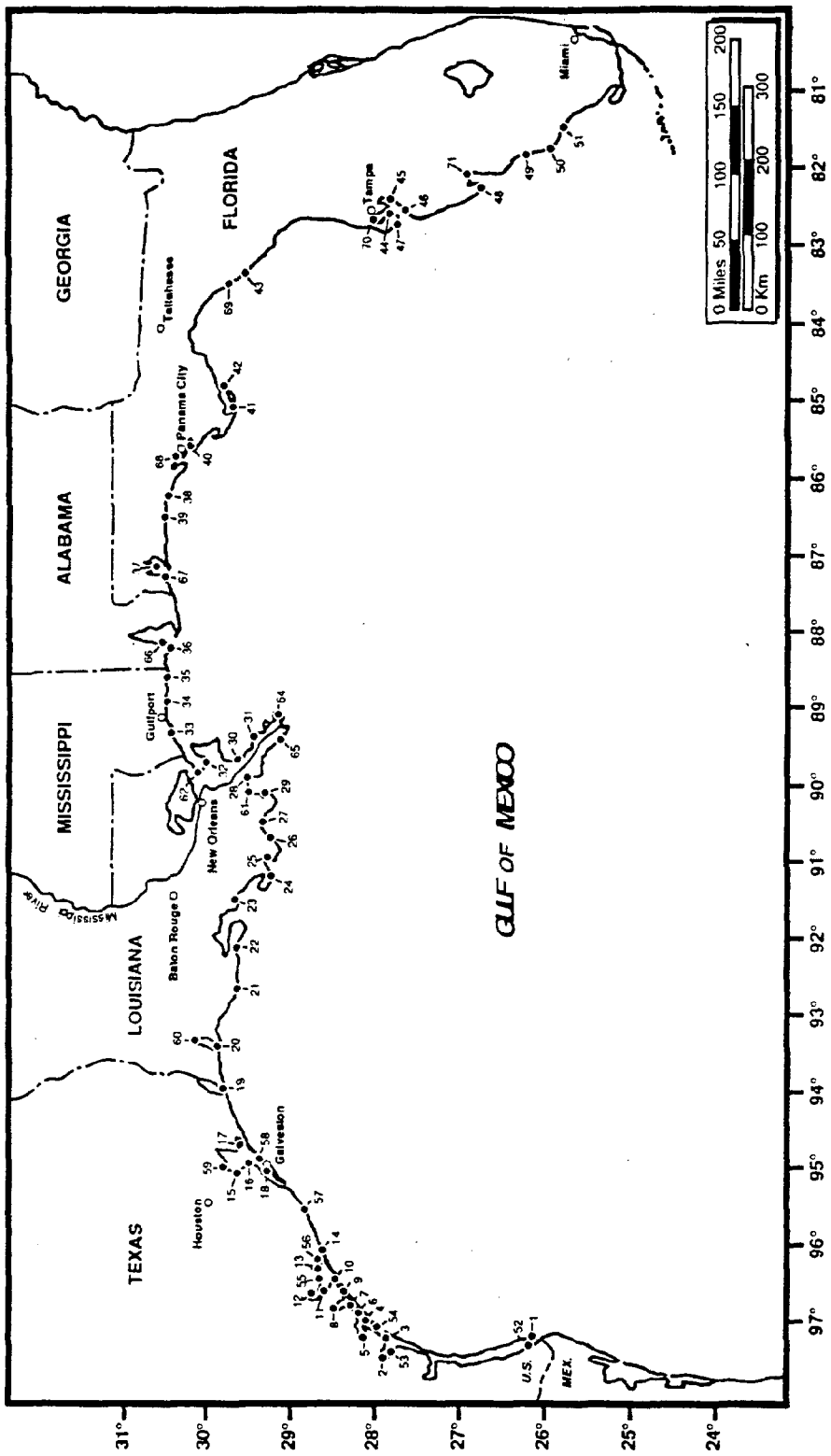

Fig. 1. Location of NS&T Mussel Watch Sites in the Gulf of Mexico (Scricano *et al.*, 1990).

established oyster site due to lack of suitable sized bivalves (Wilkinson *et al.*, 1991). The locations and designator 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 process used was adapted from a method developed by MacLeod *et al.* (1985). Approximately 15 g of wet tissue were used for the PAH analysis. After the addition of internal standards (surrogates) and 50 g 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 tube heated in a water bath (60°C) to concentrate the extract to a final volume of 2 ml. 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 h and partially deactivated with 3% distilled water (v/w). Twenty grams of silica gel were slurry-packed in dichloromethane over 10 g of alumina. Alumina was activated at 400°C for 4 h 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 × 250 mm Phenogel 100 columns (Krahn *et al.*, 1988). The purified aromatic fraction was collected from 1.5 min prior to the elution of 4,4'-dibromofluorobiphenyl to 2 min 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 10 samples. The purified aromatic fraction was concentrated to 1 ml using a Kuderna–Danish tube 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 sample 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 ×0.25 mm (0.32 μm 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 min. 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/μl to 1 ng/μl. 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 6 h 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 wt (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)

Table 2. National Status and Trends oysters polynuclear aromatic hydrocarbon analytes

Aromatic hydrocarbons	
Low molecular weight	High molecular weight
Biphenyl	Fluoranthene
Naphthalene	Pyrene
1-methylnaphthalene	Benz(*a*)anthracene
2-methylnaphthalene	Chrysene
2,6-dimethylnaphthalene	Indeo[*1,2,3-cd*]pyrene[a]
1,6,7-trimethylnaphthalene[a]	Benzo(*a*)pyrene
Acenaphthene	Benzo(*e*)pyrene
Acenaphthylene[a]	Perylene
Fluorene	Dibenz[*a,h*]anthracene
Phenanthrene	Benzo[*g,h,i*]perylene[a]
Anthracene	
1-methylphenanthrene	

[a] Analytes not used in tPAH summation.

Table 3. Total NS&T PAH concentration in oysters

No.	Site code	Median concentration of tPAH V 1990 (ng/g)	IV 1989 (ng/g)	III 1988 (ng/g)	II 1987 (ng/g)	I 1986 (ng/g)	Bay group median (ng/g)
Texas							
1	LMSB	22	20	30	20	25	
52	LMPI	—	—	3380	—	—	30 ± 58
78	LMAC	120	—	—	—	—	
53	CCBH	1 530	—	1 600	—	—	
2	CCNB	161	264	598	434	45	565 ± 725
3	CCIC	137	430	848	—	1 140	
54	ABHI	—	—	1 870	—	—	
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	1 670	682	—	—	792 ± 792
18	GBCR	370	1 170	525	478	1 070	
58	GBOB	315	593	543	—	—	
16	GBTD	25	44	20	112	149	259 ± 606
15	GBYC	247	132	207	568	1 030	
59	GBSC	1 290	1 350	3 100	—	—	
17	GBHR	20	119	34	20	31	
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	54	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	5 480	44	25	57	963 ± 1 020
29	BBMB	1 080	1 380	1 460	1 150	822	
Louisiana—*cont.*							
65	MRTP	212	310	1 410	—	—	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	1 210	893	1 500	4 310	1 600	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	1 520	—	—	—	—	
Florida							
67	PBPH	168	369	842	—	—	
37	PBIB	—	21	204	250	406	197 ± 198
80	PBSP	130	—	—	—	—	
73	CBJB	1 680	8 590	—	—	—	
39	CBSP	225	355	703	543	428	429 ± 1 140
38	CBSR	69	21	2 540	2 470	209	
74	PCLO	98	229	—	—	—	
68	PCMP	1 210	2 690	4 750	—	—	1800 ± 1 590
40	SAWB	1 150	2 090	1 990	1 970	11 800	
41	APDB	20	24	2 800	20	20	57 ± 530
42	APCP	269	1 110	740	20	109	
75	AESP	33	74	—	—	—	
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	2 150	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

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 interquartile 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,

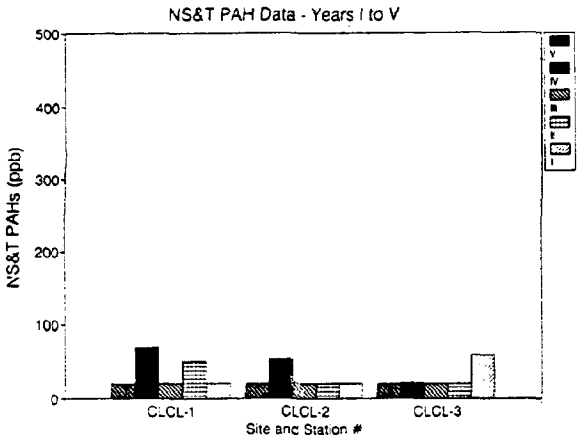

Fig. 2. Total NS&T PAH concentration distribution during the first five years for all three stations: Caillou Lake in Louisiana (Site 25—CLCL).

CLCL, LBMP, TBCB, CBBI & RBHC) with low concentration of tPAH (< 100 ng/g) and little variation in the observed values (Fig. 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 Choctawatchee Bay off Santa Rosa (CBSR, Fig. 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 IV), the oyster reefs experienced a die-off resulting in no oysters being taken from SAPP, SAMP and ESSP.

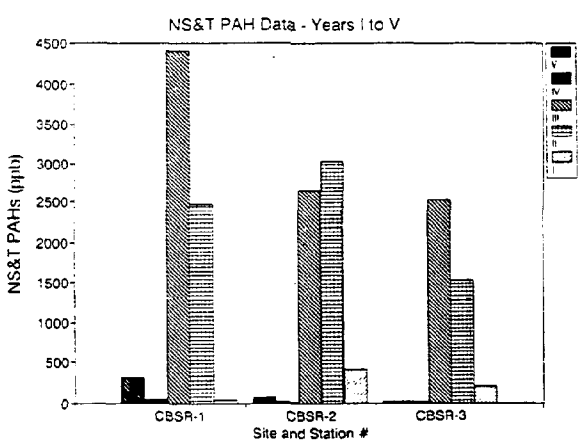

Fig. 3. Total NS&T PAH concentration distribution during the first five years for all three stations: Choctawatchee Bay off Santa Rosa (Site 38—CBSR).

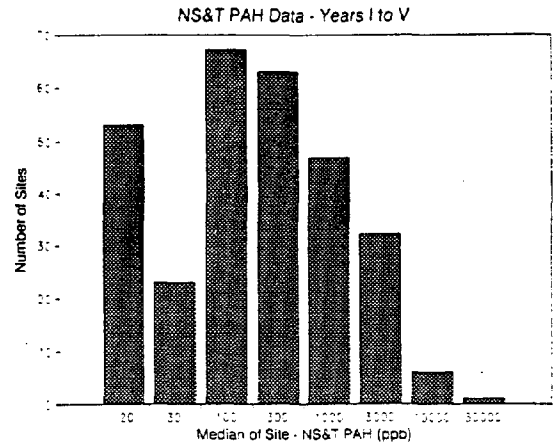

Fig. 4. Frequency distribution of the median total NS&T PAH (tPAH) concentration in the Gulf of Mexico during the first five years of the program.

Cumulative frequency model

Bar graphs (Wade *et al.*, 1990) or crossplots (Wade & Sericano, 1989) of data comparing one year's data with another have been used to display the general trend for tPAH data (Wade & 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 & 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 (Fig. 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 Fig. 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 $y = 153{\cdot}0 + 0{\cdot}9834\,x$ ($r^2 = 0{\cdot}9989$); 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

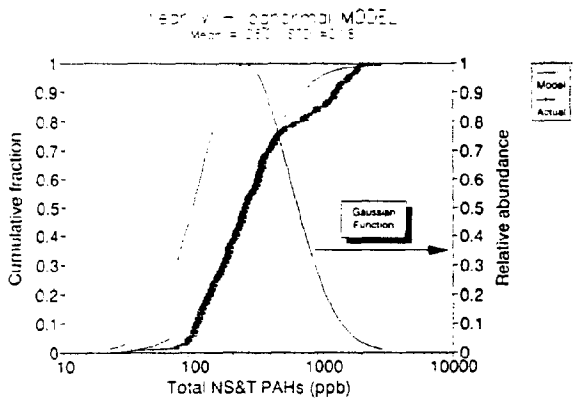

Fig. 5. Plot of the cumulative frequency distribution for Year V total NS&T PAH (tPAH) concentration, compared to the Gaussian curve and its cumulative frequency distribution generated from a lognormal model with a mean of 250 ppb and standard deviation of 218.

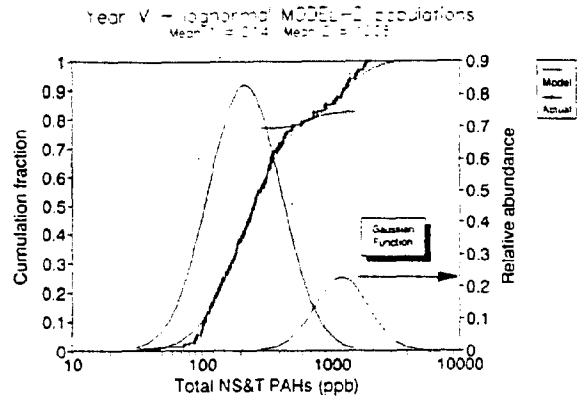

Fig. 6. Plot of the cumulative frequency distribution for Year V NS&T PAH (tPAH) concentration, compared to the Gaussian curves and their cumulative frequency distributions generated from a two population lognormal model with a mean of 214 ppb for Population 1 and a mean of 1205 ppb for Population 2.

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 & 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 the 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 interquartile range (IQR) is used 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 (Fig. 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 (Fig. 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 (Fig. 7) indicates a good correlation.

Table 4. Two population lognormal distribution model. Year V—T-PAH data (2·5% overlap)

Set	Percentile 25%	50%	75%	STD= IRQ/1·35	Log-mean	STD of log-data
1	135	214	320	137	2·330 8	0·278 3
2	801	1 210	1 530	544	3·081 0	0·209 3

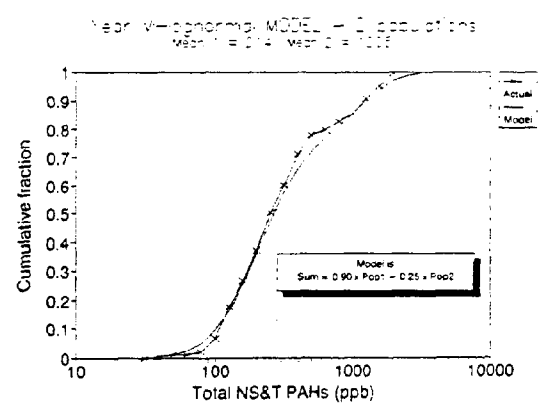

Fig. 7. Comparison of the cumulative frequency distributions for the actual Year V total NS&T PAH (tPAH) concentration data and the cumulative frequency distribution generated from the two population model.

Table 5. Two population lognormal distribution model. Corrected tPAH data—ng/g dry weight

Year	Median total data	Population 1		Population 2	
		Mean (log)	STD (log)	Mean (log)	STD (log)
I	229	197 (2·294 5)	108 (0·229 8)	1 075 (3·031 4)	714 (0·277 2)
II	208	186 (2·269 5)	87 (0·196 7)	1 150 (3·059 9)	1 100 (0·381 1)
III	345	259 (2·413 3)	216 (0·343 5)	1 910 (3·280 8)	1 190 (0·261 8)
IV	352	269 (2·429 8)	174 (0·250 0)	1 350 (3·131 6)	1 190 (0·303 9)
V	270	212 (2·326 3)	131 (0·263 9)	1 170 (3·068 9)	637 (0·243 5)

Since historical NS&T data (Table 3) is censored data (Wade *et al.*, 1988; Wade & 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 & Paterson, 1984). The summary statistics for the corrected data using the two population model for Years I–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% for 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 and 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 is a pattern which 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–V gives the percentage of sites whose PAH concentration is less than a particular concentration (Table 6). As an example, using 1000 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 had 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.

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	559	1 030	480	557
90%	1 290	1 440	2 090	1 300	1 180
95%	1 670	2 820	3 020	2 300	1 750
98%	1 920	5 530	4 550	3 740	2 450

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 concentrations 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 patter 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 heterogenous system, since the PAH concentration data for individual sites does not clearly show this pattern.

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Reprint 10

Butyltin Concentrations in Oysters from the Gulf of Mexico During 1989-1991

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BUTYLTIN CONCENTRATIONS IN OYSTERS FROM THE GULF OF MEXICO FROM 1989 TO 1991

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Abstract

Oyster samples from 53 Gulf of Mexico coastal sites were collected and analyzed for butyltins during 1989, 1990, and 1991. The geometric-mean tributylin 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 in the butyltin concentrations at sites with relatively low butyltin concentrations for 1989 compared with 1990 and 1991 was observed, and, at relatively high butyltin concentrations (>400 ng Sn/g), there was hardly any 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 that attach to the bottom of the hulls of boats and ships (Huggett *et al.*, 1992). Although an effective anti-fouling agent, tributyltin, was found to affect non-target organisms adversely (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; 1991*b*) suggests that environmental half-lives in the marine environment may be longer than reported values (Lee *et al.*, 1987; Olson & Brinckman, 1986; Seligman *et al.*, 1986*a*,*b*, 1988). After the use of TBT-based paints was limited in countries such as France, England, and the USA, the concentration of organotins in water and oysters was shown to decline (Short & Sharp, 1989; Wade *et al.*, 1991*b*; Alzieu, 1991; Page & Widdows, 1991; Valkirs *et al.*, 1991; Waite *et al.*, 1991). In the USA, 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 in 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 concentration in the environment.

Tributyltin and its degradation products were determined in oysters from 53 sites in the Gulf of Mexico from 1989 to 1991. The over-all butyltin concentrations showed a decline from 1989 to 1990 (Wade *et al.*, 1991*a*,*b*). If this decline resulted from the implementation of the limitations on the use of TBT in the USA 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 winters of 1989, 1990, and 1991. Table 1 shows the geographic location of the sites sampled and the symbols used to identify each site. Although known point sources of TBT such as marinas 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,

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Table 1. Sampling locations and site designators

Designation	Site	Location	Latitude (deg)	Latitude (min)	Longitude (deg)	Longitude (min)
		TEXAS				
LMSB	South Bay	Lower Laguna Madre	26	02·58	97	10·49
LMAC[a]	Arroyo Colorado	Laguna Madre	26	16·80	97	17·30
CCBH[a]	Boat Harbor	Corpus Christi	27	50·00	97	23·00
CCNB[a]	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[a]	Copano Reef	Copano Bay	28	08·20	97	07·58
MBAR	Ayres Reef	Mesquite Bay	28	10·30	96	49·70
SAPP[a]	Panther Pt. Reef	San Antonio Bay	28	13·20	96	43·00
SAMP[a]	Mosquito Point	San Antonio Bay	28	19·00	96	42·20
ESSP[a]	South Pass Reef	Espiritu Santo Bay	28	17·83	96	37·50
ESBD[a]	Bill Days Reef	Espiritu Santo Bay	28	25·00	96	27·00
MBGP[a]	Gallinipper Pt.	Matagorda Bay	28	35·00	96	34·00
MBLR	Lavaca River Mouth	Matagorda Bay	28	39·30	96	35·00
MBCB[a]	Carancahua Bay	Matagorda Bay	28	40·00	96	23·20
MBTP	Tres Palacios Bay	Matagorda Bay	28	39·00	96	15·50
MBEM	East Matagord[a]	Matagorda Bay	28	42·30	95	53·00
BRCL[a]	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[a]	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	94	54·42
		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 Lek	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[a]	Pass a Loutre	Mississippi River	29	04·30	89	04·60
BSSI	Sable Island	Breton Sound	29	24·70	89	28·70
BSBG	Bay Gardeme	Breton Sound	29	35·87	89	38·50
LBMP	Malheureux Point	Lake Borgne	29	52·30	89	40·70
LPGO[a]	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[a]	Dog River	Mobile Bay	30	35·50	88	02·72
		FLORIDA				
PBPH	Public Harbor	Pensacola Bay	30	34·80	87	11·50
PBIB[a]	Indian Bayou	Pensacola Bay	30	30·83	87	04·00
PBSP[a]	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[a]	Choctawhatchee Bay	30	23·50	86	10·60
PCLO	Little Oyster Bay	Panama City	30	15·00	85	40·87
PCMP[a]	Municipal Pier	Panama City	30	08·20	85	37·50
SAWB	Watson Bayou	St. Andrew Bay	30	0·850	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 Baypu	Tampa Bay	27	50·72	82	36·75
TBOT	Old Tampa Bay	Tampa Bay	28	01·48	82	37·95
TBKA[a]	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[a]	Henderson Creek	Rookery Bay	26	01·83	81	43·75
EVFU	Faka Union Bay	Everglades	25	54·27	81	30·60
BHKF[a]						

[a] Sites that were not sampled consecutively from 1989 to 1991.

spiked with a surrogate standard, extracted with 0·2% tropolone in methylene chloride, hexylated, purified by using Si/Al columns, and analyzed by gas chromatography with a tin-selective-flame photometric detector. Quality control consisted in using 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 was 5 ng Sn/g for TBT and DBT and 10 ng Sn/g for MBT.

RESULTS AND DISCUSSION

Annual variation of butyltins at individual sites

Oyster butyltin concentrations determined in 1989,

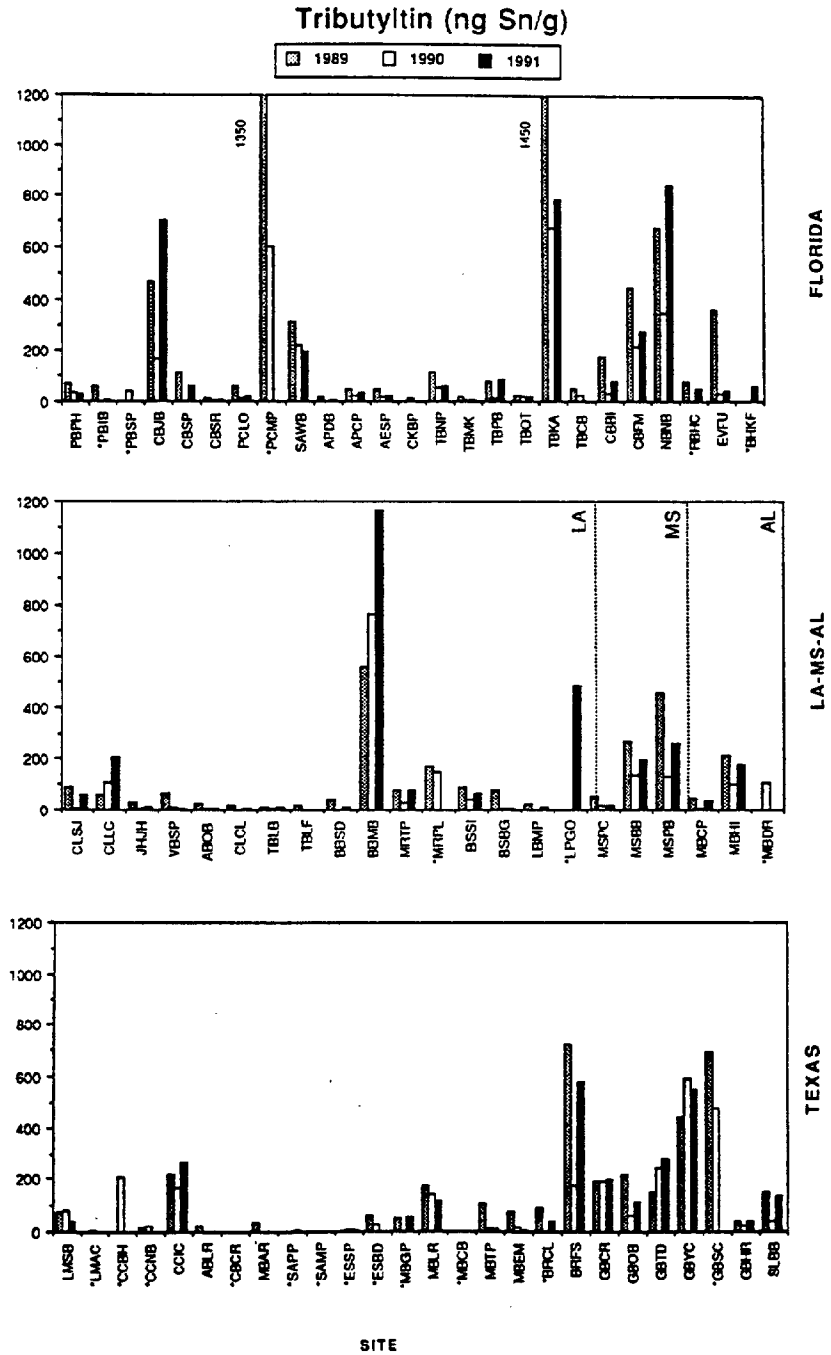

Fig. 1. Geographical distribution of tributyltin concentrations in oysters (*Crassostrea virginica*) from the Gulf of Mexico coast. Asterisks indicate those sites which were not sampled in consecutive years.

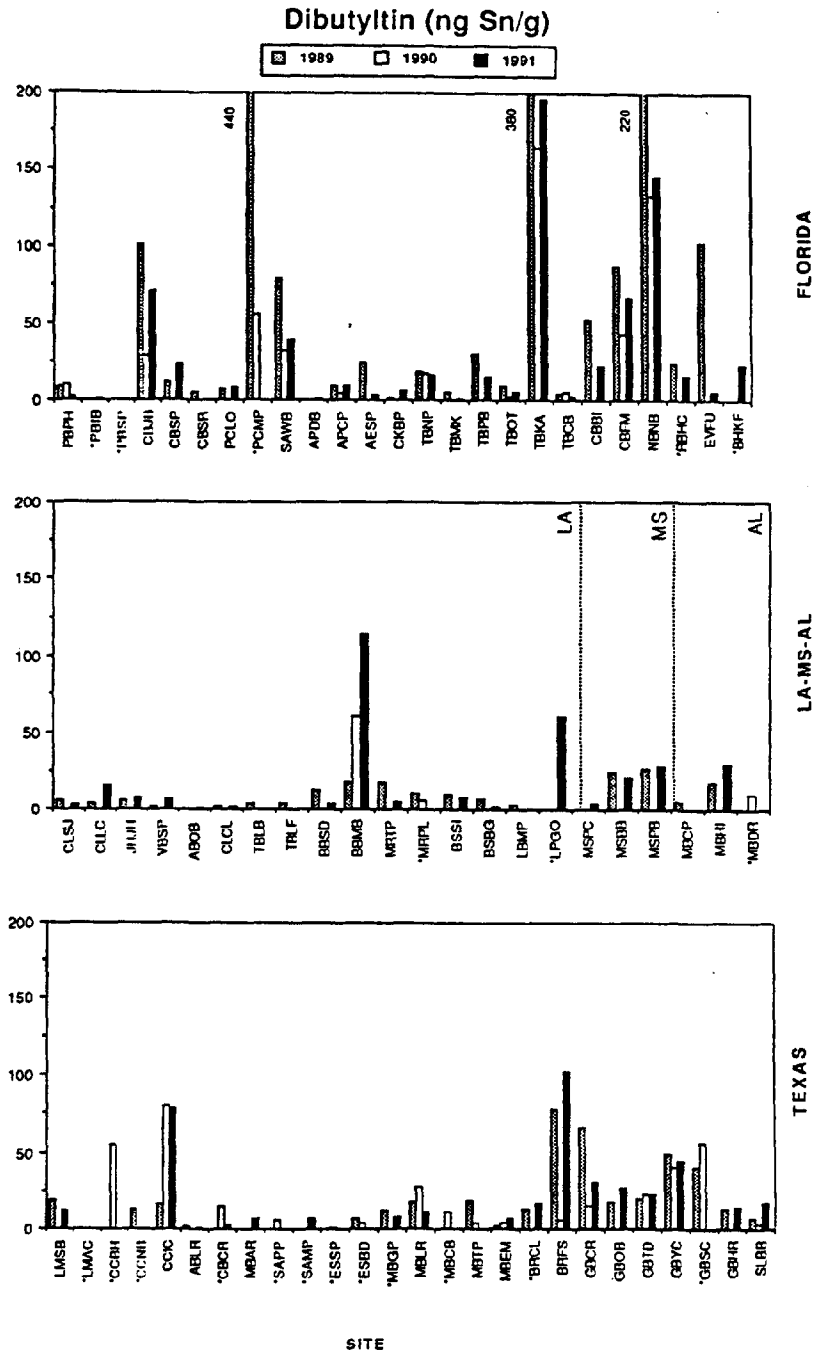

Fig. 2. Geographical distribution of dibutyltin concentrations in *C. virginica* from the Gulf of Mexico coast. Asterisks indicate those sites which were not sampled in consecutive years.

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 an asterisk in Table 1 and Figs 1, 2 and 3.

The concentrations 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, 1991, respectively. In general, the butyltin concentrations decreased from 1989 to 1990 and then increased slightly between 1990 and 1991.

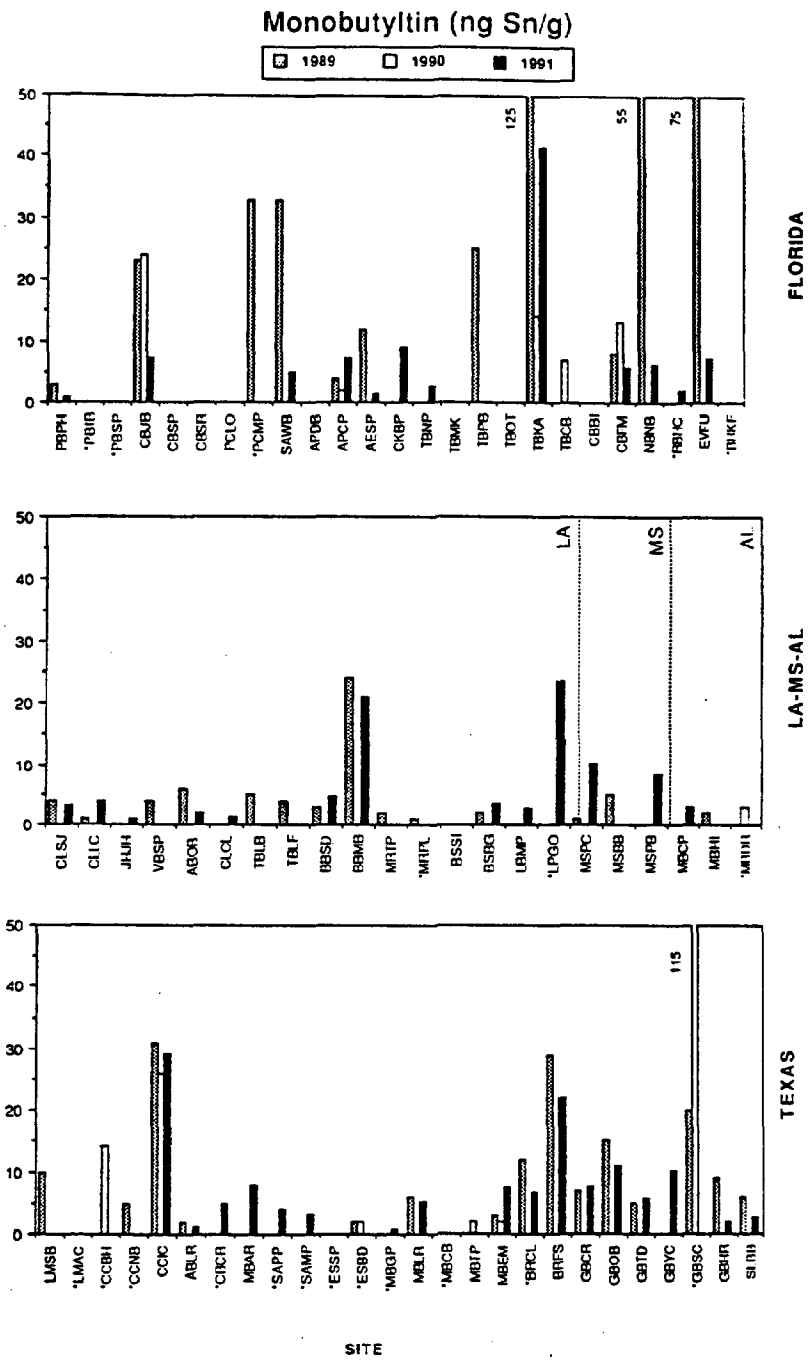

Fig. 3. Geographical distribution of monobutyltin concentrations in *C. virginica* from Gulf of Mexico coast. Asterisks indicate those sites which were not sampled in consecutive years.

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.*, 1986*a*; 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. The fluctuation of percentage TBT around 85% is therefore 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 Fig. 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 increase monotonically at some sites from 1989 to 1991, whereas, at other sites, concentrations decreased monotonically. For example, oyster TBT concentrations increased from 1989 to 1991 at CLLC, BBMB, and GBTD (Fig. 1). Decreasing TBT concentrations from 1989 to 1991 were observed for oysters from PBPH, SAWB, TBCB, MBLR, and MBEM (Fig. 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 one of 53 sites in 1989 and at ten and eleven 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 Fig. 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), the annual variation of DBT concentrations did not mimic the annual variation of TBT concentrations. Ship and boating activities have been cited as potential factors that may affect DBT fluctuations (Short and Sharp, 1989; Uhler *et al.*, 1989). Furthermore, 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, Figs 1 and 2) not only showed increasing concentrations of TBT during the three-year sampling period but also showed a steady increase in DBT in the sample period. DBT was detected in 39, 38, and 33 out of the 53 sites sampled in each of the three years. In many instances, DBT was not detected in any of the sampling years.

Regional MBT concentrations are shown in Fig. 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, four, and nineteen of the 53 sites during 1989, 1990, and 1991, respectively. During all three years, MBT was detected at only 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 anti-fouling 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 were 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 Fig. 4. The graph is a plot of 1989 concentrations against those of 1990 and 1991. The x and y scales are identical. If no change occurs in the TBT concentration at a site, those data will be plotted on the center line. Sites that fall below the line show a decrease, whereas points that rise above the line show an increase compared with 1989. Two other lines also appear in Fig. 4. These are the lines that form the boundary of sites with a factor of two increase (top line) or decrease (bottom line). Only six sites for 1990 and eight for 1991 of the 53 sites plotted for each year are above the center line. Hence, 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 two.

In order to detect temporal trends, the butyltin oyster concentrations for the entire Gulf of Mexico from 1989 to 1991 are compared. Annual variations of butyltins for the entire Gulf of Mexico are not readily apparent in Figs. 1, 2, or 3, where only annual concentrations at individual sites are compared. Comparisons of arithmetic mean, geometric mean, and medians (Table 2) for butyltin concentrations determined during 1989, 1990, and 1991 are based only on the 53 sites that were sampled in all three years. All these parameters were calculated by assigning 5 ng Sn/g to all those samples with concentrations below the limit of detection. The percentage of samples below the detection limit is listed in Table 2. The median and geometric means are similar in all cases, whereas the arithmetic mean is always higher. The median or the geometric mean appears to be the better estimator of the central tendency of the data. On the basis of the median or the geometric mean, there was a decrease in TBT oyster concentrations when 1989 is compared with 1990 or 1991.

A complete view of butyltin concentrations for the whole Gulf of Mexico for a given year can be achieved

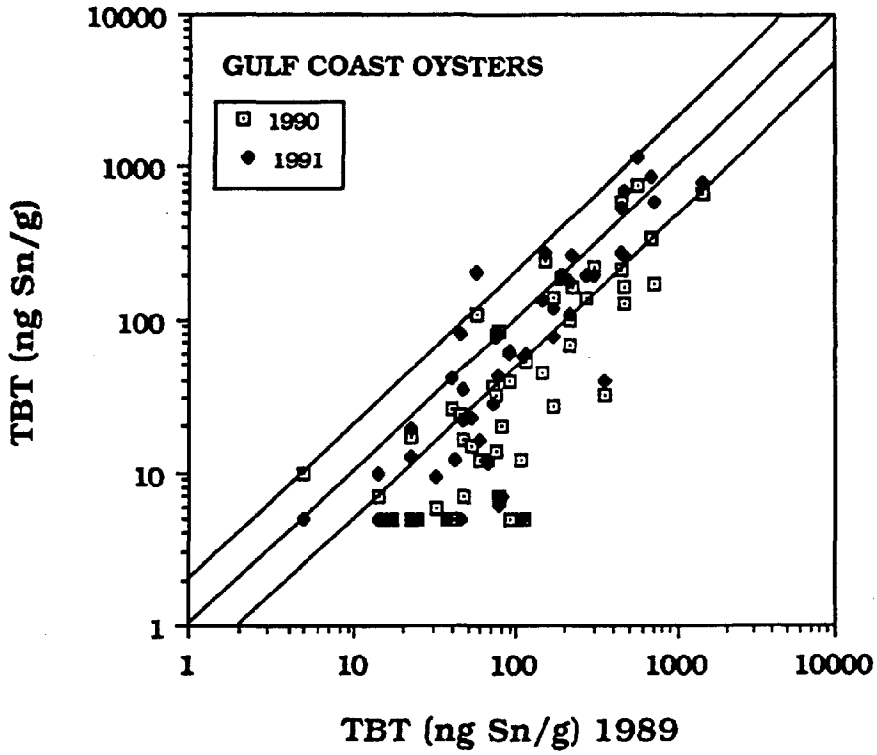

Fig. 4. Tributyltin concentrations determined in 1989 plotted against the tributyltin concentrations determined in 1990 and 1991. Points falling along the center line have equal concentrations, colateral lines indicate a factor of two greater or less than the concentrations determined in 1989.

by using either cumulative-percentage-distribution or probability-distribution curves (Mackay & Paterson, 1984; O'Connor & Ehler, 1990; Jackson *et al.*, in the press). Although both types of curve 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 the press).

TBT log (distribution) curves are shown in Fig. 5 for 1989, 1990, and 1991. These curves were obtained by using the following equation (Milton & Arnold, 1986):

$$f(x) = \{s[\sqrt{(2\pi)}]\}^{-1} \exp - \{0{\cdot}5[(x - X)/s]^2\} \quad (1)$$

where $f(x)$ is the distribution probability of the log (butyltin concentration), s is the standard deviation, x is the log of the butyltin concentration, and X is the geometrical mean. Each $f(x)$ was then divided by the sum of the $f(x)$ values shown by eqn (2):

$$f'(x)_i = f(x)_i / \sum f(x)_i \quad (2)$$

in such a way that

$$\sum f'(x)_i = 1 \quad (3)$$

TBT-concentration curves from 1989, 1990, and 1991 (Fig. 5) are Gaussian with some degree of skewness. DBT had a log–normal distribution only in 1989 and 1990, whereas 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 lines for 1990, and dashed lines for 1991 (Fig. 5) and are also reported in Table 2a. The TBT, DBT, and MBT concentrations for ±1 standard deviation from the geometric mean are listed in Table 2b. 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. The TBT concentration decreased from 1989 to 1990 at all concentrations, whereas it decreased from 1989 to 1991 at low and medium concentrations but was similar for the two years at high concentrations (Fig. 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 facts that newer TBT-containing paints are rated to be good for up to five years and that TBT was not banned but its use limited probably lead to decreased TBT inputs. This may have resulted in the observed decreases in TBT concentrations in 1990 and 1991. The decrease observed at high

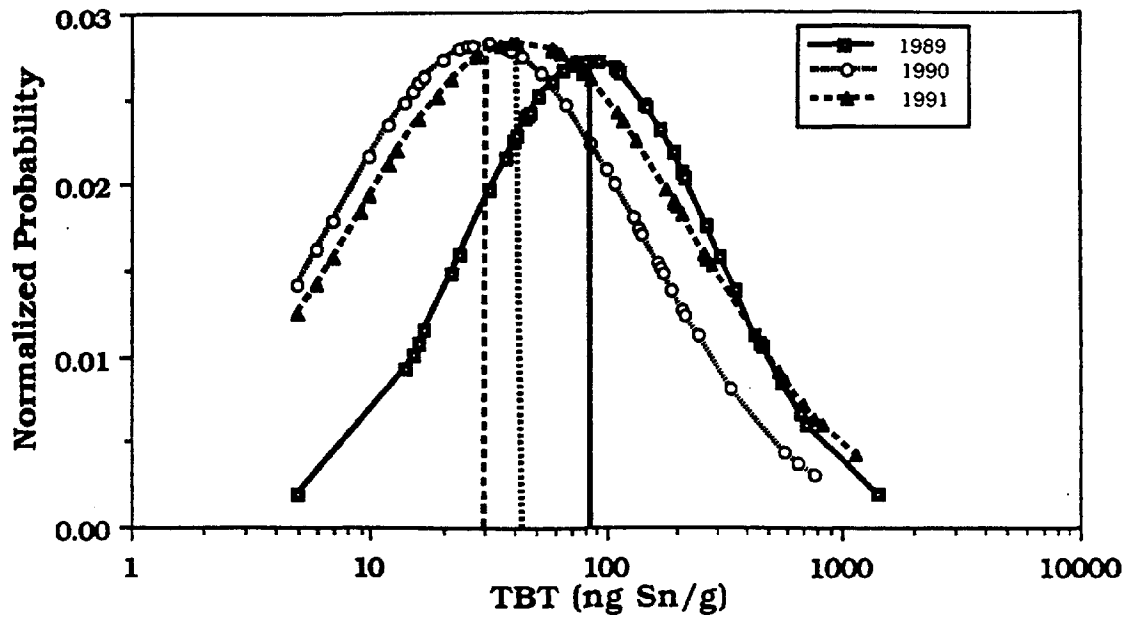

Fig. 5. Log-normal distribution of tributyltin concentrations determined in oysters in 1989, 1990, and 1991.

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). TBT lower-concentration ranges may therefore have decreased as a consequence of TBT regulations or changes in TBT-based paint formulations, but the effects 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 showed annual variations. This may be due to the high percentage of values below the MDL (Table 2).

Table 2a. Arithmetic and geometric means and medians (ng Sn/g)[a]

	TBT	DBT	MBT
1989			
Arithmetic mean	176	32	13
Geometric mean	85	14	8
Median	77 (2%)	12 (26%)	5 (60%)
1990			
Arithmetic mean	96	17	6
Geometric mean	30	8	6
Median	24 (17%)	5 (72%)	5 (90%)
1991			
Arithmetic mean	150	25	8
Geometric mean	43	13	6
Median	42 (17%)	8 (40%)	5 (66%)

[a] Numbers in parenthesis indicate percentage of samples below MDL.

Table 2b. Geometric mean ±1 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

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 in 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 or 1991 may have resulted from previous changes in anti-fouling paint formulation with lower TBT-release rates or the suspension of painting activities by individual boat owners after 1988. Because the newer TBT paints were formulated to last five 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.

ACKNOWLEDGEMENT

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Reprint 11

The American Oyster (*Crassostrea virginica*) as a Bioindicator of Trace Organic Contamination

José L. Sericano

THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA) AS A BIOINDICATOR OF TRACE ORGANIC CONTAMINATION

A Dissertation

by

JOSE LUIS SERICANO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

May 1993

Major Subject: Oceanography

THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA) AS A BIOINDICATOR OF TRACE ORGANIC CONTAMINATION

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ABSTRACT

The American Oyster (*Crassostrea virginica*) as a Bioindicator of Trace Organic Contamination. (May 1993)

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This study was designed to examine the uptake and depuration of trace organic contaminants, e.g. polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), including planar congeners, and butyltin species, by oysters (*Crassostrea virginica*) through transplantation experiments in Galveston Bay, Texas.

PAHs, low molecular weight PCBs and tributyltin (TBT) were rapidly bioaccumulated by transplanted oysters and apparent equilibrium concentrations were reached after 20 to 30 days of exposure. In contrast, high molecular weight PCBs did not reached an equilibrium plateau at the end of the seven-week exposure period. When oysters were back-transplanted to their former location, PAHs, low molecular weight PCB congeners and TBT were depurated at similar rates while the high molecular weight PCBs were depurated at considerably slower rates. The original background

concentrations were not reached after the 50-day depuration period. Chronically contaminated Ship Channel oysters, simultaneously transplanted to the uncontaminated area, showed lower clearance rates than those encountered for originally uncontaminated bivalves.

Oysters exposed in the laboratory to PCBs and PAHs, preferentially bioaccumulated four to six chlorine-substituted PCBs and four- and five-ring PAHs. Oysters exposed simultaneously to PAHs plus PCBs depurated PAHs at a faster rate than oysters that were exposed solely to PAHs. The half-lives for individual PAHs encountered in the first group of oysters were similar to those found in the field.

The present study presents evidence to substantiate the theory that bioconcentration and clearance of different PCB congeners by oysters appear to be more affected by molecular size than by hydrophobicity. The influence of the chlorine substitution patterns in the bioaccumulation of PCBs by oysters is particularly evident in the case of the highly toxic planar congeners.

Indigenous oysters can be valuable bioindicators of environmental contamination by trace organic compounds only if their limitations are fully understood. Within these limitations, transplanted oysters can be succesfully used to monitor environmental contamination by PAHs and TBTs in areas lacking indigenous bivalves if deployed *in-situ* for a period of time of at least 30 days; for PCBs, however, a much longer time period, i.e over 6 months, may be required.

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TABLE OF CONTENTS

		Page
ABSTRACT		iii
ACKNOWLEDGEMENTS		v
TABLE OF CONTENTS		vi
LIST OF TABLES		xi
LIST OF FIGURES		xiii
CHAPTER		
I	INTRODUCTION	1
	Statement of Purpose	1
	Research Objectives	5
	Galveston Bay System	7
II	BIOAVAILABILITY OF PAHs TO THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA): A FIELD STUDY	10
	Introduction	10
	PAHs: A Review	11
	Background Information	11
	Distribution and Occurrence in Galveston Bay	14
	Bivalve Uptake and Depuration Studies	20
	Uptake and Depuration of PAHs	22
	Experimental Design, Sample Collection and Methods	22
	Extraction and fractionation of PAHs	24
	Instrumental analysis	28
	Ancillary parameters	28
	Statistical analysis	29
	Uptake of PAHs by Transplanted Oysters	29

CHAPTER Page

Depuration of PAHs by Newly and Chronically Contaminated Oysters 39
Concluding Remarks 45

III UPTAKE, RETENTION AND RELEASE OF PCBs BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA) 48

Introduction 48
PCBs: A Review 49
Background Information 49
Distribution and Occurrence in Galveston Bay 53
Bivalve Uptake and Depuration Studies 56
Uptake and Depuration of PCBs 58
Experimental Design, Sample Collection and Methods 58
Extraction and sample fractionation of PCBs 58
Instrumental analysis 58
Ancillary parameters 60
Statistical analysis 60
Uptake of PCBs by Transplanted Oysters 60
Depuration of PCBs by Newly and Chronically Contaminated Oysters 70
Concluding Remarks 76

IV UPTAKE AND DEPURATION OF PLANAR PCB CONGENERS BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA): A SPECIAL CASE OF PCBs 80

Introduction 80
Planar PCBs: A Review 81
Background Information 81
Distribution and Occurrence in Galveston Bay 83
Bivalve Uptake and Depuration Studies 84
Uptake and Depuration of Planar PCBs 85

CHAPTER		Page
	Experimental Design, Sample Collection and Methods	85
	Extraction and initial sample fractionation	85
	Isolation of planar PCB congeners	85
	Instrumental analysis	86
	Planar PCB Congener Analysis	86
	Uptake of Planar PCBs by Transplanted Oysters	90
	Depuration of Planar PCBs by Newly Contaminated Oysters	95
	Concluding Remarks	96
V	UPTAKE AND DEPURATION OF TRIBUTYLTIN BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)	97
	Introduction	97
	TBT: A Review	97
	Background Information	97
	Distribution and Occurrence in Galveston Bay	99
	Bivalve Uptake and Depuration Studies	99
	Uptake and Depuration of TBT	101
	Experimental Design, Sample Collection and Methods	101
	Extraction and sample fractionation	101
	Instrumental analysis	102
	Ancillary parameters	102
	Statistical analysis	102
	Uptake of TBT by Transplanted Oysters	102
	Depuration of TBT by Newly and Chronically Contaminated Oysters	105
	Concluding Remarks	106
VI	MECHANISM OF THE UPTAKE AND RELEASE OF TRACE ORGANIC CONTAMINANTS BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)	107
	Introduction	107

CHAPTER Page

Mechanisms of Bioconcentration 108
Kinetics 108
Polynuclear aromatic hydrocarbons 111
Polychlorinated biphenyls 116
Tributyltin 120
The Octanol-to-Water Partition Coefficient 122
The Two-Compartment Model Approach 125
Depuration versus Degradation 127
Concluding Remarks 128

VII SIMULTANEOUS UPTAKE AND DEPURATION OF PAHs AND PCBs BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA) 130

Introduction 130
Simultaneous Exposure to PAHs and PCBs: A Laboratory Study 131
Aquarium Exposure 131
Extraction, fractionation and instrumental analyses of PAHs and PCBs 135
Polynuclear Aromatic Hydrocarbons 135
Polychlorinated Biphenyls 141
PAH-PCB Interactions 148
Concluding Remarks 151

VIII NOAA'S NATIONAL STATUS AND TRENDS "MUSSEL WATCH" PROGRAM 153

Introduction 153
Polynuclear Aromatic Hydrocarbons 155
Polychlorinated Biphenyls 157
ΣPCB Congeners/Total PCB Relationship 161
Planar PCB Congeners 168

CHAPTER Page

Butyltin Species 177

IX SUMMARY AND PROSPECTIVES 182

REFERENCES 187

APPENDIX 209

VITA 242

LIST OF TABLES

TABLE		Page
1	Hydrocarbon Concentrations in Samples from the Galveston Bay Area. Except Where Indicated, Concentrations in Organisms Are Expressed in ng g^{-1} on a Wet-Weight Basis. Concentrations in Sediment and Water Samples Are Expressed in ng g^{-1}, on a Dry-Weight Basis, and in ng l^{-1}, Respectively. Ranges in Parenthesis	15
2	Biological Half-Lives (Days) of PAHs in Hanna Reef and Ship Channel *Crassostrea virginica* Oysters	46
3	Polychlorinated Biphenyl Concentrations in Samples from the Galveston Bay Area. Except Where Indicated, Concentrations in Organisms Are Expressed in ng g^{-1} on a Wet-Weight Basis. Concentrations in Sediment and Water Samples Are Expressed in ng g^{-1}, on a Dry-Weight Basis, and in ng l^{-1}, Respectively	54
4	Biological Half-Lives (Days) of PCBs in Hanna Reef and Ship Channel *Crassostrea virginica* Oysters	77
5	Recoveries of Four Planar PCB Congeners from Spiked Aroclor 1254 and Dolphin Blubber Samples Using Activated Carbon:Silica Columns	91
6	TBT, DBT and MBT Concentrations (in ng Sn g^{-1} on a Dry-Weight Basis) in Oyster and Sediment Samples from the Galveston Bay Area	100
7	Estimated Days to 90% Uptake Equilibrium ($t_{90\%}$), Bioconcentration Factors (BCF), Depuration Rates (k_d) and Biological Half-Lives (BHL) for PAHs and PCB Congeners in Hanna Reef and Ship Channel Oysters During the Field Studies	112

TABLE Page

8 Characteristics of the Relationships Between log K_b and log K_{ow} for Bioconcentration of Trace Organic Contaminants in Different Organisms . 126

9 Biological Half-Lives of PAHs in *Crassostrea virginica* Oysters Exposed in the Laboratory to Particle-Associated PAHs Alone (Aquarium C) and PAHs + PCBs (Aquarium D)..... 140

10 Biological Half-Lives of PCBs in *Crassostrea virginica* Oysters Exposed in the Laboratory to Particle-Associated PCBs Alone (Aquarium B) and PCBs + PAHs (Aquarium D)..... 149

11 PCB Congeners/Total PCB Relationships in Gulf of Mexico Oyster Samples..... 164

12 Planar and Total PCB Concentrations in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays..... 170

13 2,3,7,8-TCDD Equivalents (pg g^{-1}) Corresponding to Non-*Ortho* Substituted PCB in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays..... 172

14 Selected Mono- and Di-*Ortho* Substituted PCB and Total PCB Average Concentrations (ng g^{-1}) in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays..... 174

15 Average 2,3,7,8-TCDD Equivalents (pg g^{-1}) Corresponding to Selected Mono- and Di-*ortho* Substituted PCBs in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays..... 175

LIST OF FIGURES

FIGURE		Page
1	Galveston Bay, Texas	8
2	Structures and common names of selected aromatic hydrocarbons discussed in the text	12
3	Galveston Bay transplantation sites	23
4	Exposure (a) and depuration (b) sites, respectively. Approximately 250 adult oysters (c) were transplanted in net bags (d)	25
5	Trace organic analytical scheme	26
6	Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings, in transplanted Hanna Reef and indigenous Ship Channel oysters during the 48-day exposure period near the Houston Ship Channel. Ship Channel oysters were not sampled on day 7	31
7	Concentrations of selected polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef area	32
8	Concentrations of individual polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters at the end of the 48-day exposure period	36
9	Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Ship Channel sediment samples	37

FIGURE Page

10 Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Ship Channel seawater samples..... 38

11 Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings, in back-transplanted Hanna Reef and transplanted Ship Channel oysters during the 50-day depuration period in the Hanna Reef area..... 40

12 Concentrations of individual polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters at the end of the 50-day depuration period..... 41

13 Comparison of the concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in tissues of Hanna Reef oysters before exposure to the Ship Channel contaminant levels and after depuration at the Hanna Reef site..... 42

14 Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Hanna Reef sediment samples..... 44

15 General formula of polychlorinated biphenyls and examples of major congeners commonly found in environmental samples..... 50

16 Examples of high-resolution gas chromatograms of Hanna Reef oysters transplanted to the Ship Channel area during different stages of the 48-day exposure period. PCB congeners are numbered according to Ballschmitter & Zell, 1980..... 61

17 Concentrations of polychlorinated biphenyl congeners, grouped by level of chlorination, in transplanted Hanna Reef and indigenous Ship Channel oysters during the 48-day exposure period near the Houston Ship Channel. Ship Channel Oysters were not sampled on day 7..... 62

FIGURE Page

18 Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef area 64

19 Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters at the end of the 48-day exposure period 67

20 Percent differences in concentrations of selected polychlorinated biphenyls between Hanna Reef and Ship Channel oyster tissues at the end of the 48-day exposure period. Positive values indicate congeners with greater accumulation in Ship Channel oysters 69

21 Concentrations of polychlorinated biphenyls, grouped by level of chlorination, in Ship Channel sediment and seawater samples 71

22 Concentrations of polychlorinated biphenyl congeners, grouped by level of chlorination, in back-transplanted Hanna Reef and transplanted Ship Channel oysters during the 50-day depuration period in the Hanna Reef area 73

23 Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters at the end of the 50-day depuration period 74

24 Comparison of the concentrations of selected polychlorinated biphenyl congeners measured in tissues of Hanna Reef oysters before exposure to the Ship Channel contaminant levels and after depuration at the Hanna Reef site 75

FIGURE Page

25 Concentrations of polychlorinated biphenyls, grouped by level of chlorination, in Hanna Reef sediment samples 78

26 General formula of polychlorinated biphenyls. Three of the most toxic planar PCB congeners, i.e. PCB 77, 126 and 169, are shown together with the compounds they mimic in toxic effects..... 82

27 High-resolution gas chromatographic analyses of (a) Aroclor 1254 spiked with planar PCB congeners (i.e., 77, 81, 126 and 169), (b) PCB congeners recovered in Fraction 1, and (c) planar PCB congeners eluted in Fraction 2. PCB congeners 103 and 198 are external standards. PCB congeners are numbered according to Ballschmitter & Zell, 1980 88

28 High-resolution gas chromatographic analyses of (a) dolphin blubber extract spiked with planar PCB congeners 77, 81, 126 and 169, (b) chlorinated hydrocarbons recovered in Fraction 1, and (c) planar PCB congeners eluted in Fraction 2. PCB congeners are numbered according to Ballschmitter & Zell, 1980 89

29 Example of high-resolution gas chromatograms obtained from an extract of indigenous Ship Channel oysters. PCB congeners are numbered according to Ballschmitter & Zell, 1980..... 92

30 Concentrations of planar polychlorinated biphenyl congeners 77 and 126 in tissues of Hanna Reef oysters during the uptake and depuration phases of the transplantation experiments at Galveston Bay..... 94

31 Concentrations of tributyltin in tissues of Hanna Reef and Ship Channel Oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef area 104

FIGURE Page

32 Depuration constant (k_d) and biological half-lives (BHL) of planar PCB congeners compared to ranges of values calculated for *non*-planar PCBs.. 117

33 Bioconcentration factors of six selected PCB congeners in relation to their liphophilicity and size..... 119

34 Relationship between chlorine-substitution patterns in PCBs and their depuration half-lives. See text for explanation..... 121

35 Bioconcentration factors of polynuclear aromatic hydrocarbons and polychlorinated biphenyls calculated for transplanted Hanna Reef and indigenous Ship Channel oysters during the exposure period versus log octanol to water partition coefficients (K_{ow})..... 123

36 General laboratory set-up (a), details of an aquarium and water recirculation system (b), dosing and feeding system (c), and oysters used during the experiment (d)..... 133

37 Concentrations of selected polynuclear aromatic hydrocarbons in tissues of oysters during exposure to particle-associated PAHs alone (Aquarium C) and PAHs + PCBs (Aquarium D) and following transplant to contaminant-free aquariums. Aquarium A was used as control..... 136

38 Concentrations of individual polynuclear aromatic hydrocarbons in tissues of laboratory exposed oysters after the 30-day exposure period to particle-associated PAHs (Aquarium C) and PAHs + PCBs (Aquarium D)..... 138

39 Concentrations of individual polynuclear aromatic hydrocarbons in tissues of oysters previously exposed in the laboratory to particle-associated PAHs (Aquarium C) and PAHs + PCBs (Aquarium D) after the 30-day depuration period in contaminant-free aquariums..... 139

FIGURE Page

40 Concentrations of selected polychlorinated biphenyls in tissues of oysters during exposure to particle-associated PCBs alone (Aquarium B) and PCBs + PAHs (Aquarium D) and following transplant to contaminant-free aquariums. Aquarium A was used as control 142

41 Comparison of the distribution of PCB congeners, grouped by level of chlorination, encountered in laboratory exposed oysters at the end of the 30-day exposure period with that of the exposure mixture (i.e. 1:1:1:1 Aroclor 1242, 1248, 1254 and 1260). Distribution of PCB congeners in individual Aroclor mixtures are also shown..... 144

42 Selective accumulation or depletion of PCB congeners in laboratory exposed oysters relative to the exposure Aroclor mixture 145

43 Concentrations of selected PCB congeners in tissues of laboratory exposed oysters after the 30-day exposure period to particle-associated PCBs (Aquarium B) and PCBs + PAHs (Aquarium D)..... 146

44 Concentrations of selected PCB congeners in tissues of oysters previously exposed in the laboratory to particle-associated PCBs (Aquarium B) and PCBs + PAHs (Aquarium D) after the 30-day depuration period in contaminant-free aquariums 147

45 NOAA's National Status and Trends sampling locations in the Gulf of Mexico..... 154

46 Average distributions of PCB congeners, grouped by level of chlorination, in Gulf of Mexico sediments and oysters..... 158

47 Average distributions of selected PCB congeners in Gulf of Mexico sedimens and oysters 160

FIGURE Page

48 Relationships between the sum of 18 selected PCB congeners and the total PCB load encountered in Gulf of Mexico oysters for the first year of NOAA's National Status and Trends Program. See text for discussion... 163

49 Three different examples of the bias introduced in the report of total PCB concentrations by using the regression equation (see text) compared to the total PCB load calculated as the sum of all measurable individual congeners..... 167

50 NOAA's National Status and Trends sampling locations in Galveston and Tampa Bays..... 169

51 Toxic equivalents corresponding to three planar PCBs and selected mono- and di-*ortho* chlorine-substituted congeners in oyster samples collected from six different locations in Galveston and Tampa Bays 176

52 Contribution of planar and selected mono-ortho chlorine substituted PCB congeners to the total toxicity in oysters..... 178

53 Total butiltin concentrations at selected sites in the Gulf of Mexico sampled between 1986 and 1992 as part of NOAA's National Status and Trends Program 180

CHAPTER I

INTRODUCTION

STATEMENT OF PURPOSE

Many toxic organic compounds of both synthetic and natural origin, such as polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) and butyltin compounds, e.g. tributyltin (TBT), can be present at high levels, i.e. ppm, in the coastal marine environment (e.g. Kerkhoff *et al.*, 1982; Malins *et al.*, 1984, 1987; Wade *et al.*, 1988a) and may not only affect the productivity of marine organisms but may ultimately be hazardous to human health.

PCBs and PAHs enter the marine environment from several sources including precipitation, land runoff, atmospheric fallout, industrial and municipal waste discharge and accidental spills (e.g. Hoffman *et al.*, 1984; Prahl *et al.*, 1984). PAHs are also known to enter the marine environment from natural oil seepage (Venkatesan & Kaplan, 1982; Anderson *et al.*, 1983; Kvenvolden & Harbough, 1983; Venkatesan *et al.*, 1983). The major source of tributyltin (TBT), the most toxic of the butyltin species (Davis & Smith, 1980), to the marine environment is the use of antifouling paints containing this compound.

This dissertation follows the format of the *Marine Environmental Research* journal.

Because of their low aqueous solubilities (e.g. Mackay *et al.*, 1980; Whitehouse, 1984), PCBs, PAHs and butyltin compounds are rapidly adsorbed onto particulate matter, which can result in their deposition with estuarine and coastal sediments (Herbes, 1977; Pavlou & Dexter, 1979; Means *et al.*, 1980; Langston *et al.*, 1987). Sediments may serve as a storage compartment for long-term release of contaminants by biogeochemical processes (Sodergren & Larsson, 1982; Prahl & Carpenter, 1983; Coates & Elzerman, 1986; Unger *et al.*, 1987); however, the extent of residue accumulation in sediments is largely determined by the chemical nature of the compounds and the sediment characteristics, e.g. texture and organic matter content (Choi & Chen, 1976; Karickhoff *et al.*, 1979; Chiou *et al.*, 1983; MacIntyre & Smith, 1984). Natural organic materials can enhance the partition of hydrophobic compounds into the bottom sediments and pore water (Brownawell & Farrington, 1985, 1986; Brownawell, 1986; Chin & Gschwend, 1992). This partition process influences the availability of PCBs, PAHs and butyltin compounds to the overlying seawater and, in turn, to the aquatic organisms where these compounds can accumulate by passive adsorption directly from water or by partitioning into food. Both routes have been shown to contribute significantly to levels found in fishes (Rubistein *et al.*, 1984; Malins *et al.*, 1987; Oliver & Niimi, 1983; Opperhuizen & Schrap, 1988) and benthic organisms (Clement *et al.*, 1980; Stekoll *et al.*, 1980; Laughlin *et al.*, 1986; Salazar, 1986; Oliver, 1987).

A considerable body of knowledge exists on the dynamics of PCBs and PAHs uptake and depuration in marine species (e.g., Stegeman & Teal, 1973; Lee, 1977; Clement *et al.*, 1980; Riley *et al.*, 1981; Opperhuizen *et al.*, 1985; Jovanovich & Marion, 1987; Pruell *et al.*, 1986, 1987; Tanabe *et al.*, 1987a). Most of the previous studies, however, described the steady-state bioconcentration factors of PCBs or PAHs by a variety of marine organisms while only a few of them discussed the dynamics by which the final levels are achieved. Until recently, investigations on PCBs and PAHs focused mainly on

the commercial Aroclor mixtures or whole petroleum (e.g. Stegeman & Teal, 1973; Courtney & Denton, 1976; Shaw & Connell, 1982; Stickle *et al.*, 1984) rather than on the individual PCB congeners or specific PAHs (e.g. Duinker *et al.*, 1983; Frank *et al.*, 1986; Pruell *et al.*, 1986, 1987; Jovanovich & Marion, 1987; Tanabe *et al.*, 1987a). Now, the importance of considering individual compounds is well recognized in view of differences in both toxicity (e.g. Safe, 1984, 1985, 1990; Tanabe *et al.*, 1987b, 1987c) and physicochemical properties controlling their assimilation by organisms (e.g. Shaw & Connell, 1980, 1984; Opperhuizen *et al.*, 1985, 1988). This is particularly important in the case of planar PCB congeners (McFarland & Clarke, 1989). In a recent review on PCBs, Oliver *et al.* (1989) described the current information on the behavior of individual PCB congeners as limited; however, research on environmental pathways and hazard assessments of specific congeners can be determined with the currently available analytical procedures for PCBs. Similarly, Smith *et al.* (1990) stated that our understanding of the hazards by PCBs to various animal species and ecosystems remains inadequate. Moreover, little is known about the effects that a group of xenobiotic compounds, for example PCBs, will have on the uptake and/or depuration dynamics of other contaminants, for example PAHs. Interaction between PCBs and PAHs during uptake has been reported to occur in fish and oysters (Stein *et al.*, 1984; Collier *et al.*, 1985; Fortner & Sick, 1985).

Comparatively, the knowledge of the dynamics of butyltin compounds uptake and depuration by different marine organisms is limited. Although contamination of the coastal environment by TBT has been investigated since early 1980s, it was not until the late 1980s that this compound was considered to be a real threat to the quality of coastal waters.

Monitoring of PCBs, PAHs and butyltin compounds, at trace levels, in the aquatic environment using various organisms is well-established. Bivalves are generally

preferred for this purpose because of their wide geographic distribution, sedentary form of life, ability to bioconcentrate organic and inorganic contaminants, comparatively low enzyme activity for metabolizing xenobiotics, ability to survive under extreme pollution conditions and commercial value (Goldberg *et al.*, 1978; Burns & Smith, 1981; Farrington *et al.*, 1983). The use of bivalves as bioindicators has grown rapidly over the last decade and the "Mussel Watch" concept is now being used by many national and international programs (National Academy of Sciences, 1980; Farrington *et al.*, 1983; Risebrough *et al.*, 1983; Martin, 1985; Wade *et al.*, 1988a, 1988b; Sericano *et al.*, 1990a, 1990b, Tripp *et al.*, 1992).

Laboratory experiments have been carried out in order to have a better understanding of the uptake and depuration processes taking place in the environment; however, extrapolations from laboratory tests to natural environmental conditions are not always possible. For example, results of laboratory-based studies of PCB and PAH kinetics in bivalves and field data revealed various inconsistencies, including which PCB isomers or PAHs are preferentially accumulated and/or released by different bivalves and their half-lives (e.g. Boehm & Quinn, 1976; Fucik & Neff, 1977; Jackim & Lake, 1978; Langston, 1978; Lee *et al.*, 1978; Bjorseth *et al.*, 1979; Calambokidis *et al.*, 1979; Obana *et al.*, 1983; Pruell *et al.*, 1986, 1987; Weigelt, 1986; Jovanovich & Marion, 1987; Tanabe *et al.*, 1987a; Fox, 1988; Wade *et al.*, 1988c, Tanacredi & Cardenas, 1991). Although the causes of such disagreements are not clear, the uptake of PCBs and PAHs from solution in laboratory experiments may be different from real situations since the routes of contaminant uptake may differ. Methods using contaminants adsorbed onto particles, e.g. clay, might produce more realistic results in uptake/depuration studies since they closely simulate the manner by which filter-feeding bivalves are likely to be exposed to organic xenobiotics in the coastal marine environment. Also, the effects of using solubilizing agents and exposure concentrations much higher than those measured in the field are

difficult to evaluate and extrapolate to real situations. In the case of experiments using naturally contaminated sediments, synergistic or antagonistic effects between different organic contaminants are likely to influence the uptake kinetics of these compounds. Furthermore, certain techniques, such as breaking open the bivalve shell to permit continuous contact with the contaminated medium (see, for example, Fortner & Sick, 1985), obviously produce conditions that are not normally encountered in the environment.

Finally, marine organisms in the environment are exposed to complex contaminant mixtures rather than to individual compounds. Therefore, detailed information on uptake and depuration kinetics of xenobiotics for organisms exposed to contaminant mixtures must include both field and laboratory studies to assess the effects of anthropogenic chemicals on marine biota.

RESEARCH OBJECTIVES

In view of the preceding discussion, this study was designed to:

1. Evaluate the uptake of selected PCB congeners, PAHs and butyltin species in transplanted American oysters, *Crassostrea virginica*, under field conditions in Galveston Bay, Texas. Transplanted organisms from a clean environment, Hanna Reef, are compared to native oysters from a chronically contaminated area near the Houston Ship Channel where relatively high concentrations of PCBs, PAHs and organotin compounds are known to exist. Body burdens of both oyster populations are compared to water and sediment concentrations.

2. Evaluate the depuration of selected PCB congeners, PAHs and butyltin species in newly and chronically contaminated American oysters, *Crassostrea virginica*, under field conditions in Galveston Bay, Texas. As a continuation of the uptake experiment

mentioned above, both originally clean and chronically contaminated oysters were transplanted from the area near the Houston Ship Channel to the Hanna Reef area and their depuration kinetics were compared.

3. Evaluate the potential for highly toxic coplanar PCBs to bioaccumulate in oysters under field conditions. Depuration rates of these PCB congeners by newly and chronically contaminated individuals are compared.

4. Assess the usefulness of transplanted oysters in biomonitoring studies involving these trace organic contaminants.

5. Compare, under laboratory conditions, accumulation and depuration dynamics of selected individual PCB congeners and PAHs by the American oyster, *Crassostrea virginica*, when simultaneously exposed to particle-associated PCBs, PAHs and PCBs plus PAHs, at environmentally realistic levels.

6. Use the experimental results to better understand the PCB, PAH and butyltin data in oyster samples collected along the northern Gulf of Mexico coast during the NOAA's National Status and Trends (NS&T) "Mussel Watch" Program.

The American oyster, *Crassostrea virginica,* was proposed as the organism of interest for this study due to its wide distribution in the U.S.A. coastal areas, its importance as an economic resource, and its suitability as a sentinel organism for monitoring coastal pollution (Goldberg *et al.*, 1978; Burns & Smith, 1981; Farrington *et al.*, 1983; Wade *et al.*, 1988a). PCBs, PAHs and tributyltin species were selected for study because of their toxicity and ubiquitous distributions in the marine environment.

GALVESTON BAY SYSTEM

It is not the purpose of this section to present a thorough description of the Galveston Bay system. The intention, instead, is to briefly describe the system in order to provide a basic background for this study. Most of the following paragraphs are summarized from Stanley's work (1989).

The Galveston Bay system (Fig. 1) includes the Galveston, Trinity, East and West Bays, with a total area of nearly 1,430 km^2. Water depth through the area is very shallow. Average depths range from <1 m, in East and West Bays, to 2-4 m, in the lower Galveston Bay. Upper Galveston and Trinity Bays average about 1.6 m in depth. The maximum depths (up to 12 m) are found in the dredged channels, e.g. Houston Ship Channel.

Main freshwater inflows to the Galveston Bay system include those from the San Jacinto and Trinity River drainage areas. The Trinity River basin is the largest with a drainage area of approximately 46,540 km^2 and supplies about half of the total freshwater input to Galveston Bay. The San Jacinto River basin has a much smaller drainage area (10,230 km^2). While the San Jacinto River is generally the main source of freshwater to the lower Houston Ship Channel, the principal source of inflow during dry periods is wastewater discharges. Smaller coastal drainage areas also contribute freshwater to the bay system. Total coastal inputs represent a drainage area of about 2,000 km^2. The combined annual freshwater inflow to the system averages 11.6 km^3. In addition to these overland runoffs, there is an average input of about 1.9 km^3 each year from precipitation directly onto the bays.

Mean salinity in the Galveston Bay system is around 17‰, but is highly variable in time and space. Trinity Bay has generally the lower salinity, mainly because of the Trinity River's outflow. Salinity along the western part of Galveston Bay is typically higher than

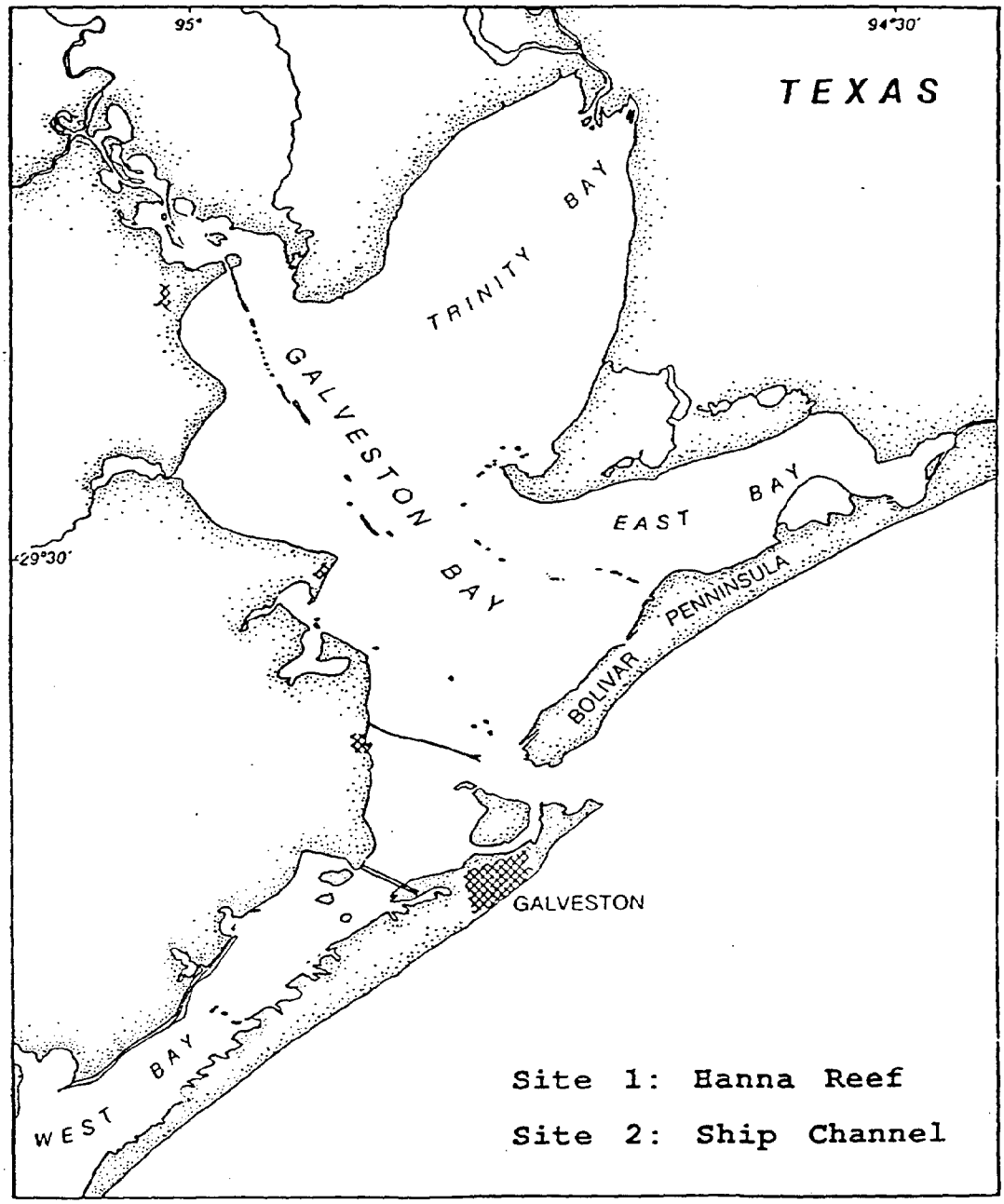


Fig. 1. Galveston Bay, Texas.

that on the eastern section. This is due to the Trinity River discharge from the east and to the barrier formed along the Houston Ship Channel. This channel is the primary path for salinity intrusion into upper Galveston Bay. Mean water temperature for the entire Galveston Bay system averages about 22°C, although the water temperature follows very closely the seasonal changes in air temperature.

The Galveston Bay system constitutes one of the largest and most economically important estuaries along the Texas Gulf coast. For many years, this area has been the recipient of various environmental injuries because of an aggressively growing urban and industrial development. 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, including petrochemical complexes and refineries, bordering the Galveston Bay estuarine system are likely to introduce significant amounts of organic pollutants into the Bay. Early ecological studies showed the damaged suffered by different areas in Galveston Bay. Hohn (1959) and Chamber & Sparks (1959) reported significant decreases in diatom species diversity and number of invertebrates and fish in the upper Houston Ship Channel. Fish species diversity indices were also used to assess the health of Galveston Bay (Betchtel & Coperland, 1970). A change in species diversity from sciaenids to anchovy was related to the influx of pollutants into the Bay. In general, these ecological studies suggested that the waters of Galveston Bay contained pollutants in sublethal amounts, which caused stress to organisms resulting in significant changes in the estuarine community structure.

CHAPTER II

BIOAVAILABILITY OF PAHs TO THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA): A FIELD STUDY

INTRODUCTION

The ability of marine invertebrates to incorporate polynuclear aromatic hydrocarbons (PAHs) from polluted aquatic environment has been documented by different authors (e.g. Mix, 1984; Pruell *et al.*, 1986, 1987; McElroy *et al.*, 1989). In this chapter, the uptake and release of PAHs, under field conditions, by two groups of American oysters (*Crassostrea virginica*) with different pollution histories, are reported and compared. Oysters from Hanna Reef, a relatively uncontaminated area in Galveston Bay, were transplanted to a site near the Houston Ship Channel, a highly polluted area, to assess the accumulation of PAHs over a period of seven weeks. Concentrations in transplanted oysters were compared to the levels encountered in indigenous Ship Channel oysters. After the uptake period, the remaining Hanna Reef oysters were back-transplanted to their original geographic location to monitor the depuration of the bioaccumulated organic contaminants. At the same time, indigenous Ship Channel organisms were transplanted to the Hanna Reef area to compare depuration rates of PAHs between both groups of oysters, i.e. newly and chronically contaminated oysters.

PAHs: A REVIEW

Background Information

The literature regarding the analytical chemistry and occurrences in environmental samples of polynuclear aromatic hydrocarbons (PAHs) has been adequately reviewed in several recent articles and books (e.g. National Academy of Sciences). Therefore, only a general discussion of the most important aspects of these trace organic contaminants related to this study is presented here.

Polynuclear aromatic hydrocarbons (Fig. 2) are one of several classes of organic pollutants that are released into the environment, due in large part to human activities, and are widely distributed in soils, waters, sediments and organisms throughout the world (e.g. National Academy of Sciences, 1975, 1985; Neff, 1979; Giesy *et al.*, 1983). PAHs are composed of carbon and hydrogen atoms arranged in the form of two or more aromatic (benzene) rings that are either fused (e.g. naphthalene) or linked (e.g. biphenyl) with occasional incorporation of cyclopentene or cyclohexene rings (e.g. indeno[1,2,3-c,d]pyrene). These hydrocarbons range in molecular weight (MW) from naphthalene ($C_{10}H_{12}$, MW 128.16) to coronene ($C_{24}H_{12}$, MW 300.26).

Until the late 1970s, it was generally considered that PAHs were formed only during high-temperature (e.g. 700°C) pyrolysis of organic materials. The discovery in fossil fuels of complex mixtures of PAHs spanning a wide molecular weight range has led to the conclusion that, given sufficient time (e.g. millions of years), pyrolysis of organic materials at temperature as low as 100-150°C can lead to production of PAHs (Blumer, 1976). In addition, there is some experimental evidence that a wide variety of organic molecules containing fused-rings polyaromatic systems are synthesized by bacteria, fungi and plants; although this contributes little to the global PAH burden in the environment (Neff, 1979).

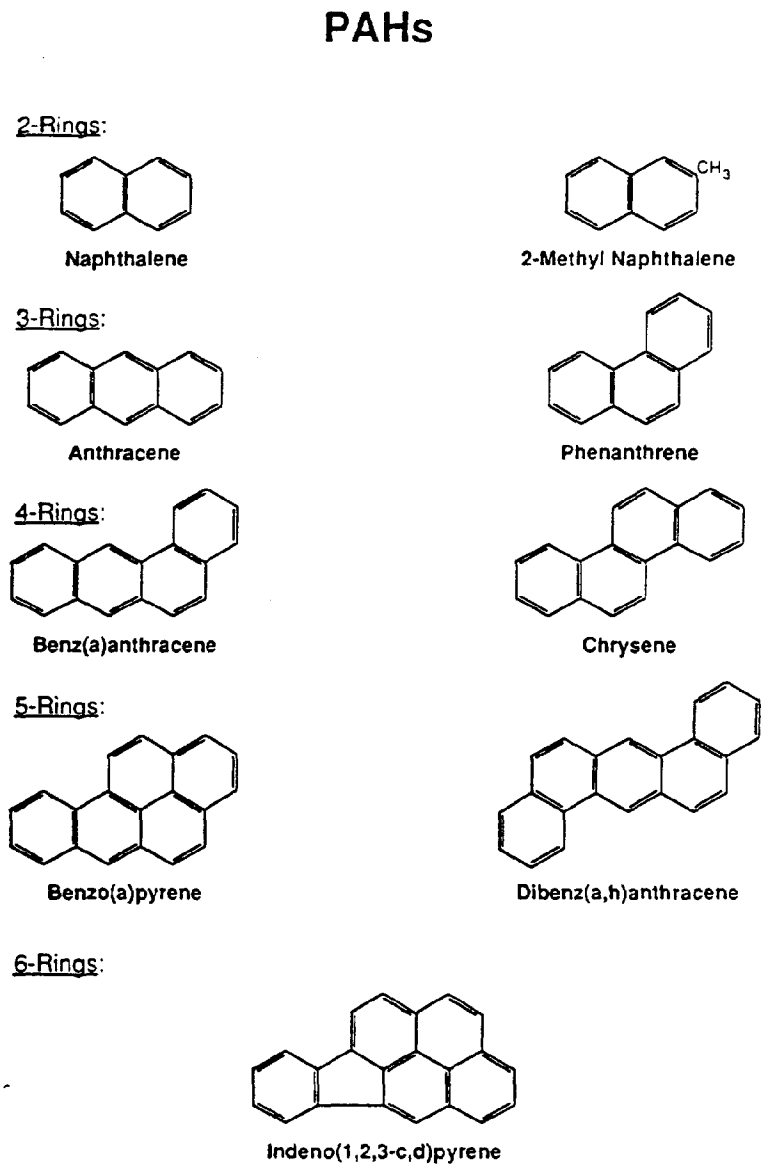


Fig. 2. Structures and common names of selected aromatic hydrocarbons discussed in the text.

Although petroleum is not the only source of hydrocarbons to an ecosystem, most of the evaluations of environmental concentrations of hydrocarbons are based on the analysis of total or selected individual compounds that are indicative of petroleum pollution. Major inputs of petroleum hydrocarbons to the coastal marine environment include drilling operations and petroleum production, transportation activities, coastal and/or riverine inputs, combustion of fossil fuels and atmospheric fallout. Cycloalkanes, branched alkanes, n-alkanes and low molecular weight aromatic compounds are the predominant hydrocarbons present in petroleum.

In addition to petroleum sources, aromatic hydrocarbons, particularly high molecular weight PAHs, are introduced into the environment from different sources, e.g. pyrolysis of organic materials, municipal incinerators, natural fires and coal production and burning. Because of the persistence and lipophilic nature of PAHs, it is not surprising that they have been frequently detected in biota, sediment and water samples from a wide variety of polluted and unpolluted habitats. In general, the presence of petroleum hydrocarbons in earlier studies has been inferred from the distribution of normal alkanes and the presence or absence of an unresolved complex mixture (UCM) in the aliphatic fractions. Since most of these studies were conducted before the introduction of capillary columns, identifications of individual aromatic compounds were not confirmed by gas chromatography/mass spectrometry (GC/MS).

It is estimated that more than 230,000 metric tons of PAHs reach the aquatic environment each year by a variety of routes and accumulate in estuaries and coastal marine areas (Giesy *et al.*, 1983). Particularly important sources of PAHs are the discharges of domestic and industrial wastes and runoff from land. For example, urban runoff entering Narragansett Bay account for 71% of the total inputs of PAHs (Hoffman *et al.*, 1984), whereas riverine contribution of PAHs to coastal sediments off Washington State was reported to be >30% of the total sediment load (Prahl *et al.*, 1984). Generally,

PAHs are detected in part per million (ppm) in organisms and sediments and in part per billion (ppb) in water samples.

Toxicity of the different PAH compounds differs. Unsubstituted two- or three-ring PAHs such as, for example, anthracenes, fluorenes, naphthalenes and phenanthrenes, exhibit significant acute toxicity and other adverse effects on organisms but are noncarcinogenic. In contrast, four- to seven-ring PAHs such as, for example, benzo(a)pyrene, are significantly less toxic but are demonstrably carcinogenic, mutagenic or teratogenic to a wide variety of animals including mammals (Kennish, 1992). Polynuclear aromatic hydrocarbons of environmental concern are those compounds having relatively high volatility and/or solubility.

Distribution and Occurrence in Galveston Bay

A number of studies have been conducted in the Galveston Bay area to establish baseline concentrations of petroleum hydrocarbons in organisms; however, reports of individual aromatic concentrations or distributions are limited (Table 1). Most of these studies were conducted with organisms, particularly bivalves.

Oysters collected from several polluted and unpolluted locations in Galveston Bay in November 1969 and January 1971 had total PAHs that ranged from 11.0 to 237 ng g^{-1} (Fazio, 1971). The highest PAHs in oyster tissues from contaminated sites were fluoranthene (7.8 ng g^{-1}), pyrene (6.5 ng g^{-1}), benzo(b)fluoranthene (2.2 ng g^{-1}), and benzo(e)pyrene (2.1 ng g^{-1}). Benzo(a)pyrene was below detection in samples from both contaminated and uncontaminated stations.

Much higher concentrations were reported for oyster samples from a heavily polluted area, Morgan's Point Reef, near the entrance of the Houston Ship Channel (Ehrhardt, 1972). Concentrations of aromatic hydrocarbons, mainly mono-, di-, and tricyclic aromatics, were higher than those of alkanes (134,000 and 102,000 ng g^{-1}, respectively).

TABLE 1

Hydrocarbon Concentrations in Samples from the Galveston Bay Area. Except Where Indicated, Concentrations in Organisms Are Expressed in ng g^{-1} on a Wet-Weight Basis. Concentrations in Sediment and Water Samples Are Expressed in ng g^{-1}, on a Dry-Weight Basis, and in ng l^{-1}, Respectively. Ranges in Parenthesis.

Location	Sample	Total HCs	Total Aromatic HCs	Individual PAHs	Reference
Galveston Bay	oysters		(11-237)	fluoranthene= 7.8	Fazio, 1971
				pyrene= 6.5	
				benzo(b)fluoranthene= 2.2	
				benzo(e)pyrene= 2.1	
				benzo(a)pyrene= n.d.	
Houston Ship Channel	oysters	236,000	134,000		Ehrhardt, 1972
Morgan's Point Reef	oysters	160,000			Anderson, 1975
Halfway Reef	oysters	26,000			Anderson, 1975
East Bay	oysters	<2,000			Anderson, 1975
West Bay	oyster	<2,000			Anderson, 1975
Galveston Bay	oysters			pyrene= 1,010	Farrington *et al*, 1980
				fluoranthene= 940	
Morgan's Point Reef	oysters			benzo(a)pyrene= 0.12	Murray *et al.*, 1980
Yacht Club	oysters		615	pyrene= 212 (55-481)	Fox, 1988(1)
			(319-1,020)	fluoranthene= 112 (55-219)	
				chrysene= 97 (<20-146)	
Todd's Dump	oysters		134	pyrene= 31 (<20-63)	Fox, 1988(1)
			(94.7-183)	fluoranthene= 12 (<20-57)	
				chrysene= <20 (<20-36)	

TABLE 1
(continued)

Location	Sample	Total HCs	Total Aromatic HCs	Individual PAHs	Reference
Confederate Reef	oysters		610 (259-1,120)	pyrene= 146 (40-293) fluoranthene= 210 (55-404) chrysene= 61 (28-87)	Fox, 1988[1]
Hanna Reef	oysters		111 (21.3-228)	pyrene= <20 (<20-25) fluoranthene= <20 (<20-37) chrysene= <20	Fox, 1988[1]
Morgan's Point Reef	oysters		5,783 (2,270-10,120)	pyrene= 2,170 (669-3,910) fluoranthene= 738 (317-1,120) chrysene= 632 (260-1,090)	Scricano[1] (unpublished data)
San Luis Pass	Fish, crab, shrimp			benzo(a)pyrene= <0.01	Murray *et al.*, 1981a
Houston Ship Channel	Cormorants			naphthalene= (20-40) fluoranthene= (n.d.-70) pyrene= (20-240) benzo(a)pyrene= (40-110) chrysene= 130 benzo(g,h,i)perylene= 590 benzo(k)fluoranthene= 40 1,2,4,5-dibenzoanthracene= 20	King *et al.*, 1987[2]

TABLE 1
(continued)

Location	Sample	Total HCs	Total Aromatic HCs	Individual PAHs	Reference
Trinity Bay	sediments	96,100	34,200	dimethylnaphthalenes= 8,000 trimethylnaphthalenes= 10,000 C_4- naphthalenes= 9,000 dimethylbiphenyls= 800	Armstrong *et al.*, 1979
San Luis Pass	sediments			benzo(a)pyrene= 2.2 (0.01-6.0)	Murray *et al.*, 1981a
Trinity Bay	water	10,500	10,500	benzene= 1,500 toluene= 3,200 C_2- benzene= 3,100 C_3- benzene= 800 dimethylnaphthalenes= 700	Armstrong *et al.*, 1979
San Luis Pass	water			benzo(a)pyrene= n.d.	Murray *et al.*, 1981a

n.d.= not detected; (1) ng g^{-1} on a dry-weight basis; (2) geometric mean

Anderson (1975) reported similar concentrations of total hydrocarbons in oysters collected at the same general location (160,000 ng g^{-1}). At Halfway Reef, a few miles farther away from the entrance of the Houston Ship Channel toward the center of Galveston Bay, 26,000 ng g^{-1}, wet weight, of total hydrocarbons were detected while oyster samples collected in the East and West Bays had less than 2,000 ng g^{-1} of total hydrocarbons in their tissues. Benzo(a)pyrene in oysters collected during May 1979 near Morgan's Point Reef ranged from 0.07 to 0.14 ng g^{-1} with a mean of 0.12 ng g^{-1} (Murray *et al.*, 1980).

In 1980, Farrington *et al.* published the hydrocarbon concentrations measured in bivalves collected from 90 to 100 stations around the U.S. coastline during the EPA "Mussel Watch" Program (1976-1978). Oysters collected in the Galveston Bay area during 1977-1978 had concentrations of 940 and 1,010 ng g^{-1} for fluoranthene and pyrene, respectively.

Fox (1988), in a study designed to examine the spatial and temporal variations in concentrations of selected organic contaminants in Galveston Bay, reported the PAHs concentrations in oysters from three stations at four sites sampled during 1986. Total PAHs were higher in samples from sites located closer to urban areas. Oysters collected in the proximity of the Houston Yacht Club (615 ng g^{-1}, range = 319-1,020 ng g^{-1}) and Confederate Reef (610 ng g^{-1}, range = 259-1,120 ng g^{-1}), near the city of Galveston, had annual average concentrations higher than samples collected in the Todd's Dump area (134 ng g^{-1}, range = 94.7-183 ng g^{-1}), located in the middle of Galveston Bay, and Hanna Reef (111 ng g^{-1}, range = 21.3-228 ng g^{-1}), in the East Bay. Pyrene, fluoranthene, chrysene, phenanthrene and 1-methyl phenanthrene were the most frequently detected analytes. Although temporal variations of individual PAHs in oysters from the Galveston Bay area did not present an easily recognizable trend during this study, it seemed evident that total PAHs in samples from the most polluted sites, i.e. Houston Yacht Club and Confederate Reef, were lower during the summer. This

observation is confirmed by data produced during a six month study with oysters from the upper part of Galveston Bay. Oysters collected monthly near the entrance to the Houston Ship Channel were analyzed for a number of organic contaminants between December 1988 and June 1989. The maximum total PAHs measured in February (10,100 ng g^{-1}, range = 9,680-10,600 ng g^{-1}) decreased to 2,270 ng g^{-1} (range = 1,840-2,710 ng g^{-1}) in May. Pyrene, fluoranthene, chrysene, benz(a)pyrene and benzo(e)pyrene were the most abundant PAHs detected during that study (Sericano, unpublished data). Temporal variations of trace organic contaminants in bivalves were also reported for DDT (Butler, 1973) and PCBs (Farrington *et al.*, 1983). Other marine organisms collected at San Luis Pass, located in West Galveston Bay at the west end of the Galveston Island, were analyzed for benzo(a)pyrene (Murray *et al.*, 1981a). In all cases, concentrations were below the detection limit (<0.01 ng g^{-1}).

In 1987, King *et al.* reported the concentrations of selected PAHs in double-crested cormorants, a fish-eating bird near the top of an aquatic food web, wintering in the Houston Ship Channel. This cormorant is rarely found in the area during summer months. Naphthalene and fluoranthene were the only PAHs present in individuals collected at the beginning of the study. After the three-month winter period, eight aromatic hydrocarbons were detected in bird carcasses (Table 1).

Reports of PAHs in sediment and water samples from the Galveston Bay area are limited. In 1979, Armstrong *et al.* reported the results of a study conducted from April 1974 to December 1975 to examine the effects of brine effluents from a producing platform in Trinity Bay on the surrounding benthic communities. Total petroleum hydrocarbons measured in sediments collected near the platform were 96,100 ng g^{-1}. Approximately one third of this total (i.e. 34,200 ng g^{-1}) were aromatic hydrocarbons, mainly dimethyl-, trimethyl-, and tetramethylnaphthalenes. Bottom water samples collected at the same site contained mostly monoaromatic compounds, e.g. toluene,

benzene, and C2-benzene, in the 200-3,200 ng l^{-1} range. Total PAHs in water was 10,500 ng l^{-1}. Sedimentary PAHs decreased with distance from the platform to near background levels (2,000-6,000 ng g^{-1}). There was a definite inverse correlation between sedimentary PAHs and the number of benthic species and individuals present. The Bay bottom was almost completely devoid of organisms within 15 m of the effluent outfall. Stations located 455 m from the platform were unaffected. Sediment samples collected in the San Luis Pass area had an average benzo(a)pyrene concentration of 2.2 ng g^{-1} (range = 0.01-6.0 ng g^{-1}; Murray *et al.*, 1981a). Benzo(a)pyrene was not detected in water samples from that area.

Bivalve Uptake and Depuration Studies

A considerable number of reports on the uptake and depuration of petroleum hydrocarbons by bivalves have been published over the last two decades. In general, bivalves can be exposed to petroleum hydrocarbons in the laboratory by any one or a combination of several methods including water-soluble fractions (Neff & Anderson, 1975; Neff *et al.*, 1976; Lee *et al.*, 1978; Nunes & Benville, 1979; Jovanovich & Marion, 1987; Axiak *et al.*, 1988), water dispersions/solutions (Boehm & Quinn, 1973; Stegeman & Teal, 1973; Stainken, 1975; Wong, 1976; Fossato & Canzonier, 1976; Stainken, 1977; Fucik & Neff, 1979; Riley *et al.*, 1981; Tanacredi & Cardenas, 1991), contaminated food (Roesijadi *et al.*,-1978; Fortner & Sick, 1985) and contaminated sediments (Palmork & Solbakken, 1981; Obana *et al.*, 1983; Pruell *et al.*, 1986, 1987). Similarly, experimental field studies include exposures to water soluble fractions (Wolfe *et al.*, 1981), water dispersions/solutions (Fucik *et al.*, 1977) and contaminated sediments (Roesijadi *et al.*, 1978). Alternatively, uptake and/or depuration studies can be performed in the field by transplanting uncontaminated bivalves to contaminated areas (e.g. Sericano *et al.*, in press) or relocating chronically contaminated bivalves into pristine environments (e.g.

Pittinger *et al.*, 1985) or in tanks in the laboratory (e.g. Boehm & Quinn, 1977). Bivalves are generally reported to preferentially bioaccumulate four-, five- and six-ring PAHs when exposed, in the laboratory, to naturally contaminated sediments (e.g. Pruell *et al.*, 1986, 1987) or in the environment (e.g. Bjorseth *et al.*, 1979), with little, if any, uptake of two- and three-ring PAHs. However, oysters from the Gulf of Mexico were reported to preferentially uptake two- and three-ring PAHs when compared to four-, five- and six-ring PAHs (Wade *et al.*, 1988c).

There is some disagreement in the published literature regarding the accumulation of individual PAHs and their half-lives in different bivalves. For example, the order chrysene > benzo(b)fluoranthene > fluoranthene > benzo(a)pyrene > benzo(a)-anthracene encountered in mussels (Pruell *et al.*, 1986) do not agree with the accumulation order pyrene > benzo(e)pyrene > benzo(b)fluoranthene > benzo(a)anthracene reported for clams (Obana *et al.*, 1983). Both bivalves were exposed in the laboratory to contaminated sediments. Similarly, the estimated half-lives for fluoranthene and benzo(a)anthracene reported for mussels (30 and 18 days, respectively; Pruell *et al.*, 1986) disagree with the half-lives encountered in oyster (5 and 9 days, respectively; Lee *et al.*, 1978)

Most previous studies have indicated significant but incomplete depurations of aromatic hydrocarbons by different bivalves (e.g. Fossato & Canzonier, 1976; Pruell *et al.*, 1986, 1987; Sericano *et al.*, in press). However, some studies reported a complete depuration of different PAHs to levels below detection limits after relatively short periods of time, i.e. less than a week. Wormell (1979), for example, reported that depuration studies with chronically contaminated oysters showed no preferential retention of saturated or aromatic hydrocarbons. Depuration was rapid and nearly complete with a biological half-life of 4.4 days for total accumulated hydrocarbons. The report suggests, however, that seasonally related conditions might be a significant factor in the ability of

oysters to clean themselves since individuals depurated in December and January retained a significant fraction of the bioaccumulated hydrocarbons.

Pittinger *et al.* (1985) indicated that contaminated oysters (*Crassostrea virginica*), transplanted to a nonimpacted site depurated PAHs to undetectable levels within four days of relocation. Other studies did not detect any depuration. Tanacredi & Cardenas (1991), for example, reported that laboratory exposed clams (*Mercenaria mercenaria*) did not show evidences of decreasing trends in accumulated PAHs after a 45-day depuration period. Similar results were reported by Boehm & Quinn (1976). In that study, chronically contaminated clams (*Mercenaria mercenaria*) failed to release the accumulated PAHs when relocated to clean seawater over a four-month period. Both reports, however, seem to be in disagreement with the vast majority of previous investigations involving bivalves.

In spite of the abundant information, it is clear from the preceding discussion that there are many contradictions in the published literature. The reasons for these disagreements are difficult to explain. It is possible, however, that the extremely high oil or individual analyte concentrations used in some studies, the presence of stressed animals and the use of different experimental designs or analytical techniques could be responsible for the observed discrepancies.

UPTAKE AND DEPURATION OF PAHs

Experimental Design, Sample Collection and Methods

In December 1988, approximately 250 oysters were collected by dredge at Hanna Reef, a relatively pristine area in Galveston Bay (Fig. 3). Within 24 hr., these oysters were transplanted live, in net bags, to a site near the Houston Ship Channel, an area where oysters have high PAH concentrations (Wade *et al.*, 1988a). Photographs of both

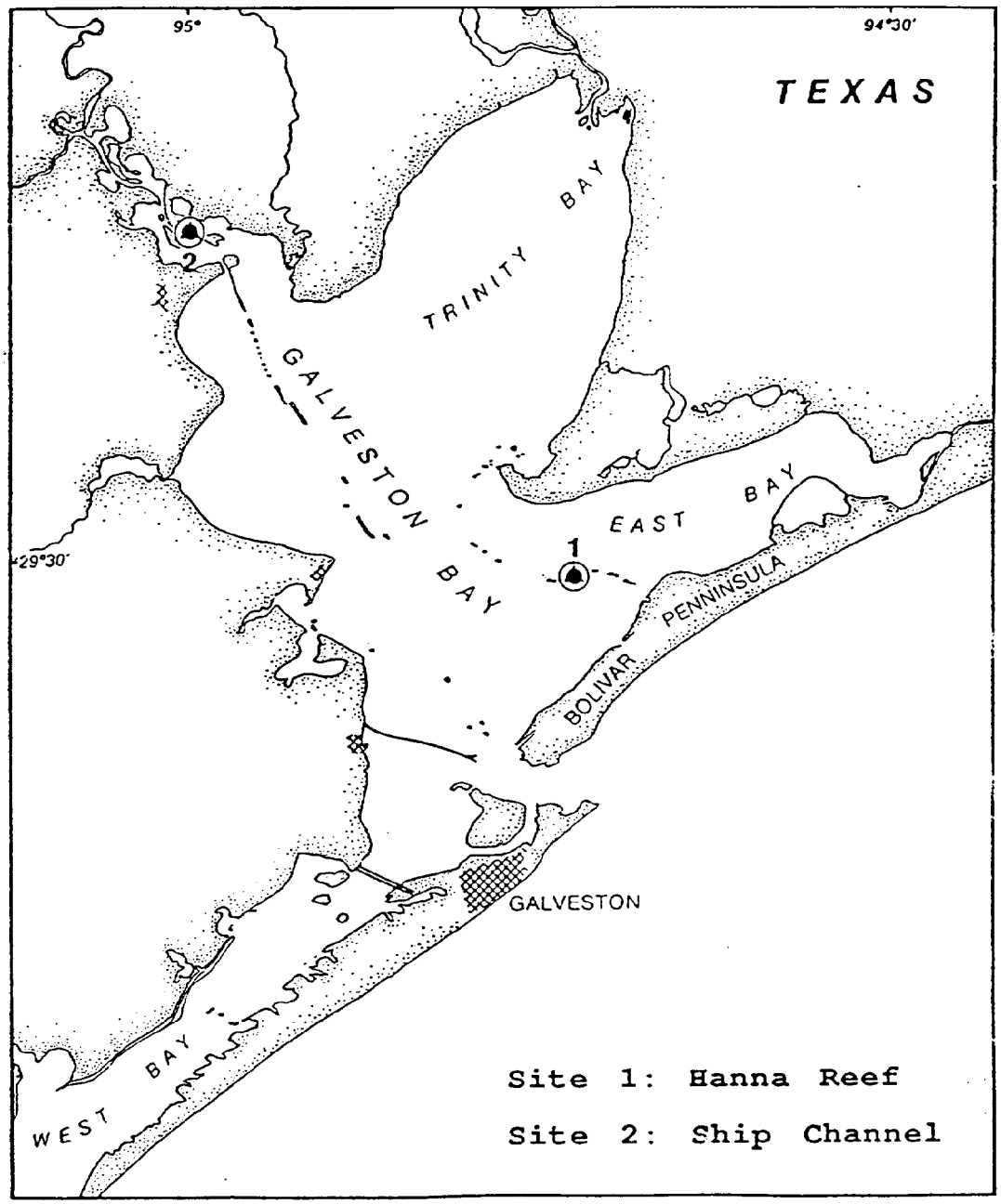


Fig. 3. Galveston Bay transplantation sites.

sites, nets and oysters are shown in Fig. 4. Thereafter, oysters were sampled in groups of 20 individuals during the 3rd, 7th, 17th, 30th and 48th days after transplantation. 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 relocated to the Hanna Reef area and sampled in groups of 20 individuals during the 3rd, 6th, 18th, 30th, and 50th days after transplantation. The transplant experiment to the Hanna Reef area was duplicated with approximately 150 native oysters from the Houston Ship Channel area in order to compare depuration dynamics in both populations. In the following sections, Ship Channel, Hanna Reef, transplanted Hanna Reef-to-Ship Channel, transplanted Ship Channel-to-Hanna Reef and relocated Hanna Reef-to-Ship Channel-back to-Hanna Reef oysters are refered as SC, HR, HRSC, SCHR and HRSCHR oysters, respectively. Sediment and water samples were collected during oyster sampling days for PAH analyses.

Extraction and fractionation of PAHs

The analytical procedure used was based on a method developed by MacLeod *et al.* (1985) with a few modifications that proved to be equivalent or superior to the original techniques. The analytical scheme is summarized in Fig. 5 Precleaning of all glassware involved extensive washing with Micro cleaning solution, rinsing with distilled water and combustion at 400°C for 4 hrs. All solvents were glass-distilled nanograde purity, e.g. Burdick & Jackson. Solvent purity was checked, after 300-fold concentration, by gas chromatography/mass spectrometry (GC/MS). Each set of samples (8-10) was accompanied by a complete system blank and spiked blank or reference material that were carried through the entire analytical procedure. Before extraction, PAH internal standards

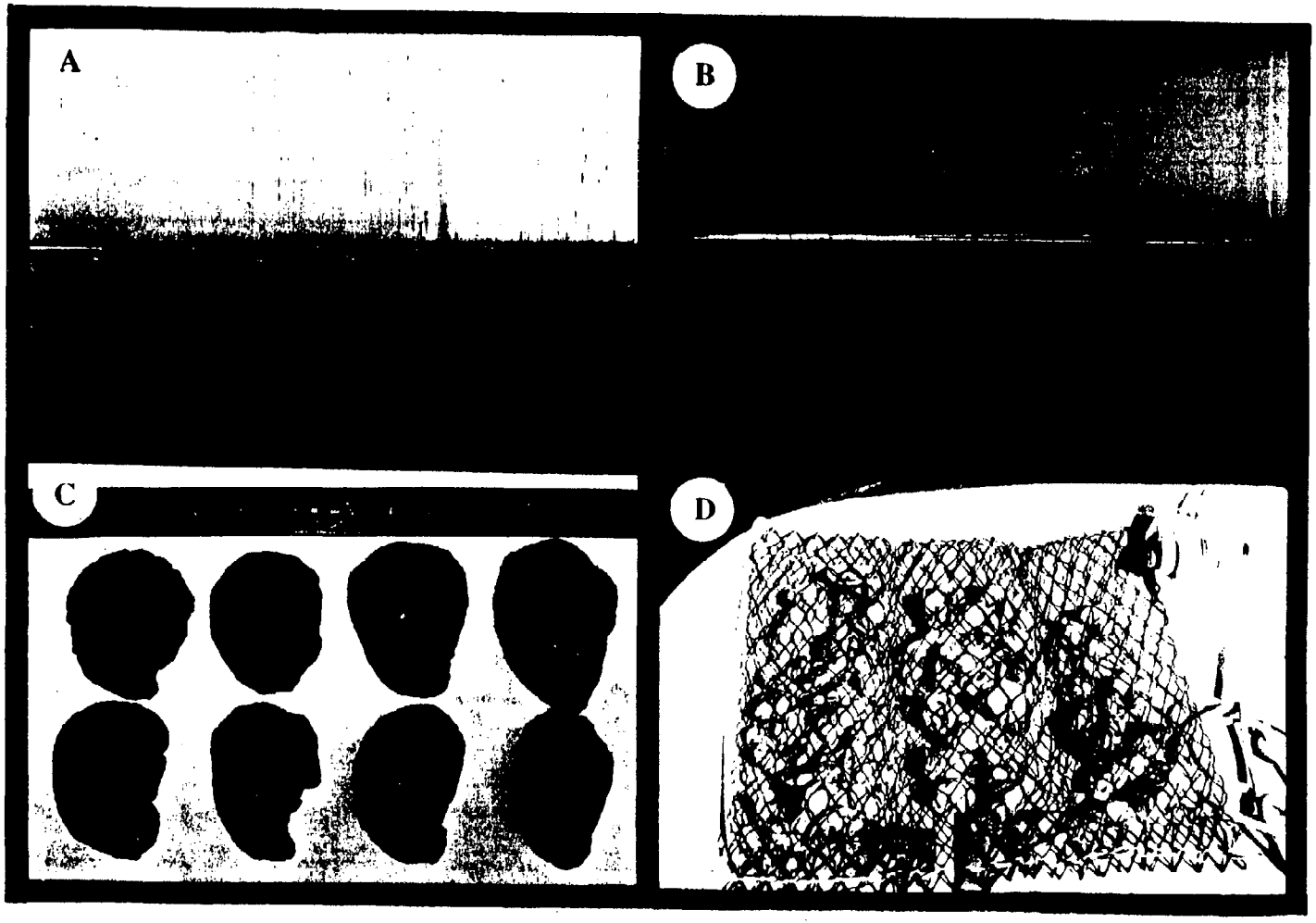


Fig. 4. Exposure (a) and depuration (b) sites, respectively. Approximately 250 adult oysters (c) were transplanted in net bags (d).

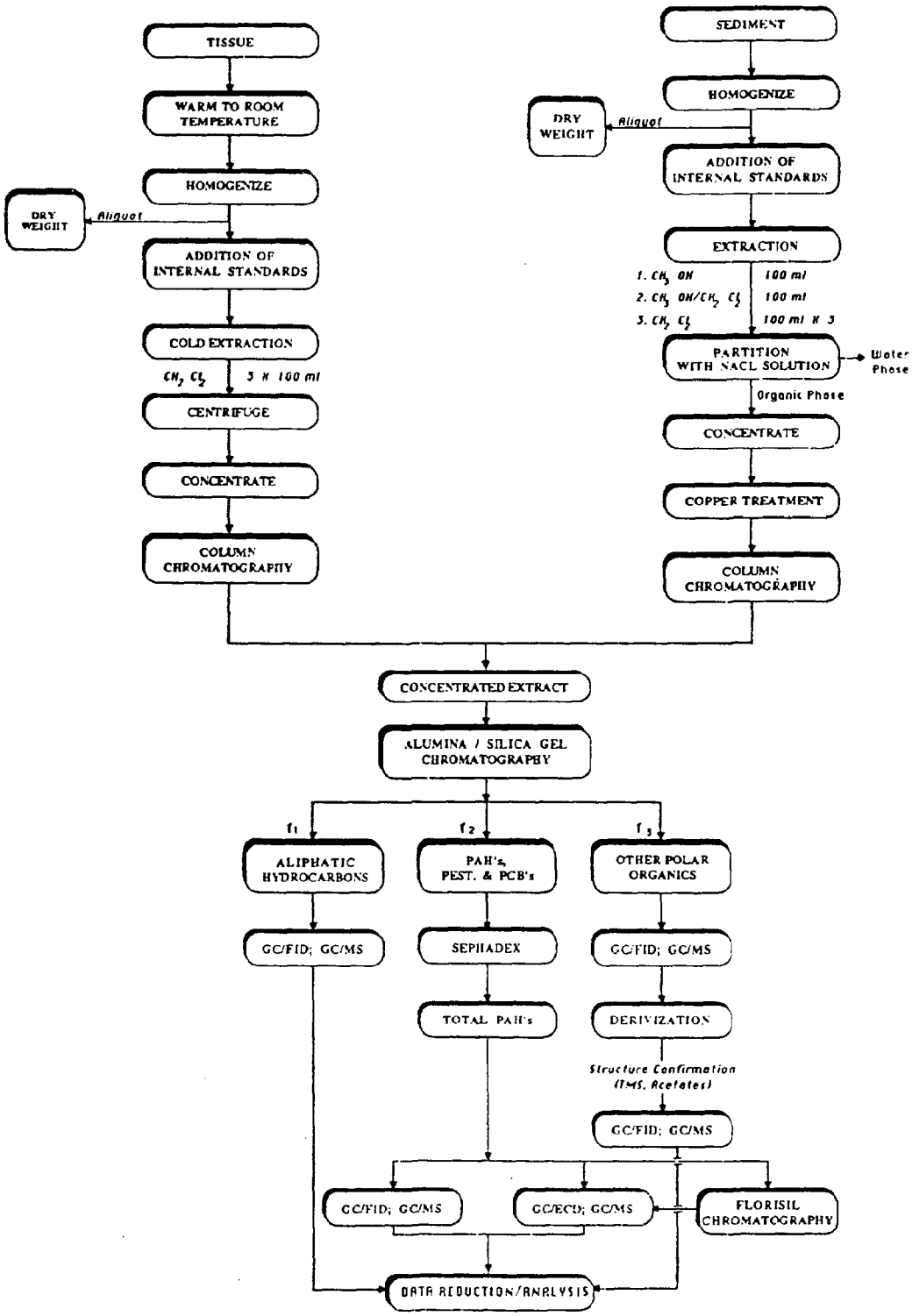


Fig. 5. Trace organic analytical scheme.

(d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenathrene, d_{12}-chrysene and d_{12}-perylene) were added to all samples, blank and spiked blank. These standards were added at a concentration level similar to that expected for the sample components of interest.

Approximately 15 g of wet tissue were used for the analysis of PAHs in oysters. After the addition of 50 g of anhydrous Na_2SO_4, the tissue was extracted three times with 100 ml of methylene chloride using a "Tissumizer" homogenizer. The organic phase was concentrated to 10-15 ml in a flat-bottom flask equipped with a three ball Snyder condenser. Kuderna-Danish tubes were heated in a water bath at 60°C, to concentrate the extracts to a final volume of 2 ml in hexane.

Approximately 50 g of sediment (wet weight) were used for analysis. The sediments were sequentially extracted on a roller table with 100 ml of methanol (1 h), 100 ml of 1:1 methanol:methylene chloride (1 h) and three portions of 100 ml of methylene chloride (16, 3 and 1 h, respectively). The combined extracts were partitioned into two phases by addition of acidic NaCl solution (10%, pH=2). The combined extracts were concentrated to 1-2 ml as previously described for oyster extracts.

Fifteen to 17 l of seawater samples were acidified with HCl (pH=2) and extracted, for 15 min, with 500 ml of methylene chloride. The extraction was repeated three times. The organic phase was partitioned against an acidic NaCl solution (pH=2). The extract was then dried with anhydrous Na_2SO_4 and concentrated to 1-2 ml as previously described for oyster extracts.

Tissue, sediment and water extracts were fractionated by alumina: silica (80-100 mesh) column chromatography. The silica gel was activated at 170°C for 12 h and partially deactivated with 5% distilled water. Twenty grams of silica gel were slurry packed in methylene chloride over 10 g of alumina. Alumina was activated at 400°C for 4 h and partially deactivated with 1% distilled water. Activated copper was added to the top of the column for sediment samples to remove any residue of elemental sulfur. The

methylene chloride was replaced with pentane and the extract was applied to the surface of the column. The column was sequentially eluted with 50 ml of pentane (f1), 200 ml of 1:1 methylene chloride:pentane (f2) and, for sediments, 50 ml of methanol (f3). The f2 fraction, which contains the polynuclear aromatic and chlorinated hydrocarbons, was concentrated as previously described. The f2 fraction from oyster samples was further purified by Sephadex LH-20 column (25-100 mesh) to remove lipids (Ramos & Prohaska, 1981). The column was eluted with 140 ml of a cyclohexane:methanol: methylene chloride (6:4:3) mixture. The first 40 ml were discarded and the next 100 ml fraction was concentrated to a final volume of 0.5-1 ml, in hexane, for gas chromatographic/mass spectrometry analysis.

Instrumental analysis

PAHs were quantitatively analyzed by GC/MS in a selected ion mode (SIM) utilizing the molecular ions of the components of interest. A 30 m DB-5 capillary column (0.31 mm i.d., 0.052 mm film thikness) was temperature programmed from 40 to 300°C at 10°C min^{-1} and hold at 300°C for 10 min. The GC/MS was calibrated by injections of standard solutions at three different concentrations. Sample analytes were quantified from a first degree calibration curve with an R^2 value equal to or greater than 0.99. Analyte identity was confirmed by their molecular weights and retention times of authentic standards.

Ancillary parameters

Grain size analysis was performed by the procedure of Folk (1974). Briefly, refrigerated samples were homogenized, treated with 30% H_2O_2 to oxidize organic matter and washed with distilled water to remove soluble salts. Sodium hexametaphosphate was added to deflocculate each sample before they were wet-sieved though a 62.5 micron (4.0

phi) sieve to separate gravel and sand from the silt and clay fraction. The total gravel and sand fraction was then oven dried at 40°C and weighed. The silt-clay fraction was analyzed for particle size distribution by the pipette (settling rate) method.

Extractable lipids in oysters were determined on an aliquot of the sample extracts. Twenty ml of the combined oyster extracts were withdrawn from the total volume and evaporated to dryness under N_2 gas. The residue was redisolved in 1 ml of methylene chloride, 0.1 ml was evaporated on a paper pad and the residual materials weighed using a Cahn 29 electrobalance.

Statistical analysis

During the uptake period, one-way analyses of variance (ANOVA) were performed on the analyte concentrations to evaluate the bioconcentration by transplanted oysters relative to indigenous individuals. Slopes of the least-square linear regressions of the logarithm of the concentrations (log C) versus time (t) for the depuration period were tested for statistical significance.

Uptake of PAHs by Transplanted Oysters

Average PAH concentrations measured in SC and HRSC oyster, sediment and water samples, during the first part of this experiment at the Ship Channel site, are reported in Tables A-2 and A-3 (Appendix).

The concentrations in Ship Channel oysters represent the time-integrated amounts of trace organic contaminants accumulated from solution, particles and/or food minus any metabolism and/or depuration of these compounds. The concentrations of most of these PAHs in SC oysters did not change significantly during the first phase of the experiment. Total aromatic hydrocarbon concentrations in SC oysters averaged 3,800±590 ng g^{-1} (range 3,200 to 4,400 ng g^{-1}) over the seven-week uptake period. The fluctuations

observed in the concentrations of the lower molecular weight PAHs with time were, however, comparatively greater than the variability encountered in the concentrations of the higher molecular weight analytes. Naphthalene, 2,6-dimethyl-naphthalene and phenanthrene, for example, had an overall average of 13±8.3, 25±16 and 54±37 ng g^{-1} during this period with coefficient of variations of 64, 64 and 69%, respectively. These coefficients of variations were larger than those observed for pyrene (1,500±290 ng g^{-1}: 19%), benzo(e)pyrene (270±65 ng g^{-1}; 24%) and perylene (130±22 ng g^{-1}; 17%) during the same period of time.

Concentrations of PAHs in HRSC oysters increased dramatically during the seven-week exposure period. Concentrations of total PAHs in transplanted HRSC oysters increased from an initial total concentration of 290 to 4,400 ng g^{-1} during this period. Four- and five-ring PAHs were rapidly accumulated by HRSC oysters; comparatively, two-, three- and six-ring PAHs were detected in low concentrations in both transplanted HRSC and indigenous SC oysters (Fig. 6). One month after the experiment started, no statistically significant differences were observed in the distributions of PAHs, by ring number, between HRSC and SC oysters.

Generally, the PAHs measured in HRSC oysters had similar concentrations to those found in SC oysters in less than 20 days (Fig. 7). Four- and five-ring PAHs had increased in the HRSC oysters while the two- and three-ring PAHs concentrations either did not change (e.g. naphthalene, 2-methylnaphthalene) or increased only slightly (e.g. 2,3,5-trimethylnaphthalene, 1-methylphenanthrene) during the seven-week exposure period. A decrease was observed in the concentrations of some lower molecular weight PAHs in transplanted oysters during the first week of exposure. These decreases in concentrations were not observed in indigenous SC oysters. However, since this initial decrease in the concentrations of low molecular weight hydrocarbons was not observed for the higher molecular weight PAHs, it is unlikely that it was a consequence of stressed

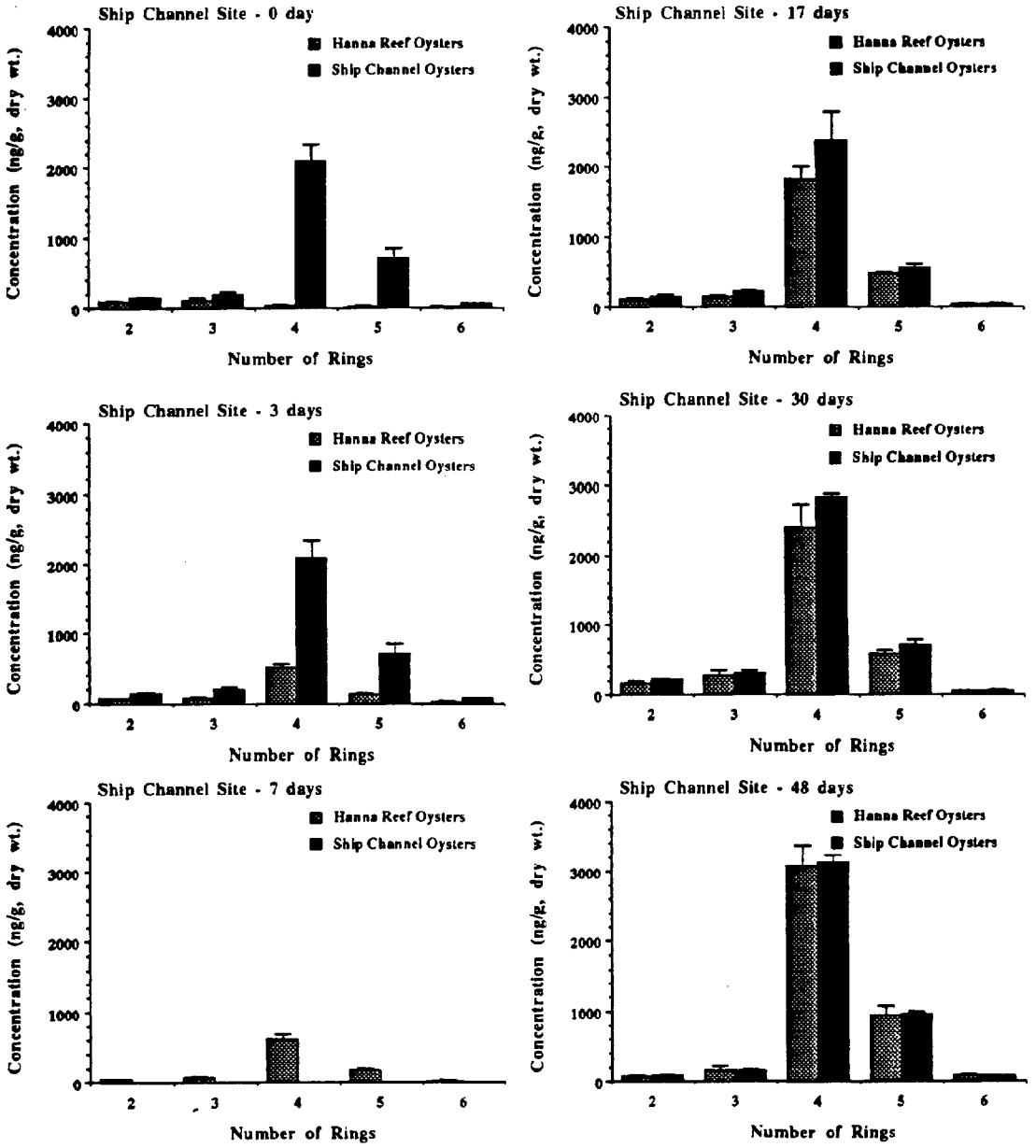


Fig. 6. Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings, in transplanted Hanna Reef and indigenous Ship Channel oysters during the 48-day exposure period near the Houston Ship Channel. Ship Channel oysters were not sampled on day 7.

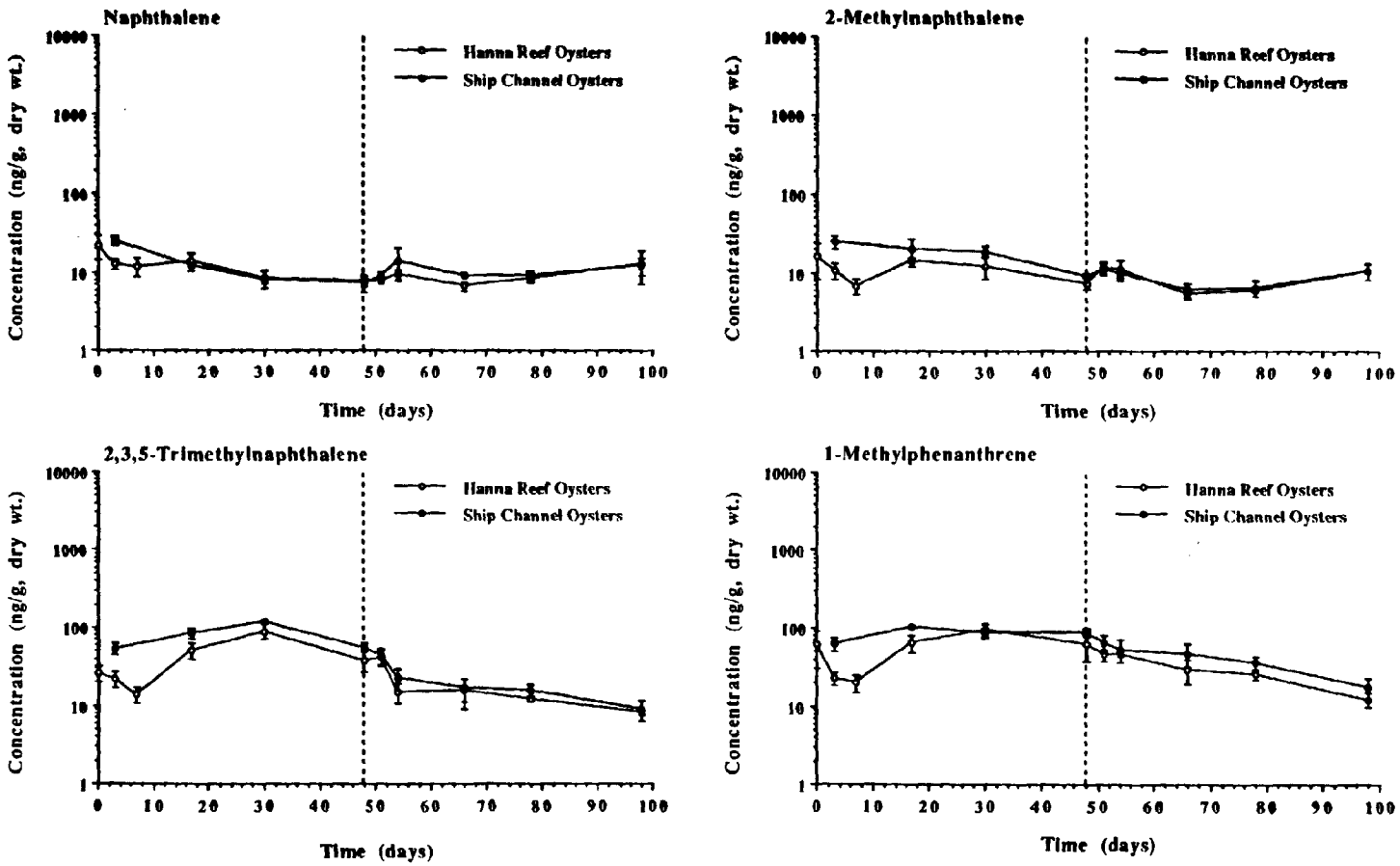


Fig. 7. Concentrations of selected polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef area.

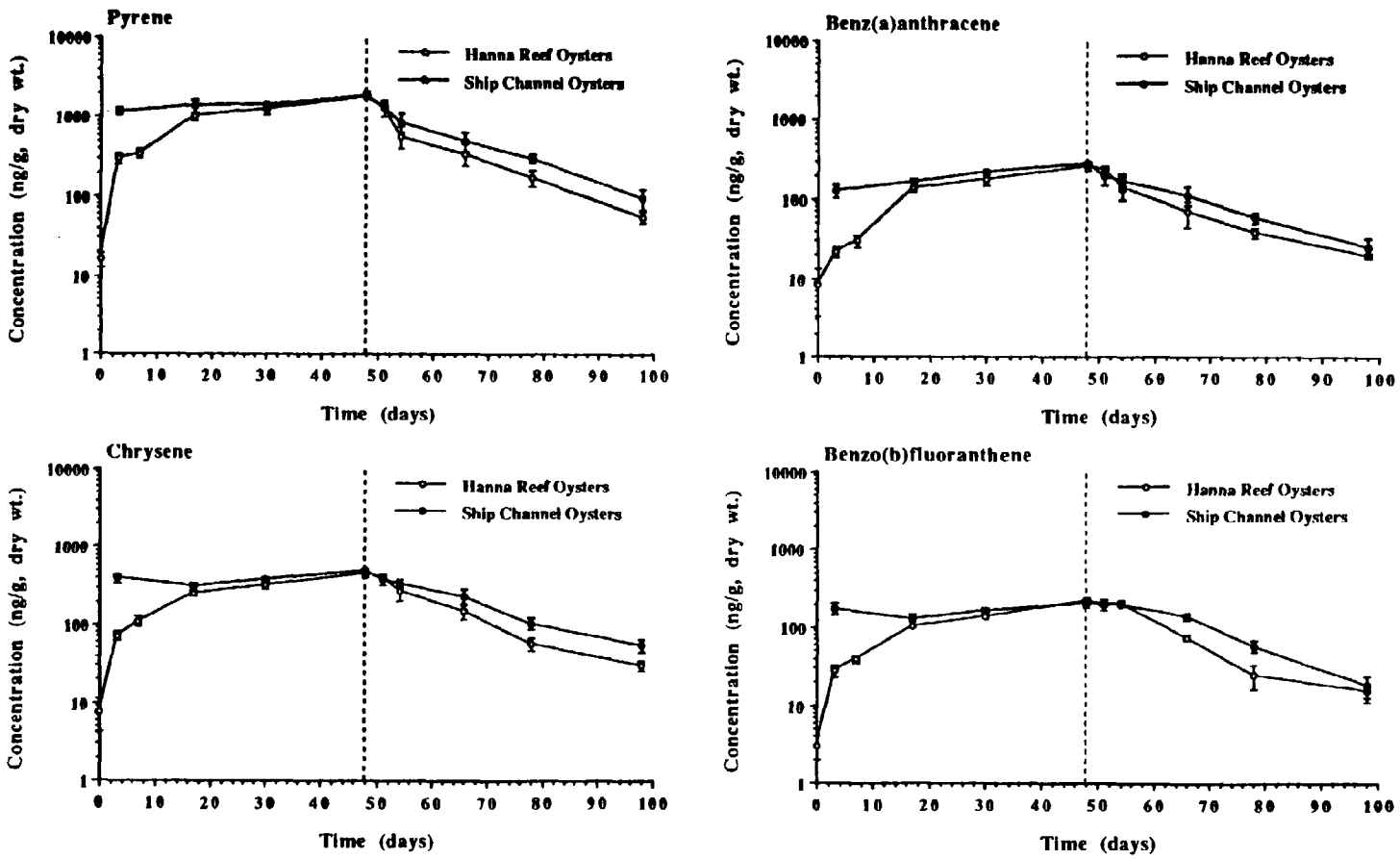


Fig. 7. (Continued)

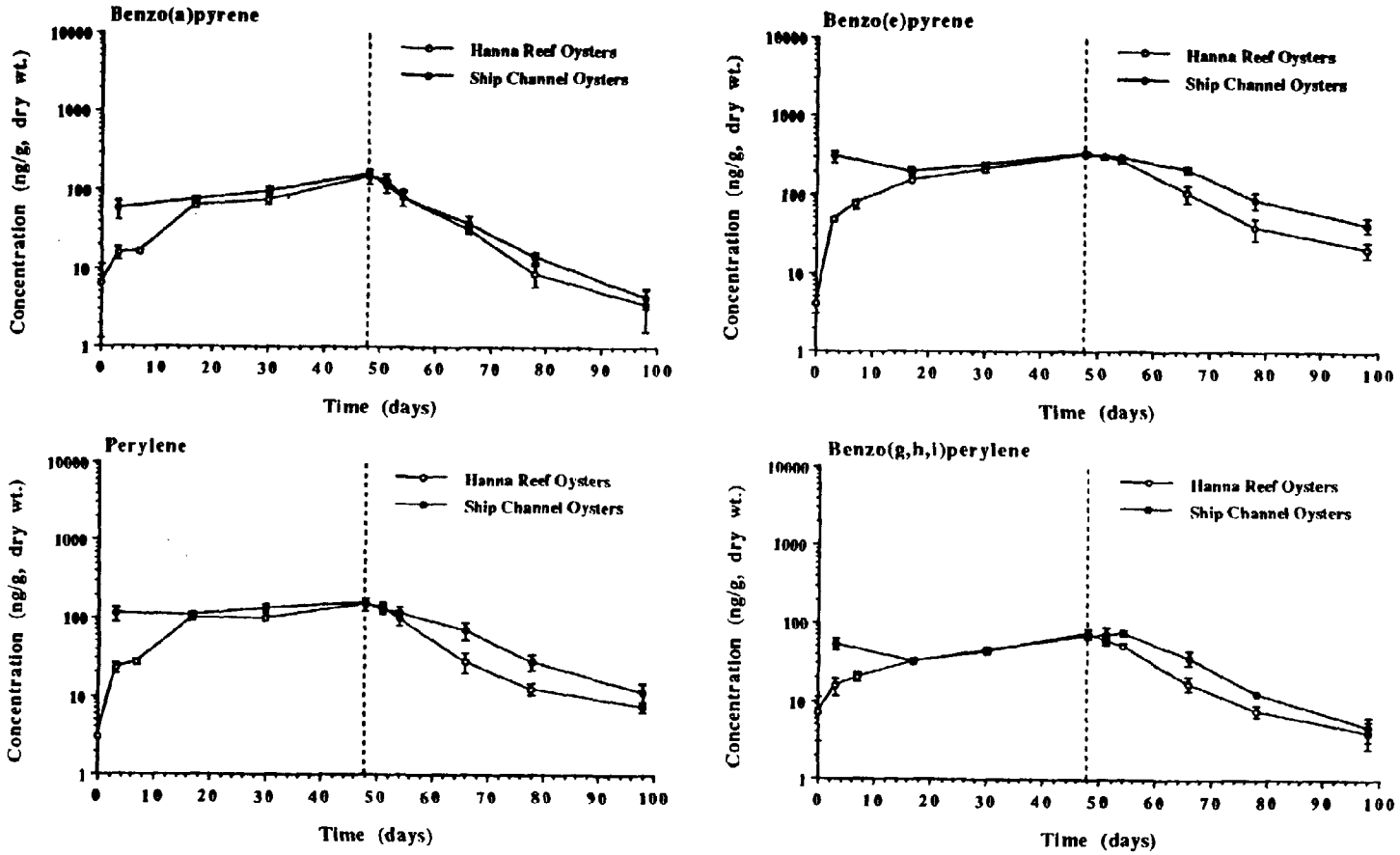


Fig. 7. (Continued)

animals. By the end of the exposure period, the concentrations of all the individual PAH measured in transplanted oysters were not statistically differentiable from those encountered in indigenous oysters (Fig. 8). The observed rapid bioconcentration of PAHs is similar to the uptake curves reported in different studies involving bivalves either in laboratory experiments (Nunes & Benville, 1979; Pruell *et al.*, 1986; Tanacredi & Cardenas, 1991) or in transplantion studies (Pittinger *et al.*, 1985). The PAHs accumulated to the highest concentrations in SC and HRSC oysters were: pyrene > fluoranthene > chrysene > benzo(e)pyrene > benzo(b)anthracene. Three PAHs, pyrene, fluoranthene and chrysene, accounted for about 60% of the total PAH load measured in these oysters. Other uptake studies, using a variety of organisms exposed to different sources of PAHs, produced a different order for the concentration of four- and five-ring PAHs. For example, the relative abundances reported by Pruell *et al.* (1986) for mussels (chrysene > benzo(b)fluoranthene > fluoranthene > benzo(a)pyrene > benzo(a)anthracene) or by Obana *et al.* (1983) for clams (pyrene > benzo(e)pyrene > benzo(b)fluoranthene > benzo(a)anthracene) are diferent from that found in this study for oysters. These discrepancies might reflect the different PAH compositions in the sources or a different uptake ability of the organisms.

The average concentrations of PAHs, by number of rings and individually, in Ship Channel sediment and water samples are shown in Fig. 9 and 10, respectively. Water and sediment samples were collected each time the oysters.were collected. Sediment samples had higher relative concentrations of four- and five-ring PAHs when compared to seawater samples. The relative abundances of PAHs in sediments were pyrene > benzo(b)fluoranthene > benzo(e)pyrene > chrysene > benzo(a)pyrene > fluoranthene > benzo(k)fluoranthene (Fig. 9). Two-ring PAHs and most of the three-ring PAHs were detected at low concentrations in sediments. In contrast, two-ring PAHs, i.e. naphthalenes, were the predominant PAHs in the seawater samples (Fig. 10). The

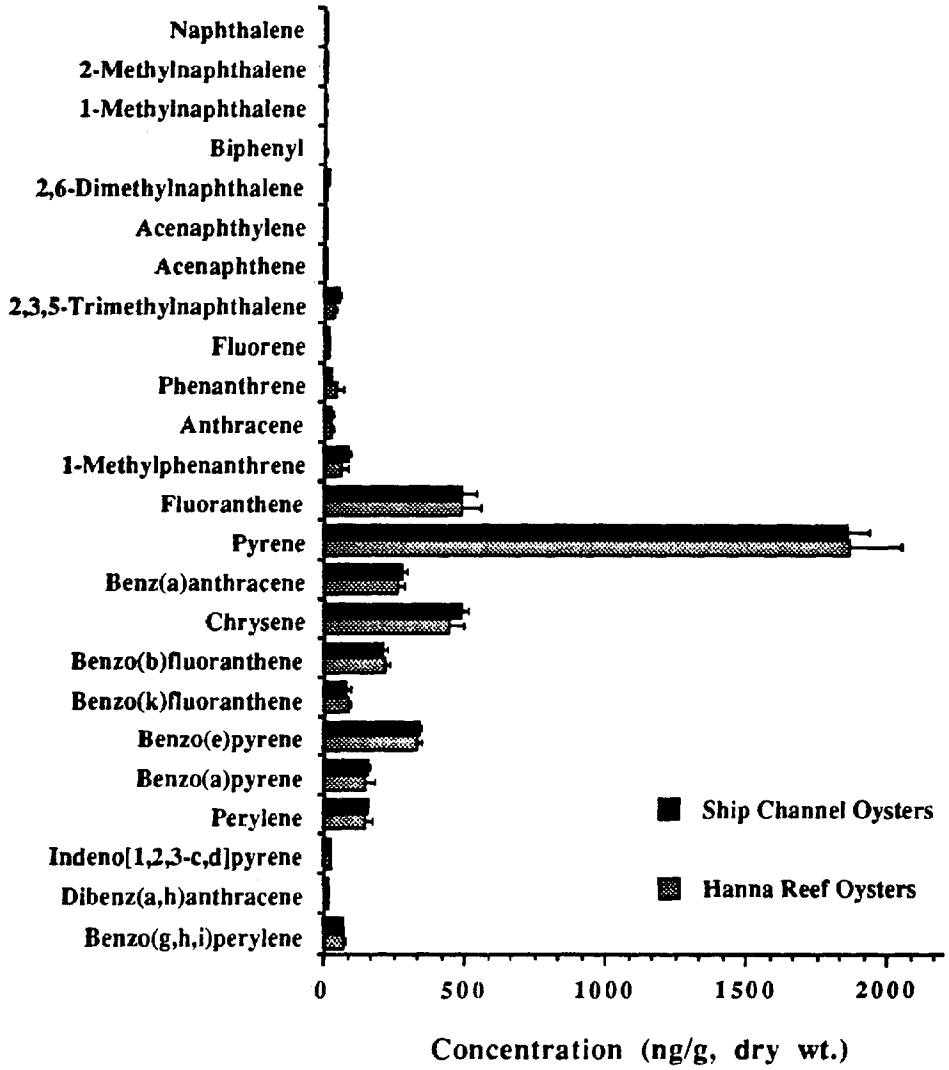


Fig. 8. Concentrations of individual polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters at the end of the 48-day exposure period.

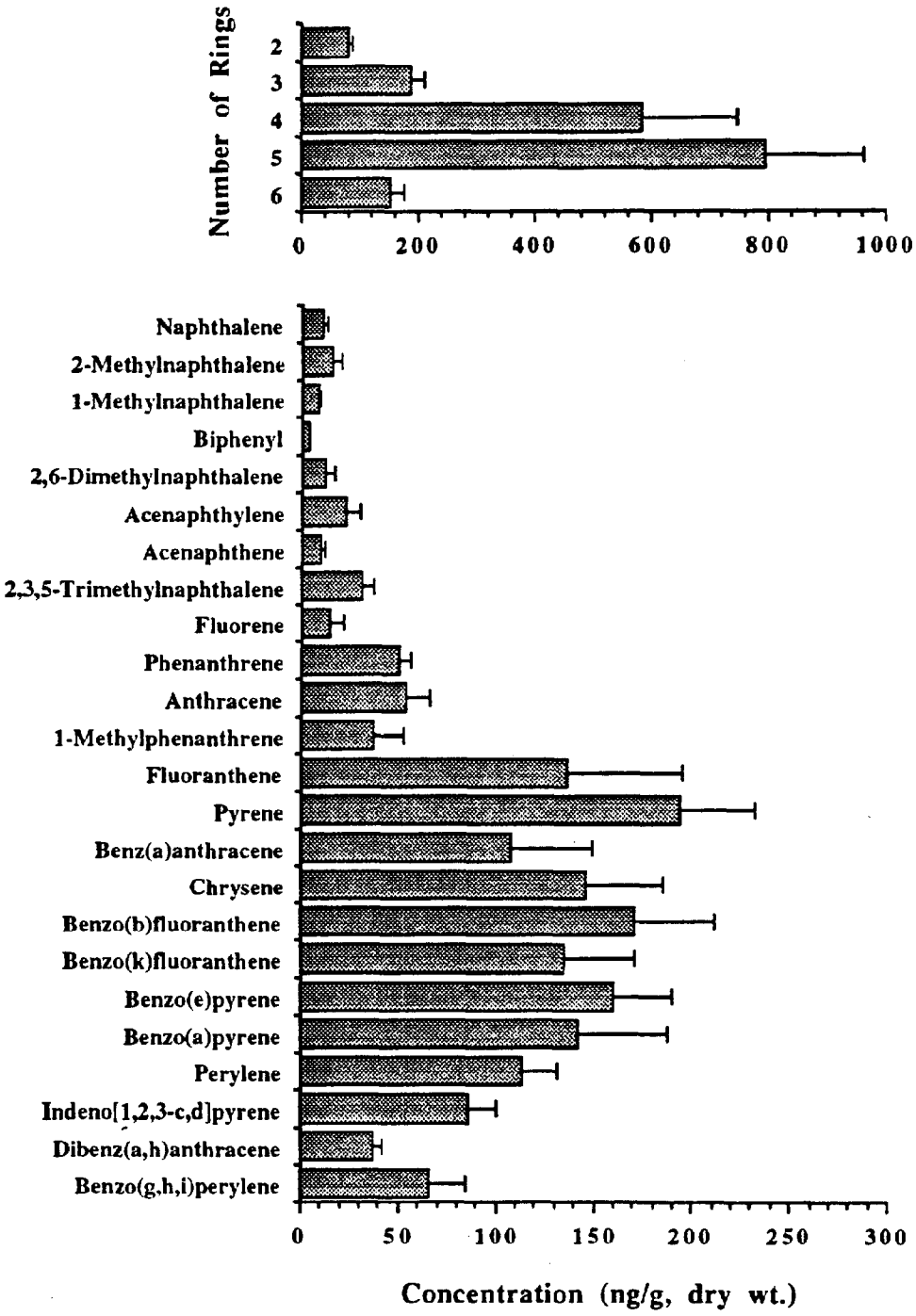


Fig. 9. Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Ship Channel sediment samples.

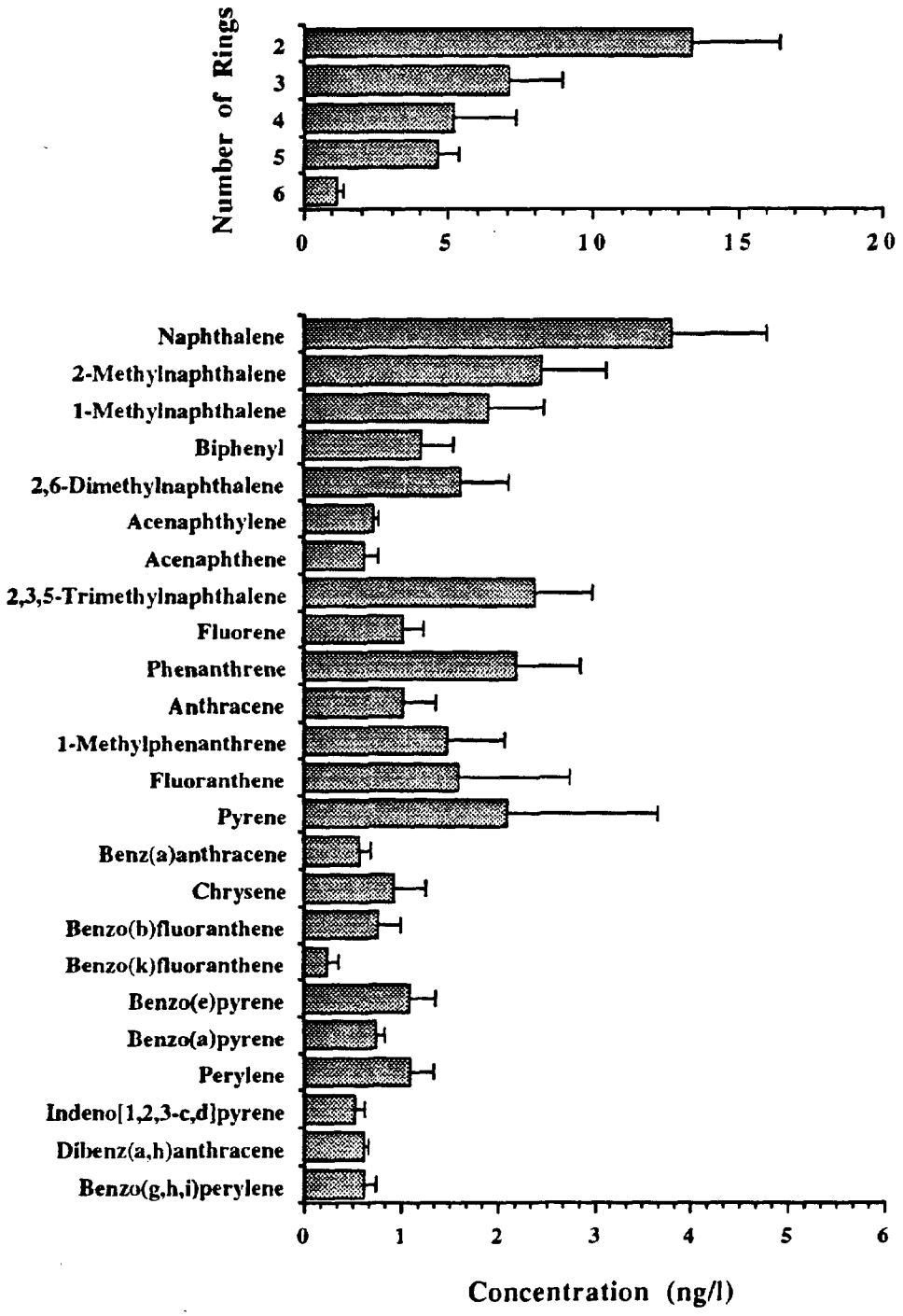


Fig. 10. Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Ship Channel seawater samples.

observed decreasing PAH concentrations in seawater with increasing molecular weight is consistent with published solubility data (e.g. Whitehouse, 1984). The distribution of PAHs by ring numbers in oyster tissues showed lower concentrations of five-ring PAHs relative to the surrounding sediments. Compared to the seawater PAH distribution, oyster tissues were depleted in the more soluble two- and three-ring aromatic hydrocarbons. The PAH distribution for oysters is intermediate when compared to sediments and seawater.

Depuration of PAHs by Newly and Chronically Contaminated Oysters

Average concentrations of selected PAHs measured in HRSCHR and SCHR oyster and sediment samples from the Hanna Reef area are shown in Table A-4 and A-5 (Appendix). Sediment concentrations were normalized by dividing by the percentages of silt and clay in the samples to decrease the variability observed among the samples. After relocation to the Hanna Reef area, HRSCHR and SCHR oysters showed statistically significant depuration of accumulated PAHs. At the end of the depuration period, the total PAH concentrations in HRSC oysters were about 40% higher than the final concentrations in HRSCHR individuals in the same period of time. Total PAH concentrations decreased from 4,400 to 360 ng g^{-1}, in HRSCHR oysters, and from 4,400 to 500 ng g^{-1}, in SCHR oysters. In both cases, most of the decreases in concentrations were due to the depuration of four- and five-ring PAHs (Fig. 11). The diffences in the final concentrations of some individual three-, four- and five-ring hydrocarbons between SCHR and HRSCHR oyster populations at the end of the 50-day depuration period are evident (Fig. 12). The largest percent differences were observed for fluoranthene (38%), pyrene (70%), chrysene (80%) and benzo(e)pyrene (110%).

Although HRSCHR and SCHR oysters significantly depurated most of the bioaccumulated individual PAHs, their concentrations did not decrease to the levels encountered for HR oysters at the begining of this study. For example, Fig. 13 shows

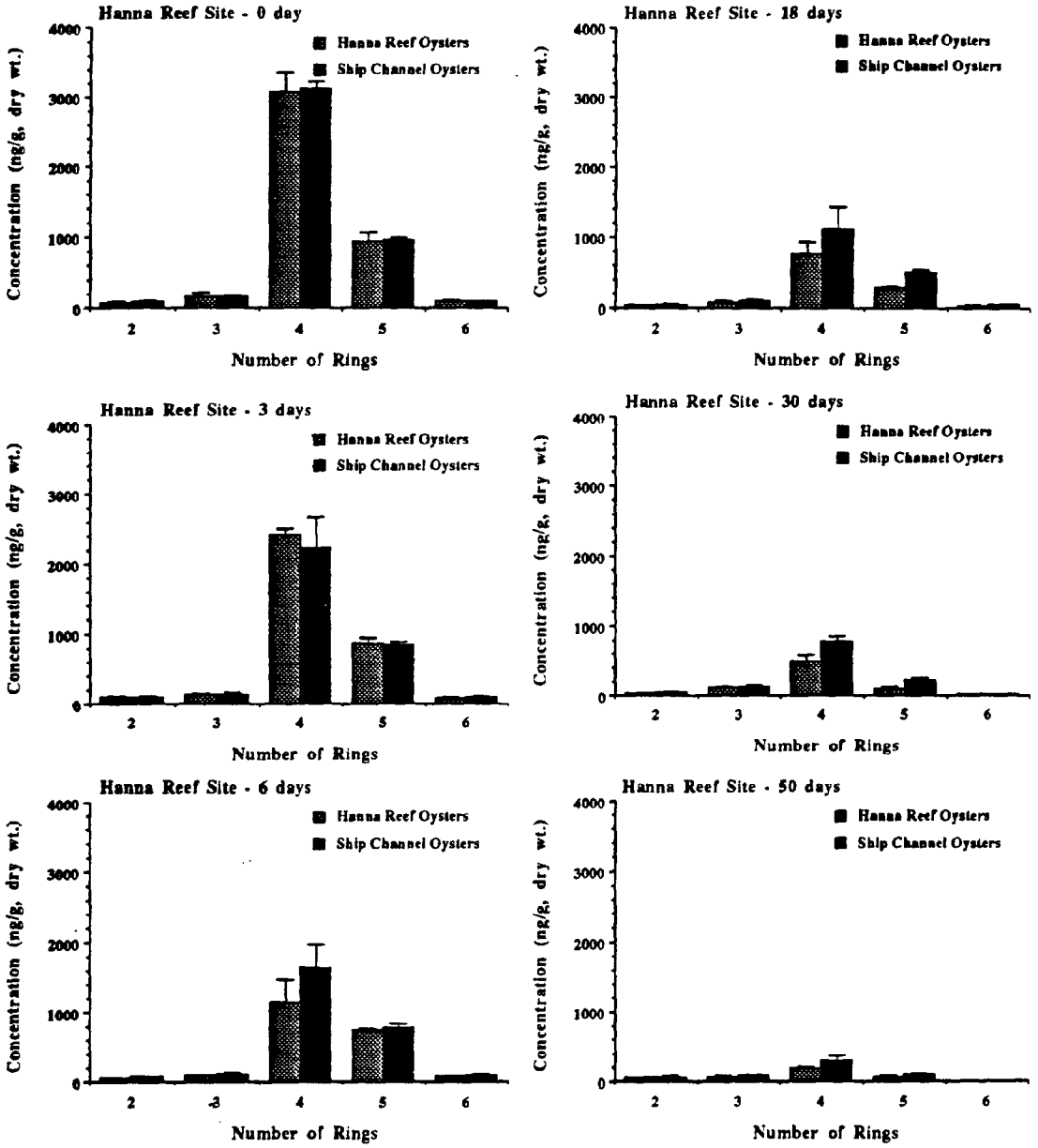


Fig. 11. Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings, in back-transplanted Hanna Reef and transplanted Ship Channel oysters during the 50-day depuration period in the Hanna Reef area.

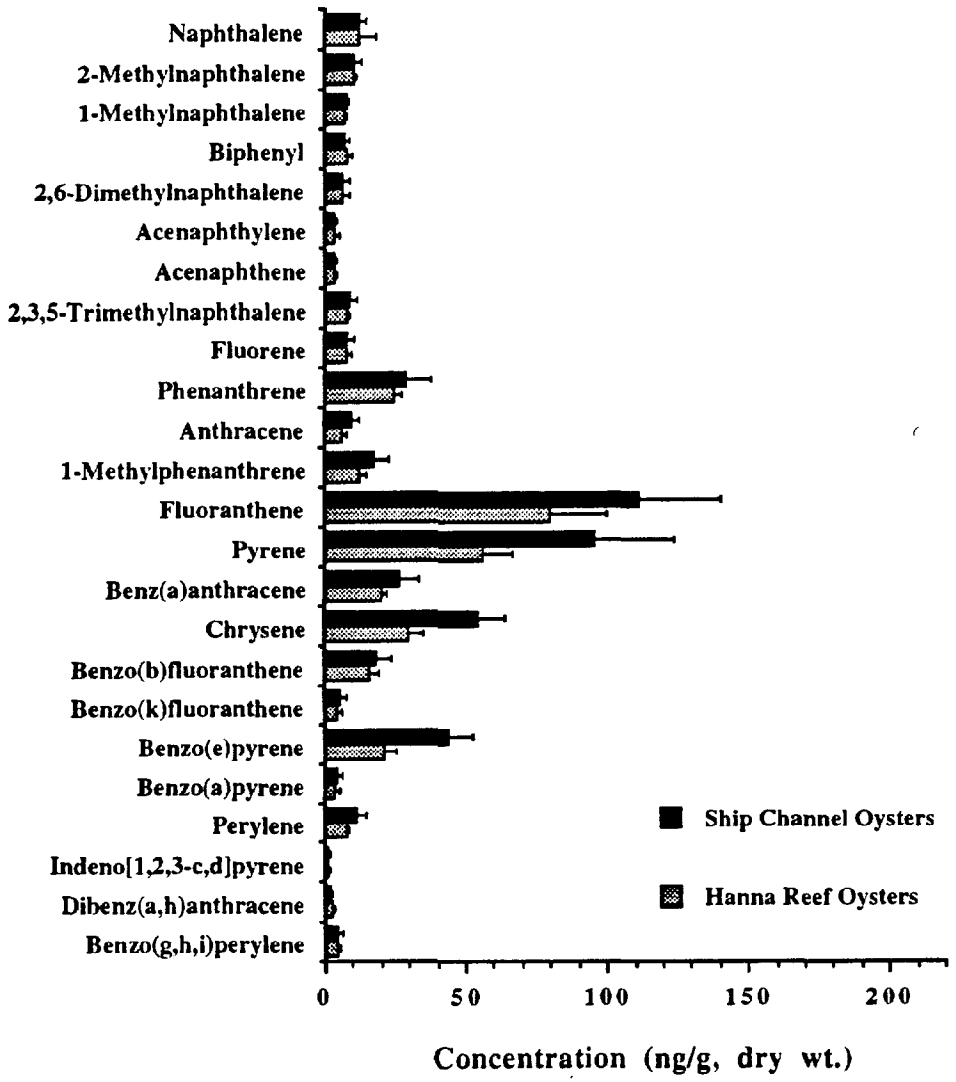


Fig. 12. Concentrations of individual polynuclear aromatic hydrocarbons in tissues of Hanna Reef and Ship Channel oysters at the end of the 50-day depuration period.

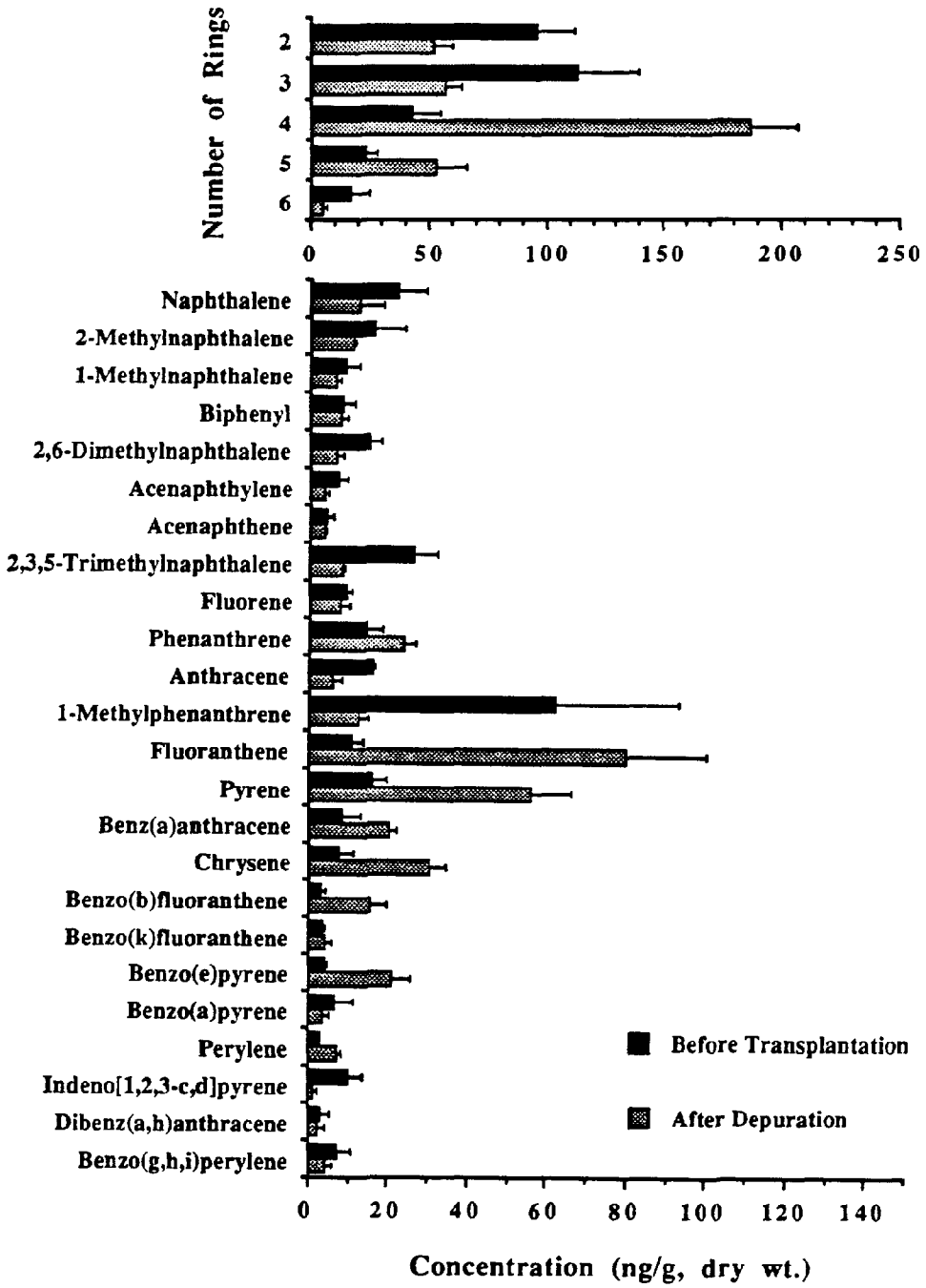


Fig. 13. Comparison of the concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in tissues of Hanna Reef oysters before exposure to the Ship Channel contaminant levels and after depuration at the Hanna Reef site.

the concentrations of PAHs, according to the number of rings and individually, in Hanna Reef oysters before transplantation to the contaminated site and after depuration for 50 days at the Hanna Reef site. The original distribution of PAHs in Hanna Reef oysters showed a predominance of the more volatile and soluble compounds, i.e. two- and three-ring PAHs. When these oysters were exposed to higher PAH concentrations at the Ship Channel area, they bioconcentrated four- and five-ring PAHs. This resulted in higher concentrations for total PAHs as well as in a different distribution of PAHs when the oysters were back-transplanted to the Hanna Reef location. These different distributions are probably the consequences of two different sources of PAHs. In the first case, the PAH distribution reflects the remote location of the Hanna Reef site and indicates atmospheric inputs and water transport of the more soluble PAHs as their most probable sources. A slight increase in the concentration of naphthalenes with time in SCHR and HRSCHR oysters during the depuration part of this study was observed. When oysters are exposed to the significantly higher PAH concentrations, over a wider molecular weight range, present in the Ship Channel area, they readily bioconcentrate the higher molecular weight PAHs. Because of the low water solubility of the predominant compounds encountered in Ship Channel oysters, it is probable that the main route of uptake is through food ingestion. In contrast, most of the uptake in the Hanna Reef area probably occurs through gills. Unfortunately, the concentrations of PAHs in Hanna Reef water samples were below the detection limit; therefore, no firm conclusion can be drawn.

Sediment samples collected from the Hanna Reef area had a distribution of PAHs, by ring numbers, similar to that encountered near the Houston Ship Channel; however, the concentrations of individual PAHs were about an order of magnitude lower (Fig. 14). With the exception of the high concentration of perylene, a compound with natural as well as combustion sources, in Hanna Reef sediments, the differences in concentrations

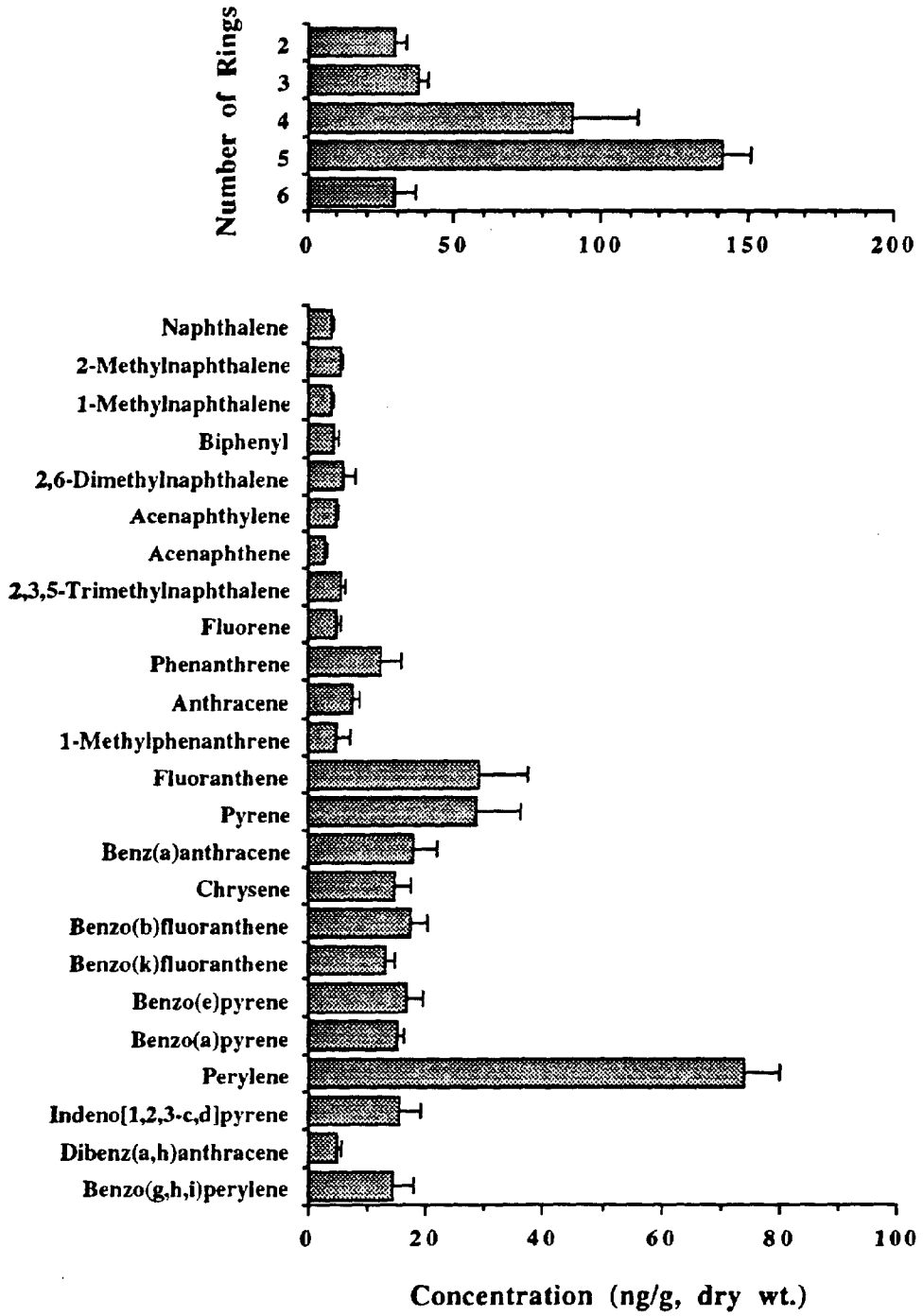


Fig. 14. Concentrations of polynuclear aromatic hydrocarbons, grouped by number of rings and individually, in Hanna Reef sediment samples.

between the lower and higher molecular weight PAHs in these samples is less marked than in the case of the Ship Channel sediment samples.

Clearance rates of aromatic compounds by both groups of oysters were approximately exponential. This is indicated in Fig. 7 where the concentrations of selected PAHs, during the depuration phase of this study, plotted on semi-log plots, approximate straight lines. Original Hanna Reef oysters depurated PAHs at a faster rate than Ship Channel oysters. Differences in the slopes of the depuration curves are reflected in the lower PAH half-lives for SCHR oysters. Calculations to estimate the half-lives and related kinetic parameters for the different trace organic pollutants by *Crassostrea virginica* oysters will be discussed in more details in Chapter VI. The depuration half-lives for PAHs ranged from 9 to 24 days and from 10 to 24 days in originally uncontaminated Hanna Reef and chronically exposed Ship Channel oysters, respectively. Most of PAH half-lives were between 10 and 13 days and 13 and 16 days for HRSCHR and SCHR oysters, respectively. These values are within the ranges of previously reported PAH half-lives (Table 2). A few studies report complete depuration of PAHs by bivalves after they are relocated to a clean environment over short periods of time (less than a week). However, the reported minimum detection limits for PAHs in those studies were generally high. For example, Pittinger *et al.* (1985) reported minimun detection limits for PAHs ranging from 93 to 222 ng g^{-1}. If those minimum detection limits are applied to this study, most of the measured PAHs, in both groups of oysters, would be below detectable levels after one week of relocation to the Hanna Reef area.

CONCLUDING REMARKS

PAHs were rapidly accumulated by uncontaminated oysters to final concentrations that were statistically undistinguished from the concentrations encountered in indigenous Ship

TABLE 2

Biological Half-Lives (Days) of PAHs in Hanna Reef and Ship Channel *Crassostrea virginica* Oysters.

Analyte	Hanna Reef Oysters	Ship Channel Oysters	Dun & Stich (1976) Mussels	Lee *et al.* (1978) Oysters	Pruell *et al.* (1986) Mussels
2,3,5-Trimethylnaphthalene	24	22	-	-	-
Anthracene	24	42	-	3	-
1-Methylphenanthrene	23	24	-	-	-
Fluoranthene	26	32	-	5	30
Pyrene	10	12	-	-	-
Benz(a)anthracene	13	15	-	9	18
Chrysene	12	16	-	-	14
Benzo(b)fluoranthene			-	-	17
Benzo(k)fluoranthene			-	-	12
Benzo(e)pyrene	12	16	-	-	14
Benzo(a)pyrene	9	10	16	18	15
Perylene	11	13	-	-	-
Indeno[1,2,3-c,d]pyrene	10	11	-	-	16
Dibenz(a,h)anthracene	16	14	-	-	-
Benzo(g,h,i)perylene	11	12	-	-	15

Channel oysters within 20 to 25 days after transplantation. The PAHs accumulated to the highest concentrations in SC and transplanted HRSC oysters were: pyrene > fluoranthene > chrysene > benzo(e)pyrene > benzo(a)anthracene. Although there are some discrepancies when comparing the order of uptake of these PAHs, encountered in the present study, with previously published works using different bivalves, these discrepancies might reflect the different PAH compositions in the sources or a different uptake ability of the organisms. The final distributions of individual PAHs in transplanted and indigenous oysters during the uptake phase of this experiment were intermediate between the profiles encountered in sediment and seawater samples from the Ship Channel area.

When transplanted to the relatively uncontaminated Hanna Reef area, both groups of oyster depurated the bioaccumulated PAHs. Calculated depuration rates were higher for the originally uncontaminated oysters. Most of individual PAH depuration half-lives were between 10 and 13 days and 13 and 16 days for HRSCHR and SCHR oysters, respectively. The depuration of individual PAHs by HRSCHR oysters was, however, not complete and the concentrations encountered at the end of the depuration period were higher than the levels measured before their exposure to the Ship Channel concentrations. Comparing the distribution profiles of PAHs encountered in HRSCHR oysters at the end of the depuration period with the distribution they had before the transplant experiment, i.e. HR oysters, seems to indicate that the sources of PAHs to Hanna Reef and Ship Channel are different. While the original distribution of PAHs in Hanna Reef oysters showed a predominance of the more volatile and soluble compounds, i.e. two- and three-ring PAHs, the distribution of PAHs after the depuration phase of this experiment shows predominance of four- and five-ring PAHs. It can be speculated that petroleum background and water transport are the most probable sources for the predominance of the lower molecular weight PAHs to the Hanna Reef area.

CHAPTER III

UPTAKE, RETENTION AND RELEASE OF PCBs BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

INTRODUCTION

Polychlorinated biphenyls (PCBs) are of particular concern in pollution studies because of their widespread occurrence, environmental persistence and bioaccumulation properties. For these reasons, these compounds have been included as analytes of interest in many national and international programs (see, for example, Farrington *et al.*, 1980; Sericano *et al.*, 1990a). In most of these monitoring programs, bivalves were preferred as sentinel organisms.

Despite the overwhelming popularity that the "Mussel Watch" concept has obtained since its introduction in the 1970s, both monitoring data and, particularly, laboratory-generated data on PCB kinetics in bivalves show discrepancies. For example, there are disagreements over which PCB congeners are preferentially accumulated by bivalves and the length of time bivalves need to reach an equilibrium with environmental concentrations or the time needed for different PCB congeners to be depurated, if they are depurated, when the environmental concentration is reduced. Such inconsistencies decrease the usefulness of the Mussel Watch concept in environmental studies.

This chapter reports the uptake and release of PCBs by two groups of American oysters (*Crassostrea virginica*) with different pollution histories. Oysters from Hanna

Reef, a relatively uncontaminated area in Galveston Bay, were transplanted to a site near the Houston Ship Channel, a highly polluted area, to assess the accumulation of PCBs over a period of seven weeks. Concentrations in transplanted oysters were compared to the levels encountered in indigenous Ship Channel oysters. After the uptake period, the remaining Hanna Reef oysters were back-transplanted to their original geographic location to monitor the depuration of the bioaccumulated organic contaminants. At the same time, indigenous Ship Channel oysters were transplanted to the Hanna Reef area in order to compare depuration rates of PCBs between the two groups of oysters, i.e. newly and chronically contaminated.

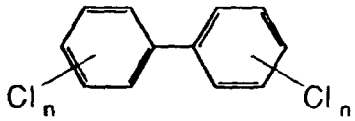
PCBs: A REVIEW

Background Information

PCBs are the subject of several monographs and books (e.g. Safe, 1984; Erickson, 1986; Safe *et al.*, 1987; Tanabe & Tatsukawa, 1986; Voogt & Brinkman, 1989; Lang, 1992). Polychlorinated biphenyls (PCBs), systematically called 1,1'-biphenyl, chloro derivatives, is the generic name of many isomers and congeners with 1 (monochlorobiphenyls) to 10 (decachlorobiphenyl) chlorine atoms substituted on both biphenyl rings (Fig. 15). The synthesis of PCBs was first described by Schmidt & Schultz (1881); however, commercial production in the U.S.A. did not begin until 1929. The rings in the biphenyl molecule are joined by a single carbon-carbon bond allowing free rotation of both rings. The presence of one or more chlorine in *ortho* positions (2, 2', 6 and/or 6') results in an inter-ring angle of up to 90° (McKinney *et al.*, 1983). Although there are 209 possible PCB congeners, the catalytic electrophilic substitution of chlorines is favored at the *ortho* and *para* positions on the biphenyl molecule. Thus, several congeners have been found to be absent (or present at levels below 0.05% total

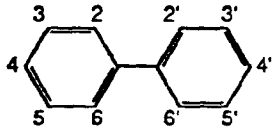
PCBs

General Formula:

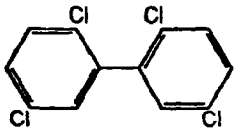


$C_{12}H_{10-n}Cl_n$
(n = 1 to 10)

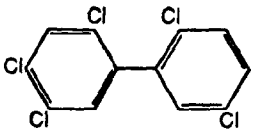
Nomenclature:



Examples of Major Congeners in Environmental Samples:



PCB #52



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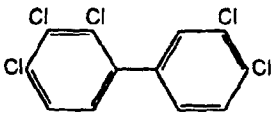

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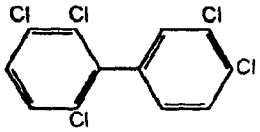

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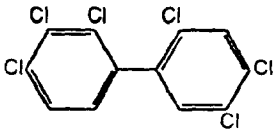
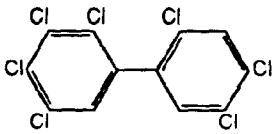


PCB #138



PCB #180

Fig. 15. General formula of polychlorinated biphenyls and examples of major congeners commonly found in environmental samples.

concentration) from technical PCB mixtures (Schulz *et al.*, 1989). Unique properties, including thermal stability and resistance to oxidation, resulted in the use of PCBs as adhesives, heat transfer fluids, wax extenders, hydraulic fluids, lubricants, flame retardants and as dielectric fluids in transformers and capacitors.

Different PCB formulations were graded and marketed according to their chlorine content. Monsanto Chemical Corporation produced, for example, Aroclor 1221, 1232, 1254 and 1260, which contained 21, 32, 54 and 60 percent of chlorine by weight, respectively. Many comparable commercial PCB formulations have been produced by different chemical companies in several countries including Kanegafuchi Chemical Co. in Japan (Kaneclor), Prodelec in France (Phenoclor), Bayer in West Germany (Clophen), Deutchen Solvay Werken in East Germany (Orophene), Caffaro in Italy (Fenclor) and Soval in the U.S.S.R. (Sovol and Sovtol) (Onuska & Comba, 1980). It has been reported that between 1930 and 1975 the U.S.A. production of PCBs was 570×10^3 tons (U.S. Environmental Studies Board, 1979) whereas the total worldwide production of PCBs through 1980 was estimated to be 1100×10^3 tons (Erickson, 1986). In 1977, the major U.S. producer, Monsanto Chemical Corporation, ceased manufacturing PCBs partly due to their widespread detection in the environment. A recent estimation, however, indicates that more than two-thirds of the cumulative world PCB production may still be in use mainly in older transformers and capacitors (Tanabe, 1985).

Dr. Soren Jensen, a Swedish environmental chemist, first reported the presence of several unknown peaks that interfered with quantitative determinations of DDT in environmental samples (Jensen, 1966); those peaks were soon identified as a complex mixture of PCBs by GC and GC/MS (Widmark, 1967). The earliest analyses of PCBs, e.g. before 1980, were performed with packed columns. The results of these studies have provided valuable information on hot spots and general trends in concentration distributions; however, because of the low-resolution chromatograms most of the

information regarding individual congeners, which are important when determining source identification, sink, toxicity and biological uptake or depuration, was limited. The importance of considering individual PCB congeners in view of their differences in both toxicity and physico-chemical properties is well recognized (see, for example, Shaw & Connell, 1984; Opperhuizen *et al.*, 1985, 1988; Tanabe *et al.*, 1987b, 1987c). Although a complete separation of all 209 PCB congeners with a single gas chromatographic run has not been achieved yet, the introduction of the capillary column greatly improved the separation of individual congeners.

The synthesis and chromatographic properties of all 209 PCB congeners have been reported (Mullin *et al.*, 1984). Certain PCB congeners are considered to be the most toxic because they can attain a planar stucture similar to the highly toxic dibenzo-p-dioxins and dibenzofurans (McKinney *et al.*, 1976, 1985; Hansen, 1987; McFarland & Clarke, 1989). Because of their environmental significance, these PCB congeners will be discused separately in Chapter IV.

PCBs are ubiquitous contaminants of the global environment. The physicochemical properties of these components vary widely depending on the number and position of chlorine atoms in the biphenyl rings. In general, vapor pressure, water solubility and biodegradability decrease with increasing number of chlorine atoms, whereas lipophilicity and adsorption capacity show a reverse trend (Tanabe *et al.*, 1984). Large variations of PCB compositions are found in different environmental compartments resulting from this wide range of properties. PCBs have been found in air, water, soil and sediment samples throughout the world (e.g. Atlas & Giam, 1981; Tanabe *et al.*, 1983a). Nearly all marine plant and animal specimens, fish, mammals, birds (especially fish-eating birds), bird eggs and humans have measurable PCB concentrations (Tanabe *et al.*, 1983b, 1986, 1987c). In general, PCBs are detected in parts per billion (ppb) in organism, soil and

sediment samples and in parts per trillion (ppt) in water samples; however, concentration levels vary over a large range from highly polluted to pristine.

Distribution and Occurrence in Galveston Bay

A variety of organochlorine residues have been determined in organisms, e.g. bivalves and various species of fishes and birds, sediment and water samples, from the Galveston Bay area. PCB congeners were one of the most commonly found compounds in Galveston Bay samples (Table 3).

The ubiquity of PCBs in Galveston Bay was demonstrated by Fox (1988). Oyster samples were collected from four different sites. PCBs were detected in every sample analyzed during the study. Concentrations measured in oysters collected near the Houston Yacht Club were higher than the levels found in samples from Hanna Reef, Todd's Dump and Confederate Reef areas.

A number of different species of fish were also analyzed for PCBs. Concentrations ranged over two orders of magnitude. Finfishes such as mullet, croaker and Florida pompano, collected in the vicinity of a power plant (Houston Lighting and Power Company) near the upper Trinity Bay, contained PCB concentrations in the range of 50-500 ng g^{-1} (Strawn *et al.*, 1977). Lower concentrations were reported in juvenile croakers (9-43 ng g^{-1}; Stahl, 1980). Similar ranges to those published by Strawn *et al.* (1977) were reported for tidewater silverside, sheepshead minnow and striped mullet (King, 1989a, 1989b). These fishes are the food source of some birds such as black skimmer and olivaceous cormorant.

A few waterbirds, e.g. olivaceous and double-crested cormorants, laughing gulls and black skimmers, nesting in the upper Galveston Bay were also analyzed for chlorinated hydrocarbons (King & Krynitsky, 1986; King *et al.*, 1987). Average concentrations and concentration ranges encountered in these birds were similar. PCB average

TABLE 3

Polychlorinated Biphenyl Concentrations in Samples from the Galveston Bay Area. Except Where Indicated, Concentrations in Organisms Are Expressed in ng g^{-1} on a Wet-Weight Basis. Concentrations in Sediment and Water Samples Are Expressed in ng g^{-1}, on a Dry-Weight Basis, and in ng l^{-1}, Respectively.

Location	Sample	PCBs	Range	Reference
Yacht Club	oysters	966	120-4,025	Fox, 1988(1)
Todd's Dump	oysters	155	47.4-283	Fox, 1988(1)
Confederate Reef	oysters	131	94.7-171	Fox, 1988(1)
Hanna Reef	oysters	59.4	32.5-107	Fox, 1988(1)
Trinity Bay	fish		50-160	Strawn *et al.*, 1977
	fish		50-500	Strawn *et al.*, 1977
	fish		60-150	Strawn *et al.*, 1977
Galveston Bay	fish		9-43	Stahl, 1980
	crab		18-42	
Galveston Bay	fish	310	70-540	King, 1989a(2)
Galveston Bay	fish	350	100-620	King, 1989b(2)
Galveston Bay	cormorants	6,990	2,600-24,000	King & Krynitsky, 1986(2)
	gulls	4,210	1,500-11,000	
	skimmers	3,880	800-11,000	
Galveston Bay	cormorants	1,580	1,100-3,300	King *et al.*, 1987(2)

TABLE 3
(continued)

Location	Sample	PCBs	Range	Reference
Houston Ship Channel	sediments	3,250		Salch & Lee, 1976
Texas City Channel	sediments	2,860		Salch & Lee, 1976
Galveston Bay	sediments		15-68	Stahl, 1980
San Luis Pass	sediments	0.52	0.25-0.78	Murray *et al.*, 1981a
Morgan's Point	sediments	1.5	<0.14-3.3	Murray *et al.*, 1981b
Trinity Bay	sediments	1.2	<0.14-7.1	Murray *et al.*, 1981b
Texas City Channel	sediments	2.8	<0.14-5.6	Murray *et al.*, 1981b
Galveston Bay	water		2-15	Stahl, 1980
Morgan's Point	water	1.1	<0.01-4.6	Murray *et al.*, 1981b
Trinity Bay	water	1.8	<0.01-4.1	Murray *et al.*, 1981b
Texas City Channel	water	18	<0.01-70	Murray *et al.*, 1981b

n.d.= not detected; (1) ng g^{-1} on a dry-weight basis; (2) geometric mean

concentrations ranged from 1,580 to 6,990 ng g^{-1}, respectively. These concentrations are one order of magnitude higher than concentrations listed on Table 3 for Galveston Bay fish samples. Since these fish-eating birds are at the top of an aquatic food chain, a bioaccumulation of organic contaminants is seen. With overall average PCB concentrations of 4,170 ng g^{-1} in waterbirds and 330 ng g^{-1} in fishes, a biaccumulation factor of 13 can be calculated for PCB residues in Galveston Bay waterbirds.

Reports of PCBs concentrations in sediment and water samples from the Galveston Bay area are limited. In general, PCB concentrations in sediments were in the <0.14 to 7.1 ng g^{-1} range (Murray *et al.*, 1981, 1981b). Stahl (1980) reported a slightly higher concentration range for PCBs in sediments (15-68 ng g^{-1}). These concentrations are two to three orders of magnitude lower than those previously reported in dredged sediments from the Houston Ship and Texas City Channels (Saleh & Lee, 1976). The samples collected during that study corresponded to sediments disturbed by the construction of underwater pipelines; therefore, they might represent sediments deposited before the restrictions of the use of PCBs in the U.S.A. in the 1970s. Water samples collected at different locations in Galveston Bay had PCB concentrations ranging from <0.01 to 70 ng l^{-1} (Saleh & Lee, 1976). The higher PCB concentrations were measured near Texas City.

Bivalve Uptake and Depuration Studies

There are published works reporting uptake and depuration of PCBs by a variety of organisms; however, the number of studies involving bivalves are limited. The methods generally used to expose bivalves to PCB congeners in the laboratory (e.g. Lowe *et al.*, 1972; Vreeland, 1974; Courtney & Denton, 1976; Pruell *et al.*, 1986, 1987) are similar to those mentioned for petroleum hydrocarbon exposures (Chapter II). Transplanting bivalves from an uncontaminated area to contaminated areas or vice versa has also been

done in uptake and depuration studies (e.g. Calambokidis *et al.*, 1979; Tanabe *et al.*, 1987a; Kannan *et al.*, 1989; Sericano *et al.*, in press).

In a laboratory study with blue mussels *(Mytilus edulis)* exposed to contaminated sediments, Pruell *et al.* (1986) reported an equilibration time of about 20 days for four PCB congeners although this bivalve failed to accumulate the highly chlorinated congeners present in the exposure sediments after a 40-day exposure period. Similar time scales were reported for the uptake of the lower molecular weight PCB congeners, i.e. those congeners having 2, 3 or 4 chlorines in the molecule, by transplanted green-lipped mussels (*Perna viridis*) in contaminated Hong Kong waters (Tanabe *et al.*, 1987a). Lower equilibration rates, i.e. more time, for higher-chlorinated PCB congeners are reported. Vreeland (1974) suggested that, even after an equilibrium with the PCB congener concentrations is attained, the total amount of PCB per oyster increases as the oyster grows. Langston (1978) observed some differences in the depuration rates of selected PCB congeners by bivalves (*Cerastoderma edule* and *Macoma balthica*). Di-, tri- and tetrachlorobiphenyls, with half-lives ranging from 5 to 21 days, were depurated faster than hexachlorobiphenyls and some pentachloro-biphenyls. Most of the hexachlorobiphenyls did not show any decrease after 21 days. Pruell *et al.* (1986) reported that about 50% of the total PCBs accumulated by exposed blue mussels (*Mytilus edulis*) were lost after 40 days in clean seawater with half-lives for tri- to hexachlorobiphenyls ranging from 16.3 to 45.6 days. Contrasting with this study, Courtney & Denton (1976) reported that clams exposed to a PCB mixture, Aroclor 1254, in the laboratory did not depurate the accumulated PCBs during a three-month period in control seawater.

UPTAKE AND DEPURATION OF PCBs

Experimental Design, Sample Collection and Methods

The experimental design and sample collection used for the study of PCBs were the same as those discussed for PAHs (Chapter II).

Extraction and sample fractionation of PCBs

As described in the previous Chapter, the analytical procedure used in the extraction, fractionation and cleanup of PCBs in oyster, sediment and water samples, which is done concurrently with the extraction, fractionation and cleanup of PAHs (Fig. 5, Chapter II), was 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. The important steps of this method have been previously discussed for PAHs. The only differences for PCB analysis are:

a. PCB congener quantitations were done using 4,4 dibromoocta-fluorobiphenyl (DBOFB) and PCBs congeners 103 and 198 as internal standards. As previously discussed, these standards were added at concentrations similar to those expected in the samples for the compounds of interest.

b. After the final extract concentration to 1 ml, and before the addition of the GC internal standard for GC-ECD analysis, a 250 ul fraction was reserved for further planar PCB analyses.

Instrumental analysis

PCBs were analyzed by fused-silica capillary column GC-ECD (Ni^{63}) using either a Varian 3500 GC or a Hewlett Packard 5880A GC in the 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 140°C at 5°C min^{-1}, from 140 to 250°C at 1.5°C min^{-1} and from 250 to 300°C at 10°C min^{-1} with 1 min hold time at the beginning of the program and before each program rate change. A hold time of 5 min was used at the final temperature. Total run time was 94.33 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. Nitrogen or argon/methane (95:5) were used as make-up gases at a flow rate of 20 ml min^{-1}. The volume injected was 2 µl. The numbering of PCB isomers, after Ballschmiter & Zell (1980), is as follows: numbers 1-3 represent mono-, 4-15 di-, 16-39 tri-, 40-81 tetra-, 82-127 penta-, 128-169 hexa-, 170-193 hepta-, 194-205 octa-, 206-208 nonachlorobiphenyls and 209 decachloro-biphenyl. A group of PCB congeners (i.e. 8, 18, 28, 44, 52, 66, 101, 105, 110, 118, 128, 138, 153, 170, 180, 187, 195, 206 and 209) were quantitated against a set of authentic standards, which were injected at four different known concentrations to calibrate the instrument and to compensate for non-linear response of the electron capture detector. The remaining PCB congeners were quantitated by comparison to a single reference congener of the same degree of chlorination injected at four different known concentrations. The reference PCB congeners used for quantitation were 8, 28, 52, 101, 138, 170, 195, 206 and 209 for di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, nonachlorobiphenyls and decachloro-biphenyl , respectively. Tetrachloro-m-xylene (TCMX) was used as the GC internal standard to calculate the recoveries of the internal standards. The detection limits for organochlorines and individual PCB isomers, calculated on the basis of 15 g (wet weight) tissue and 50 g (wet weight) sediment sample sizes with 0.2% by volume of the extract injected into the GC-ECD, were 0.25 and 0.02 ng g^{-1} dry weight for oysters and sediments, respectively.

Ancillary parameters

Methodologies for the sediment grain-size analysis and extractable lipids percentage were discussed in the materials and methods section of Chapter II.

Statistical analysis

The statistical analyses performed on the PCB data were previously discussed in the materials and methods section of Chapter II.

Uptake of PCBs by Transplanted Oysters

In the following sections, Ship Channel, Hanna Reef, transplanted Hanna Reef-to-Ship Channel, transplanted Ship Channel-to-Hanna Reef and relocated Hanna Reef-to-Ship Channel-back to-Hanna Reef oysters are refered as SC, HR, HRSC, SCHR and HRSCHR oysters, respectively.

Average concentrations of the predominant PCB congeners found during the first part of this experiment in SC and HRSC oyster, sediment and water samples are reported in Tables A-6 and A-7 (Appendix). Total PCB concentrations in indigenous Ship Channel oysters were fairly constant during the seven-week uptake period with values fluctuating between 960 and 1,500 ng g^{-1}. In contrast, concentrations of total PCBs in transplanted HRSC oysters increased from 30 ng g^{-1} to 830 ng g^{-1} after the 48-days exposure period to the Ship Channel conditions. Typical PCB chromatograms of extracts obtained from transplanted HRSC oysters during the uptake phase of this study are shown in Fig. 16.

Pentachlorobiphenyls accumulated to the highest concentrations in HRSC and native SC oysters (Fig. 17). In comparison, practically no octa-, nona- or decachloro-biphenyls were detected in either oyster group. 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 not statistically significant differences

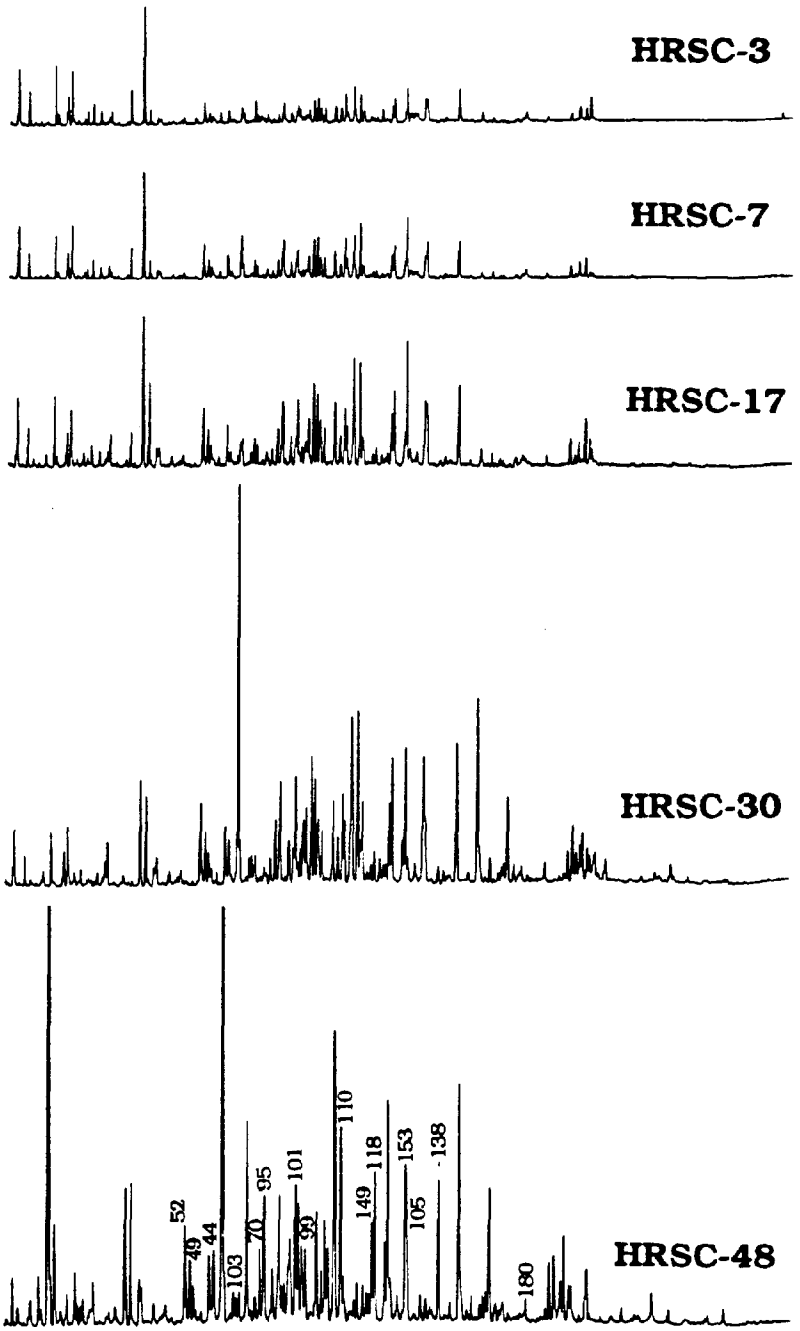


Fig. 16. Examples of high-resolution gas chromatograms of Hanna Reef oysters transplanted to the Ship Channel area during different stages of the 48-day exposure period. PCB congeners are numbered according to Ballschmitter & Zell, 1980.

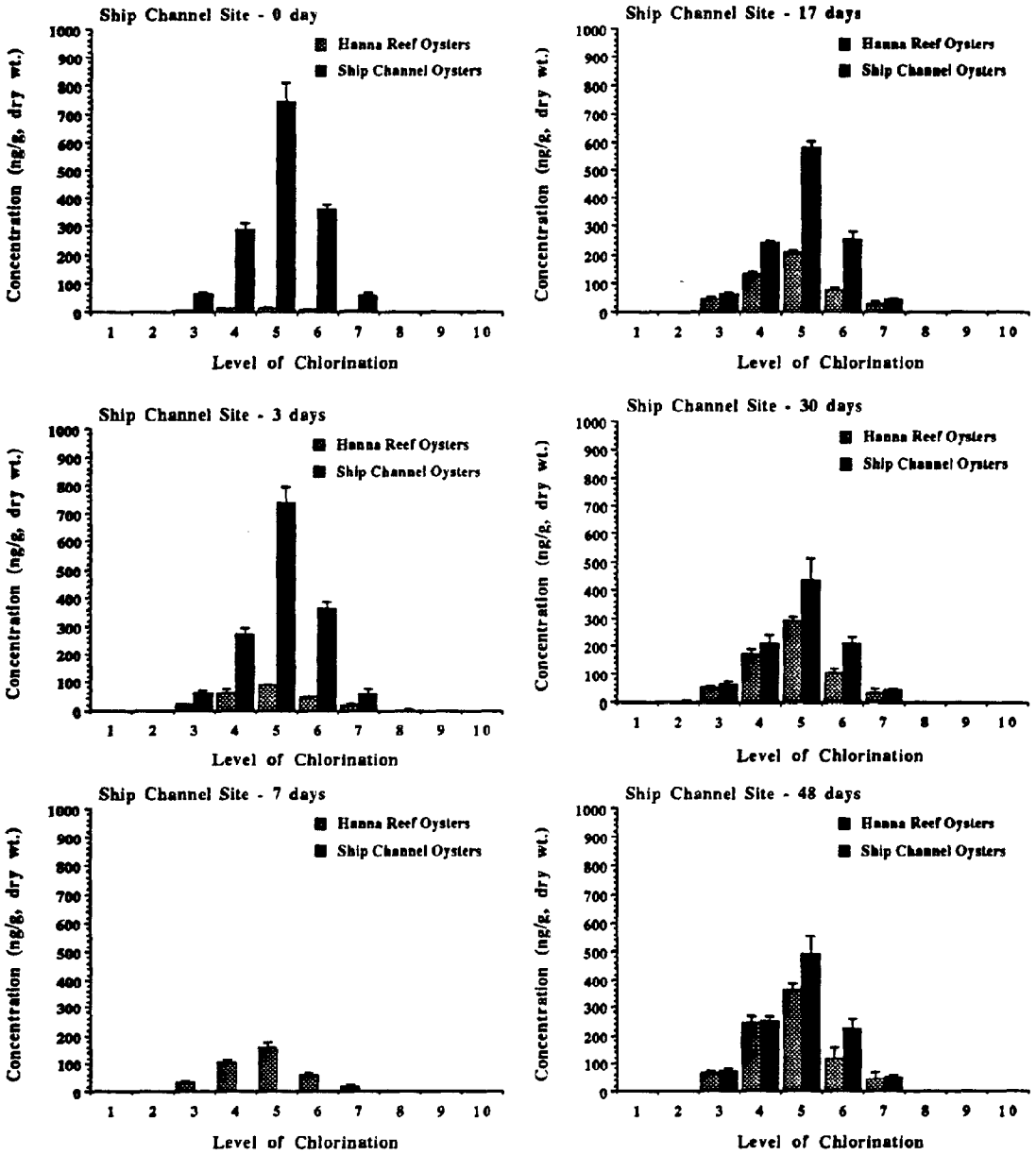


Fig. 17. Concentrations of polychlorinated biphenyl congeners, grouped by level of chlorination, in transplanted Hanna Reef and indigenous Ship Channel oysters during the 48-day exposure period near the Houston Ship Channel. Ship Channel Oysters were not sampled on day 7.

(alpha = 0.05) in the total tri- and tetrachlorobiphenyl concentrations measured in HRSC and SC oysters, significant differences were observed in the total concentrations of penta- and hexachlorobiphenyls. Concentrations of these two homolog groups in transplanted HRSC oysters, at the end of the uptake period were about 30 and 50% lower than the total concentrations measured in indigenous SC oysters, respectively.

Uptake and depuration curves observed for different PCB congeners are shown in Fig. 18. Some of these represent coeluting congeners, for example PCBs 101 and 90 or PCBs 110 and 77. However, the first congeners listed, i.e. 101 and 110, would be expected to be highly dominant over the others. For example, in one of the most common PCB mixtures, Aroclor 1254, the contribution of the PCB congener 101 to the total 101/90 peak is close to 90%; similarly, PCB congener 110 contributes almost 100% of the total 110/77 peak (Schulz *et al.*, 1989). Therefore, it is assumed that the uptake and depuration curves represent the first PCB congener; although all the co-eluting congeners are indicated. When comparing the concentrations of individual PCBs measured in transplanted and indigenous oysters after about one month of exposure, the concentrations of the lower-chlorinated congeners, i.e. tri- and tetra-chlorinated biphenyls, in HRSC oysters were similar to the levels encountered in indigenous individuals

Although increasing trends in the concentrations of all the predominant PCB congeners were observed in HRSC oyster tissues, the concentrations of the higher-chlorinated biphenyls, i.e. penta-, hexa- and heptachlorobiphenyls, did not always reached full equilibrium with the levels measured in SC oysters. This results in qualitative as well as quantitative differences between the PCB profiles in HRSC and SC oyster samples at the end of the exposure period. The total PCB concentration at the end of the exposure period in HRSC oysters (830 ng g^{-1}) was about 25% lower than the levels encountered in SC oysters (1,100 ng g^{-1}). This is clearly shown in Fig. 19 where the concentrations of selected PCB congeners measured at the end of the seven-week uptake

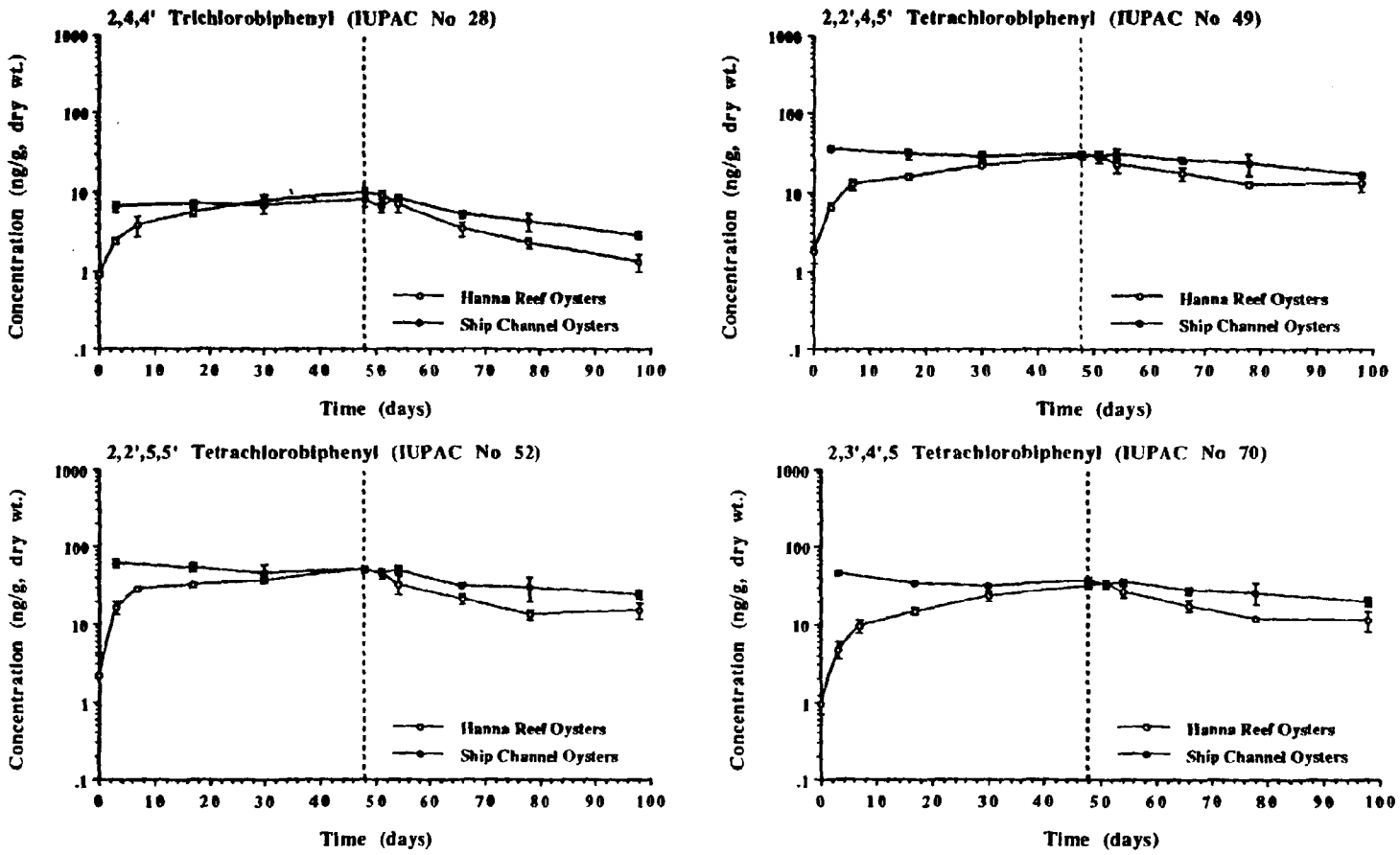


Fig. 18. Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef area.

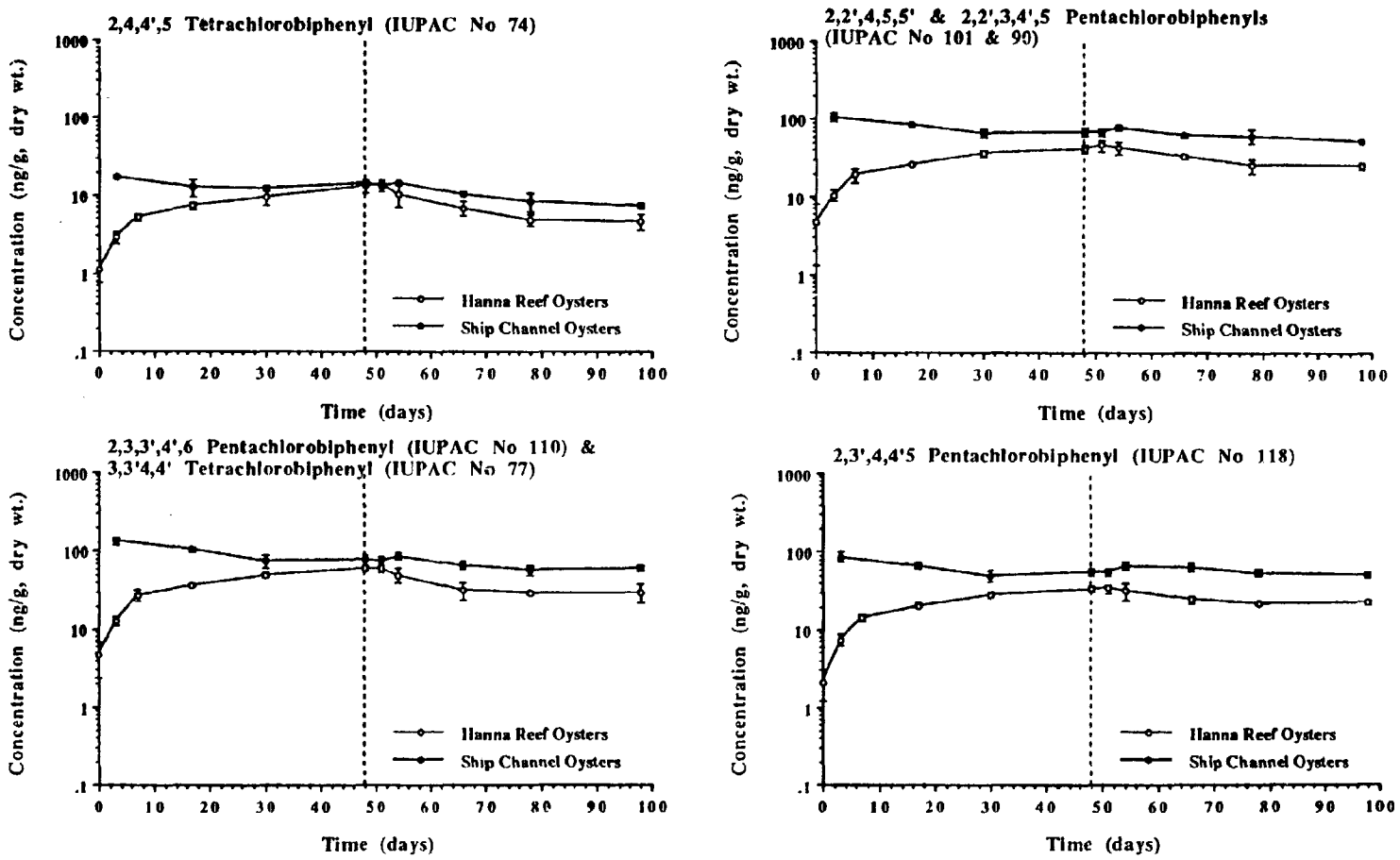


Fig. 18. (Continued)

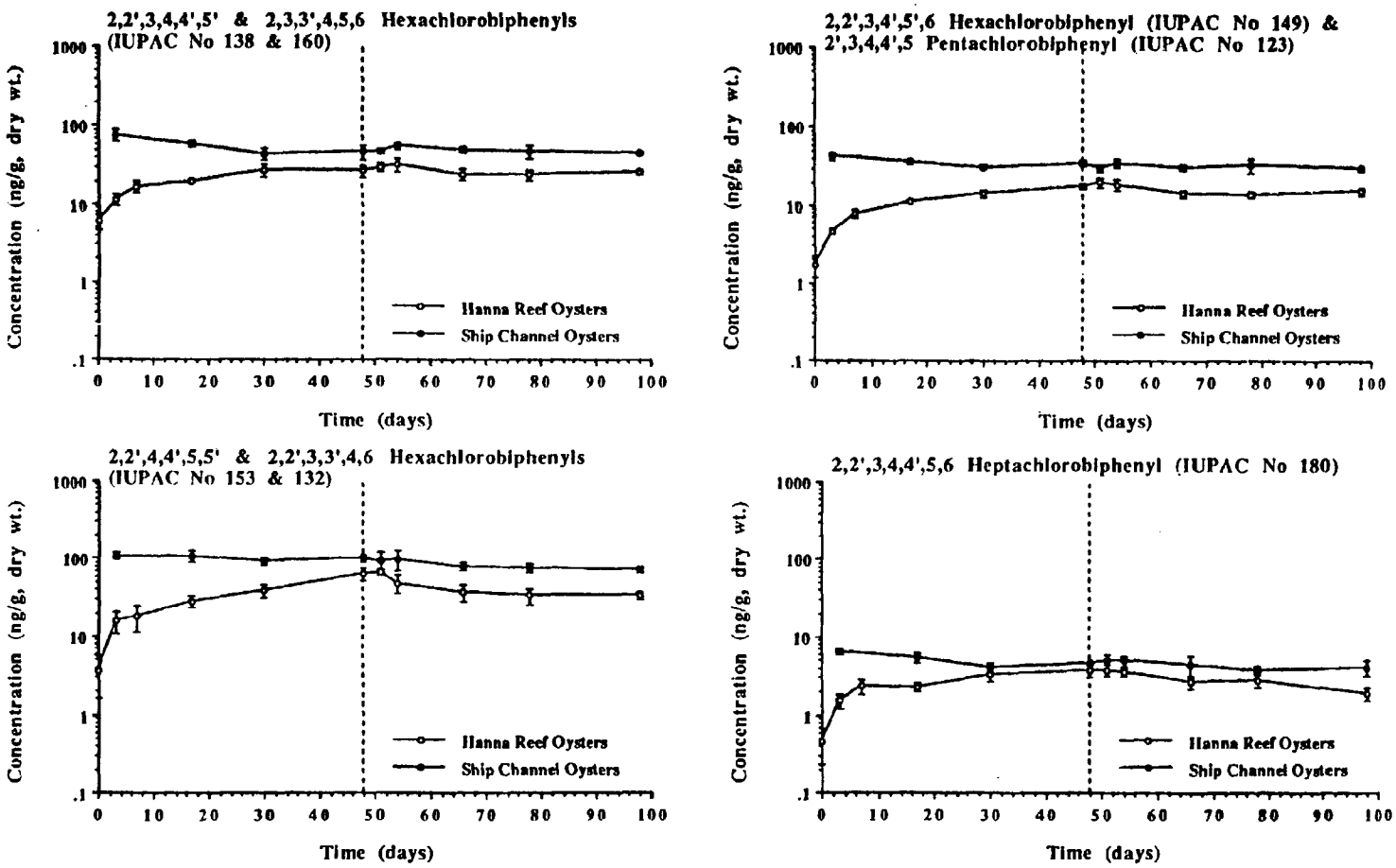


Fig. 18. (Continued)

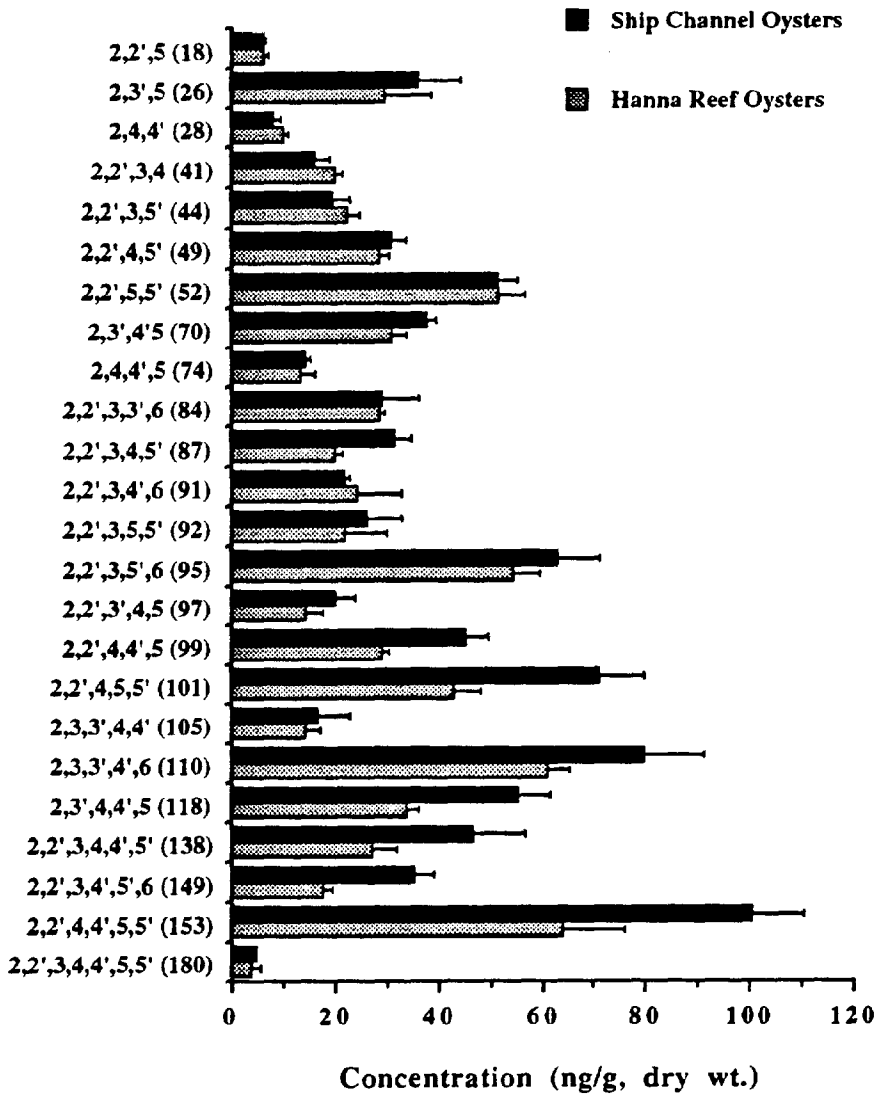


Fig. 19. Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters at the end of the 48-day exposure period.

period are presented. In general, the higher the number of chlorines substituted in the biphenyl molecule, the larger the difference between the concentrations encountered in HRSC and SC oysters. Typically, these differences, in percentages, ranged from -20% to 20% for the lower molecular weight congeners, and from 40% to 60% for the higher molecular weight PCBs (Fig. 20). A negative percentage indicates a higher concentration in HR oysters compared to SC oysters, i.e. PCB congeners 28, 41, 44 and 91. The predominant PCB congeners in HRSC individuals were 153(6)/132(6),110(5)/77(4), 95(5),52(4), 101(5)/90(5), 118(5) and 70(4) compared to 153(6)/132(6), 110(5)/77(4), 101(5)/90(5), 95(5), 118(5), 52(4) and 138(6)/160(6) in SC oysters (the "/" indicates co-eluting congeners; the numbers given in parentheses indicate the level of chlorination). Combined, these congeners accounted for more than 40% of the total PCB load.

This study confirms previously published reports which indicate that the less lipophilic congeners reach equilibrium concentrations, during both uptake and depuration, at faster rates than the more lipophilic compounds (Ellegehausen *et al.*, 1980; Bruggeman *et al.*, 1981; Tanabe *et al.*, 1987a). For example, there is an initial enrichment of the lighter PCB fraction, e.g. congeners 95 and 52, in HRSC oysters. The concentrations of these congeners, however, reached constant concentrations while the concentrations of the more lipophilic congeners, e.g. 101 and 118, continued increasing. If the oysters were allowed enough time, the final PCB distribution in HRSC oysters would probably have approximated the distribution observed in SC oysters. Despite the differences observed in equilibration rates of the various congeners, the composition of PCB homologs, in both oyster populations, were largely dominated by penta-, tetra- and hexachloro-biphenyls and had low concentrations of octa-, nona- and decachloro-biphenyls.

The dominant PCB congeners and homolog distribution encountered in newly and chronically contaminated oysters at the end of the uptake period of this study are similar to those reported for benthic invertebrates (*Macoma balthica* and *Arenicola marina*) and

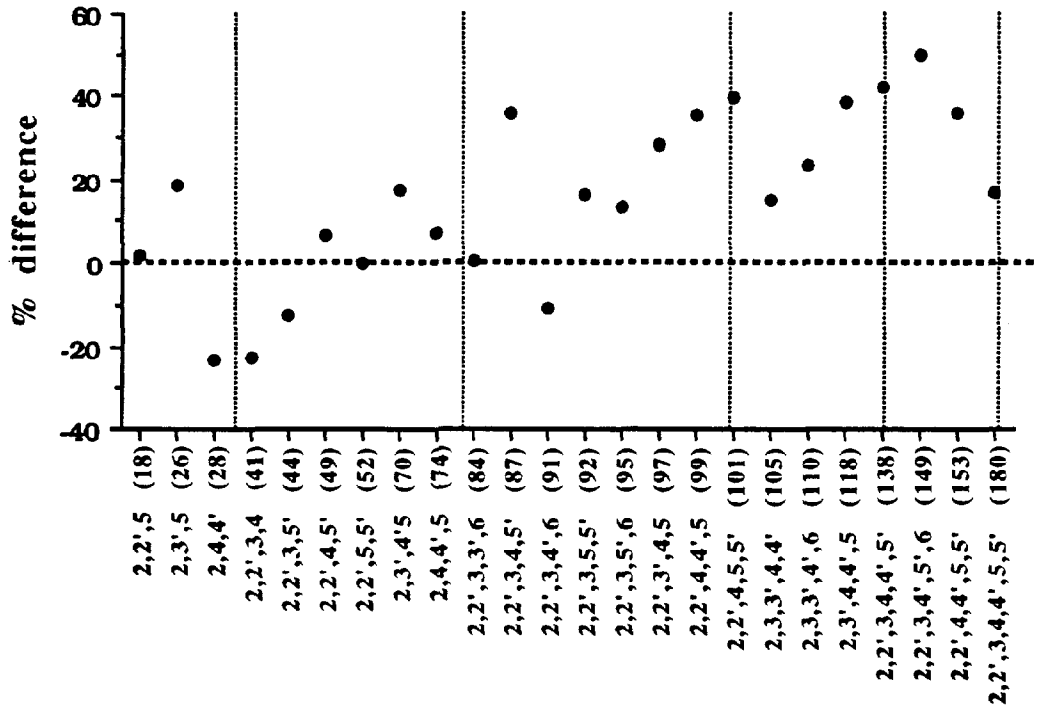


Fig. 20. Percent diferences in concentrations of selected polychlorinated biphenyls between Hanna Reef and Ship Channel oyster tissues at the end of the 48-day exposure period. Positive values indicate congeners with greater accumulation in Ship Channel oysters.

sediments from the Dutch Wadden Sea where 101, 118, 138, 149, 153, 180 and 187, and 15, 18, 28, 118, 138, 153, and 187 were the dominant PCB congeners, respectively (Duinker *et al.*, 1983). Dominant PCB congeners in benthic polychaetes (*Nephtys* spp.) from the southern North Sea were 118, 138, 149, 153 and 180, while in sediments the highest concentrations corresponded to congeners 15, 18, 118, 138 and 153 (Boon *et al.*, 1985). Recently, Niimi & Oliver (1989) reported that the 10 most common congeners detected in trout and salmon from Lake Ontario were 101, 84, 118, 110, 87/97, 153, 138, 149 and 180.

PCB congeners in Ship Channel sediments were dominated by pentachloro-biphenyls and, to a lesser extent, by hexa- and tetrachloro-biphenyls (Fig. 21). Combined, these three homologs represented more than 90% of the total sedimentary PCB load. Dominant PCB congeners in sediments were 110(5)/77(4), 138(6)/160(6), 101(5)/90(5), 153(6)/132(6) and 52(4). Each of these compounds accounted for more than 5% of the total PCB load in the average sediment sample. This sedimentary PCB distribution is similar to the distribution profiles encountered in HRSC and SC oysters.

Comparatively, PCB concentrations measured in water samples were significantly lower (Fig. 21). The homolog PCB group with six chlorines represents the largest portion of total PCBs in water, mainly because of the relatively high concentrations of PCB 138 and 153.

Depuration of PCBs by Newly and Chronically Contaminated Oysters

When relocated to the Hanna Reef area, Hanna Reef and Ship Channel oysters showed statistically significant depuration of total PCBs. Tables A-8 and A-9 (Appendix) list the average concentrations of predominant PCB congeners in HRSCHR and SCHR oysters. Also listed are the average concentrations encountered in Hanna Reef sediments. Total PCB concentration decreased from 830 to 380 ng g^{-1} and from 1,100 to 730 ng g^{-1}

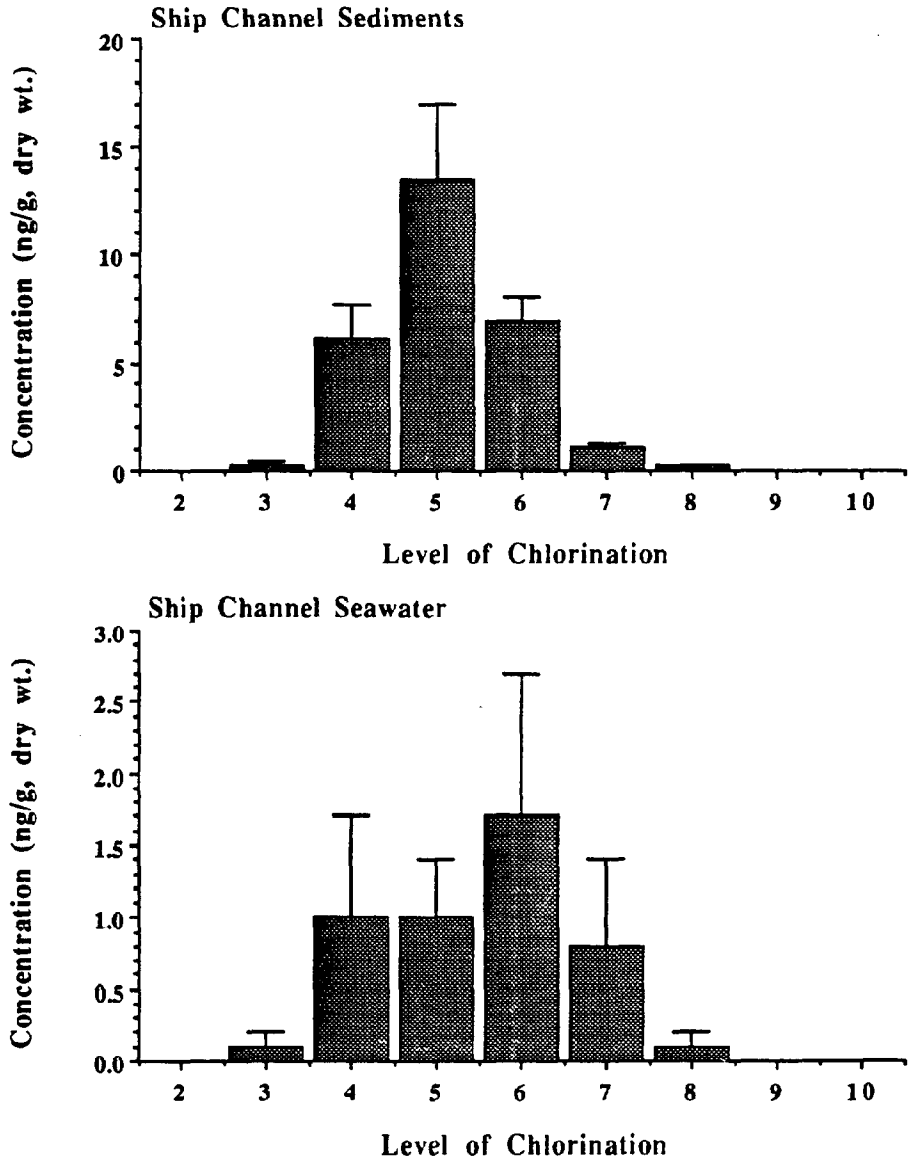


Fig. 21. Concentrations of polychlorinated biphenyls, grouped by level of chlorination, in Ship Channel sediment and seawater samples.

in HRSCHR and SCHR oysters, respectively, after seven weeks at the Hanna Reef location.

The concentrations of PCBs, grouped by level of chlorination, in both oyster populations at different stages during the 50 days depuration period are shown in Fig. 22. Different PCB congeners were depurated at different rates by SCHR and HRSCHR oysters. Also, a marked decrease in the depuration efficiencies of the bioaccumulated homologs with increasing number of substituted chlorines was observed in both groups of oysters. For example, three-, four-, five- and six-chlorine substituted homologs decreased 80, 70, 47, 20%, in HRSCHR oysters, and 73, 50, 24, 17%, in SCHR individuals, respectively. This differential depuration of the accumulated PCBs can be observed in Fig. 23 where the concentrations of selected PCB congeners in HRSCHR and SCHR oysters at the end of the depuration period are shown. This retention of the highly lipophilic congeners was more evident in chronically contaminated oysters.

Because of the incomplete depuration, the total PCB concentration in Hanna Reef oysters, after 50 days, remained one order of magnitude higher than the original levels (380 ng g^{-1} versus 30 ng g^{-1}). The concentrations of homologs and selected PCB congeners in Hanna Reef oysters before the transplantation to the polluted Ship Channel site and 50 days after their relocation to the Hanna Reef area are shown in Fig. 24. The distribution of PCBs in originally uncontaminated, i.e. HR, oysters shows a relative predominance of five- > four- > six-chlorine substituted homologs whereas the predominant homologs in HRSCHR oysters were those having five, six and four chlorines.

Depuration of PCBs by HRSCHR and HRSC oysters were approximately exponential (Fig. 18). The clearance rates for high molecular weight PCBs were significantly slower in both oyster populations. Transplanted HRSCHR oysters depurated most of the recently incorporated PCB congeners at a faster rate than SCHR oysters. Detailed

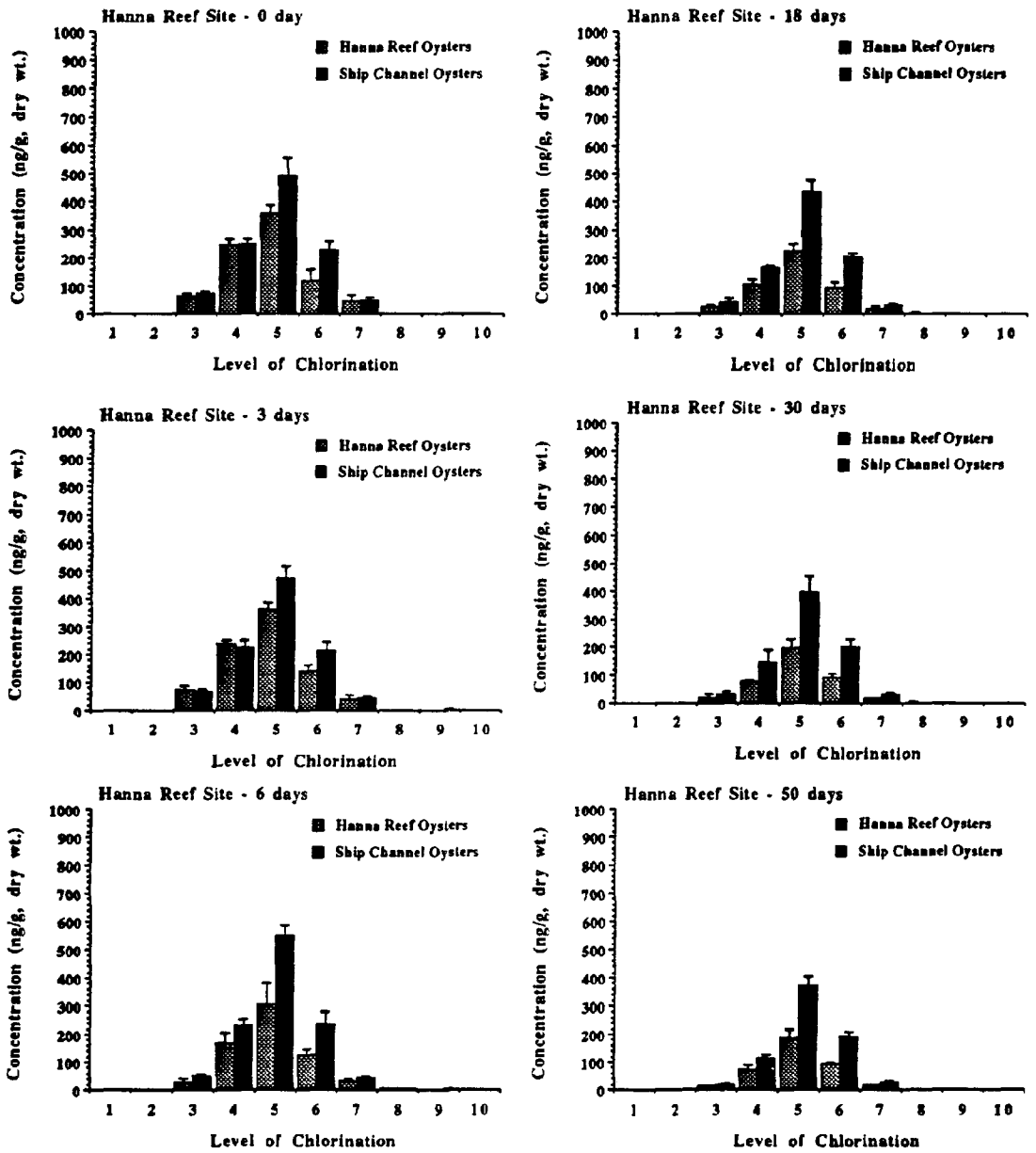


Fig. 22. Concentrations of polychlorinated biphenyl congeners, grouped by level of chlorination, in back-transplanted Hanna Reef and transplanted Ship Channel oysters during the 50-day depuration period in the Hanna Reef area.

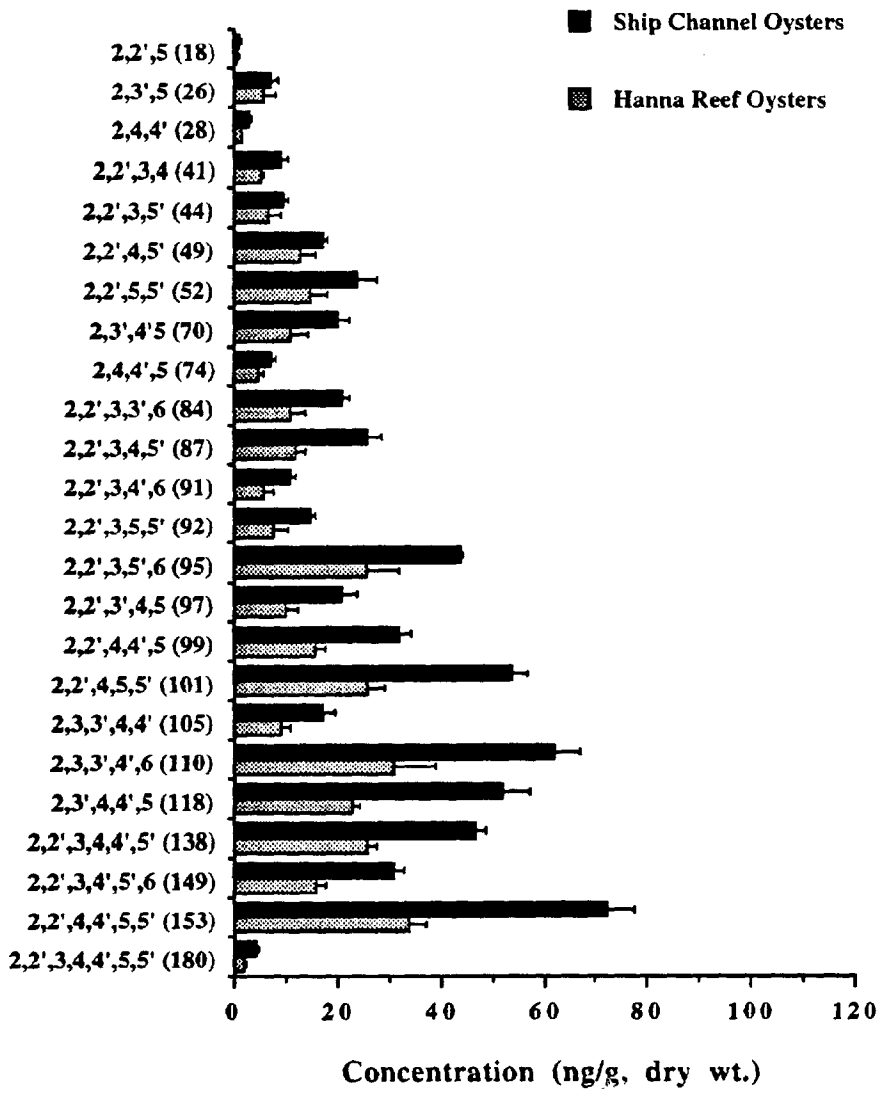


Fig. 23. Concentrations of selected polychlorinated biphenyl congeners in tissues of Hanna Reef and Ship Channel oysters at the end of the 50-day depuration period.

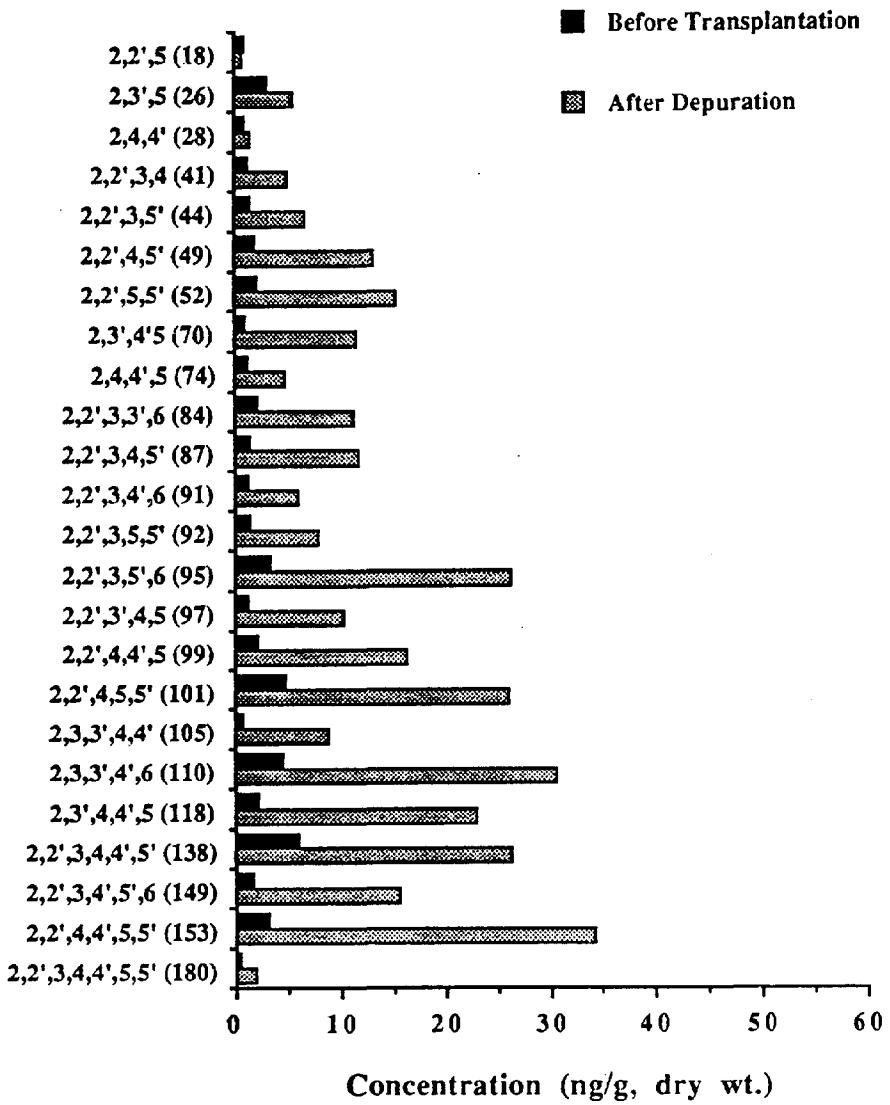


Fig. 24. Comparison of the concentrations of selected polychlorinated biphenyl congeners measured in tissues of Hanna Reef oysters before exposure to the Ship Channel contaminant levels and after depuration at the Hanna Reef site.

discussion of the PCB biological half-lives and related kinetic parameters are presented in Chapter VI. The estimated half-lives of selected PCB congeners in Hanna Reef and Ship Channel oysters are listed in Table 4 for comparison purposes. Calculated PCB biological half-lives ranged from 14 to 200 days in Hanna Reef oysters and from 18 to 595 days in Ship Channel oysters. Similarly to previous studies, the biological half-lives of PCB congeners increased with the number of chlorine atoms in the biphenyl rings. With the exception of the values reported by Tanabe *et al.* (1987a), the estimated half-lives for different PCB congeners during this study were comparable to most of the values previously reported for a number of different organisms. In Tanabe's study, most of the PCB congeners were depurated with extremely short half-lives, i.e. less than 10 days.

The average homolog concentrations in Hanna reef sediments (Fig. 25) were one order of magnitude lower than the levels encountered in the Ship Channel area. Comparing sediment samples from the Ship Channel area to the Hanna Reef location, it is possible to observe some differences in the relative contribution of the different homologs to the total sedimentary PCB load. While the average PCB distribution in Ship Channel sediments is largely dominated by pentachlorobiphenyls, sediment samples from the Hanna Reef area show a slight predominance of hexachlorobiphenyls.

CONCLUDING REMARKS

Low molecular weight PCB congeners, i.e. those substituted with two, three and four chlorines, were rapidly accumulated by transplanted oysters to final concentrations that were not statistically differentiable from the concentrations encountered in indigenous oysters. In most cases, these concentrations were reached in 30 days. Comparatively, the bioaccumulation of higher molecular weight PCB congeners was much slower. As a consequence of this slower uptake rate, the high molecular weight PCB congeners did not

TABLE 4

Biological Half-Lives (Days) of PCBs in Hanna Reef and Ship Channel *Crassostrea virginica* Oysters.

Congener	Hanna Reef oysters	Ship Channel oysters	Pruell *et al* (1986) mussels	Tanabe *et al* (1987a) mussels	Bruggeman et al. (1981) fish
2,2',5 (18)	14	19	-	6	14
2,3',5 (26)	22	22	-	-	-
2,4,4' (28)	17	34	16	7	-
2,2',3,3' (40)	14	18	-	4	-
2,2',3,4 (41)	23	55	-	5	-
2,2',3,5' (44)	27	45	-	6	-
2,2',4,5' (49)	39	61	-	5	-
2,2',5,5' (52)	27	45	-	6	46
2,3',4',5 (70)	30	58	-	6	69
2,4,4',5 (74)	30	47	-	7	-
2,2',3,3',6 (84)	37	80	-	6	-
2,2',3,4,5'/2,3,4,4',6 (87/115)	55	132	-	5	-
2,2',3,4',6 (91)	25	50	-	5	-
2,2',3,5,5' (92)	31	63	-	6	-
2,2',3',5',6 (95)	45	95	-	5	-
2,2',4,4',5 (99)	49	91	-	6	-
2,2',4,5,5'/2,2',3,4',5 (101/90)	55	116	28	6	-
2,3,3',4,4' (105)	63	120	-	6	-
2,3,3',4',5 (107)	30	46	-	-	-
2,3,3',4',6/3,3',4,4' (110/77)	45	103	-	6	-
2,3',4,4',5 (118)	73	299	-	7	-
2,2',3,3',4,4' (128)	76	229	37	7	-
2,2',3,4,4',5'/2,3,3',4,5,6 (138/160)	200	595	-	8	-
2,2',3,4',5,5' (146)	111	239	-	-	-
2,2',3,4',5',6/2',3,4,4',5 (149/123)	130	439	-	7	-
2,2',4,4',5,5'/2,2',3,3',4,6' (153/132)	51	102	46	9	-
2,2',3,3',4',5,6 (177)	52	145	-	11	-
2,2',3,3',5,5',6 (178)	52	91	-	8	-
2,2',3,4,4',5,5' (180)	50	142	-	7	-
2,2',3,4',5,5',6 (187)	70	258	-	10	-

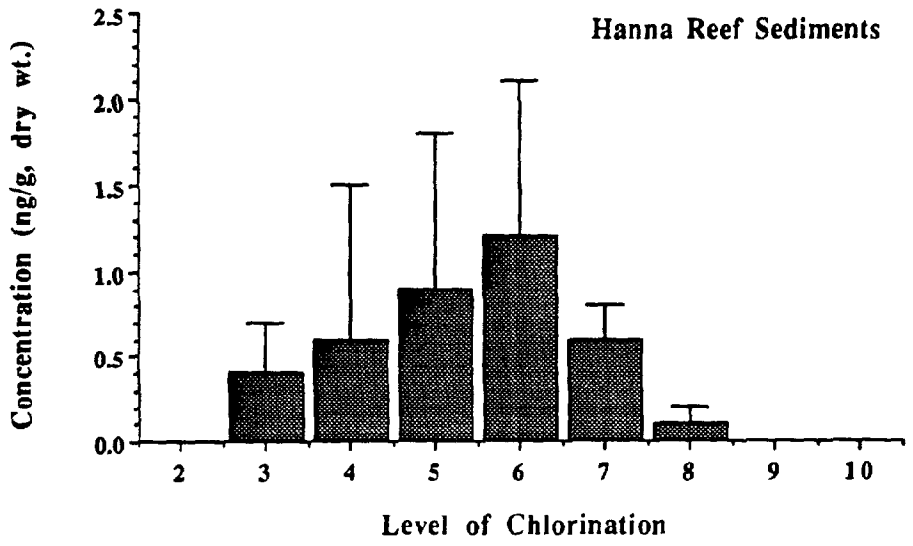


Fig. 25. Concentrations of polychlorinated biphenyls, grouped by level of chlorination, in Hanna Reef sediment samples.

attain equilibrium concentration by the end of the exposure period in this study and statistically significant differences were evident between SC and HRSC oysters. In general, the higher the number of chlorines substituted in the biphenyl molecule, the larger the difference between the concentrations found in HRSC and SC oysters. In spite of their lower uptake rates, pentachlorobiphenyls were the PCBs accumulated to the highest concentrations in HRSC and SC oysters. In comparison, practically no congeners having eight, nine or ten chlorines were accumulated by either oyster group. At the end of the seven-week exposure period, the final distribution profiles of PCB homologs and individual congeners in both transplanted (HRSC) and indigenous (SC) oysters were similar to the profile encountered in sediment samples collected in the Ship Channel area.

When transplanted to the Hanna Reef location, both groups of oysters (i.e. HRSCHR and SCHR) depurated the low molecular weight congeners at faster rates than the clearance rates observed for the heavier PCBs. However, individual tetra- and pentachlorobiphenyl congeners were depurated at a faster rate by HRSCHR than by SCHR oysters. The concentration at the end of the 50-day depuration period measured in HRSCHR oysters was about one order of magnitude higher than the original level. In both groups of oysters, the depuration efficiency decreased with the increasing number of substituted chlorines in the biphenyl rings. This observed decrease in the clearance efficiency is reflected in the estimated half-lives. In general, the less lipophilic congeners reach equilibrium concentrations, during both uptake and depuration, at faster rates than the most liphophilic PCB congeners.

CHAPTER IV

UPTAKE AND DEPURATION OF PLANAR PCB CONGENERS BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA):A SPECIAL CASE OF PCBs

INTRODUCTION

One of the objectives of this study was to evaluate the bioaccumulation of the highly toxic planar PCB congeners, i.e. PCBs 77, 126 and 169, by bivalves under environmental conditions. Most of the effort, however, was dedicated to the development of a reliable technique for the isolation of non-*ortho* substituted tetra-, penta- and hexachlorobiphenyl congeners that could be coupled to the existing cleanup procedures in the laboratory.

This chapter serves two purposes. First, a new method for the isolation of the three most toxic planar PCB congeners is presented and evaluated. As compared to previously published methods for planar PCB analysis, this methodology saves both time and materials, i.e. solvents, and eliminated the use of benzene, a highly carcinogenic solvent, that requires extreme care in handling by the analyst. Second, the uptake and depuration of these planar PCB congeners by transplanted oysters in Galveston Bay are determined and discussed.

PLANAR PCBs: A REVIEW

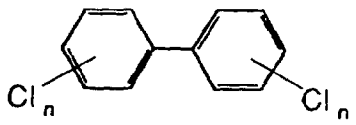
Background Information

Of the 209 possible PCB congeners, only 20 have non-*ortho* chlorine substitutions in the biphenyl rings. Some of these congeners can attain planarity, which makes them sterically similar to the highly toxic dibenzo-p-dioxins and dibenzofurans (McKinney *et al.*, 1976, 1985; Hansen, 1987; McFarland & Clarke, 1989). Particularly important within this group are the PCBs with no *ortho*, two *para* and at least two *meta* chlorines. 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), shown in Fig. 26, 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 (Goldstein & Safe, 1989; Poland & Knutson, 1982; Safe, 1984, 1986, 1990; Tanabe, 1988). These planar PCBs are the most potent pure 3-methylcholanthrene-type (3-MC-type) inducer congeners. Some studies have indicated that not only non-*ortho* chlorine substituted PCBs but also some mono- and di-*ortho* analogs of planar PCBs possess similar toxic potential (e.g. Robertson *et al.*, 1984; Safe, 1985; Bryan *et al.*, 1987; Hansen, 1987; Olafsson *et al.*, 1987; Tanabe *et al.*, 1987c; McFarland & Clarke, 1989).

In a recent review, Safe (1990) discussed the environmental and mechanistic considerations behind the development of the Toxic Equivalent Factor (TEF) concept for different PCBs. Safe proposed provisional TEF values of 0.01, 0.1 and 0.05 for planar congeners 77, 126 and 169, respectively. Recently, the validation and limitations of these factors have been reported (Safe, 1992).

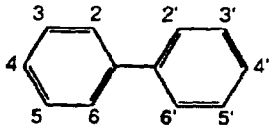
PCBs

General Formula:

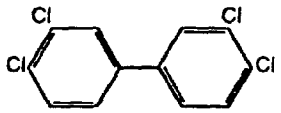


$C_{12}H_{10-n}Cl_n$
(n = 1 to 10)

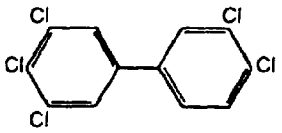
Nomenclature:



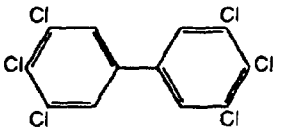
Planar Congeners:



PCB #77

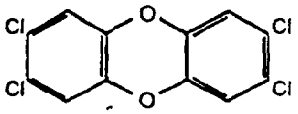


PCB #126

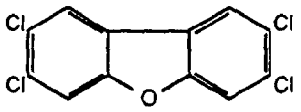


PCB #169

Related Toxic Compounds:



2,3,7,8-Tetrachlorodibenzo-p.dioxin



2,3,7,8-Tetrachlorodibenzofuran

Fig. 26. General formula of polychlorinated biphenyls. Three of the most toxic planar PCB congeners, i.e. PCB 77, 126 and 169, are shown together with the compounds they mimic in toxic effects.

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), monitoring these compounds is needed because of their high toxicity. However, quantitation of individual non-*ortho* substituted PCB congeners is very difficult because of their extremely low concentrations. Routine high-resolution capillary gas chromatography analyses fails to separate some of these planar PCBs from other *ortho*-PCB congeners, although this separation can now be achieved with more expensive and complicated techniques such as multidimensional gas chromatography (Duinker *et al.*, 1988a).

During the last decade, a wide variety of different methodologies have been reported for the separation of individual PCBs, according to the number of chlorines in the *ortho* positions, using different adsorbents, such as. florisil and activated carbon. In general, the existing methods for the separation of planar PCBs from other congeners use an extremely large volume of eluant per sample, i.e. over 1000 ml (e.g. Huckins *et al.*, 1980; Stalling *et al.*, 1980), involve a carcinogenic solvent, i.e. benzene, (e.g. Tanabe *et al.*, 1987; Hong & Bush, 1990; Kuehl *et al.*, 1991, or are extremely complicated for routine analysis (e.g. Smith *et al.*, 1984, Patterson Jr. *et al.*, 1989).

Distribution and Occurrence in Galveston Bay

Although PCB congeners have been widely reported in Galveston Bay samples (Table 3) and have been one of the most commonly found chlorinated compounds in oyster samples from Galveston Bay (Sericano *et al*, 1990a), the occurrence of planar PCB congeners in this area have not, until recently, been reported (Sericano *et al.*, 1992).

This study, which is discussed in greater details in Chapter VIII, reports the occurrence of three highly toxic PCB congeners (PCBs 77, 126 and 169) in oysters *(Crassostrea virginica)* from different locations in Galveston Bay using a newly developed

carbon chromatographic method (Sericano *et al.*, 1991). The highest concentrations of planar PCB congeners in Galveston Bay were reported in samples collected near the area where the Houston Ship Channel enters the upper Galveston Bay (2,000, 2,200 and 790 pg g^{-1} for congeners 77, 126 and 169, respectively) and decreased seaward. The second highest concentrations were encountered in samples from near the city of Galveston (500, 400 and 93 pg g^{-1} for congeners 77, 126 and 169, respectively). The lowest concentrations were measured in samples collected near Hanna Reef in East Bay (89, 110 and 89 pg g^{-1} for congeners 77, 126 and 169, respectively). The general distribution of planar PCB congeners in Galveston Bay clearly correlates high concentrations with highly populated areas. The same correlation between urban centers and concentrations was observed in Tampa Bay (Sericano *et al.*, 1992).

Bivalve Uptake and Depuration Studies

The number of studies reporting the uptake and depuration of PCBs by different bivalves is limited. Even more limited is the number of studies reporting the uptake, persistency and release of highly toxic planar PCB congeners. In one of the first reports regarding the bioconcentration of planar PCB congeners in lower aquatic organisms, e.g. Green-lipped mussels (*Perna viridis Linnaeus*) and possible transfer through food chain to higher animals, it was concluded that these compounds are highly bioaccumulated by lower organisms and, because of their persistence, they may reach higher consumers, including humans, in quantities of toxicological concern (Kannan *et al.*, 1989).

UPTAKE AND DEPURATION OF PLANAR PCBs

Experimental Design, Sample Collection and Methods

The experimental design and sample collection used for the study of planar PCBs were the same as those discussed for PAHs (Chapter II).

Extraction and initial sample fractionation

The extraction, initial fractionation and cleanup of planar PCBs were done simultaneously with the rest of the *ortho*-substituted PCBs. After the final extract concentration to 1 ml, and before the addition of the GC internal standard for GC-ECD analysis, a 250 µl fraction was reserved for the analysis of planar PCB congeners. Before proceeding to the next step, PCB 81 was added to the extracts as an internal standard.

Isolation of planar PCB congeners

The methodology to analyze planar PCBs in transplanted oyster tissues is published elsewhere (Sericano *et al.*, 1991). Glass chromatographic columns (10 mm i.d.) were packed in methylene chloride. Two g 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 column. 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 *ortho* unsubstituted 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 m long x 0.25 mm i.d. with 0.25 µm 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 before 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 the carrier gas at a flow velocity of 30.0 cm sec^{-1} at 100°C. Nitrogen or argon/ methane (95:5) were used as the make-up gas at a flow rate of 20 ml min^{-1}. The volume injected was 2 µl. Planar PCBs were quantitated against a set of authentic standards that were injected at four different known concentrations to calibrate the instrument and to compensate for a non-linear response of the electron capture detector. Tetrachloro-m-xylene (TCMX) was used as the GC internal standard to estimate the recoveries of the internal standards. The detection limits for organochlorines and individual PCB congeners, calculated on the basis of 15 g (wet weight) oyster tissue sample size with 0.2% by volume of the extract injected into the GC-ECD, was 0.05 ng g^{-1} dry weight.

Planar PCB Congener Analysis

Activated carbon has been previously used to separate chlorinated compounds based on the degree of chlorination as well as molecular planarity (e.g. Jensen & Sundström, 1974; Stalling *et al.*, 1980). In the case of PCBs, for example, the planar structure is

related to the number of chlorines in the *ortho* positions. Based on the high surface area of activated carbon and its selective adsorptive capacity of planar structures, this adsorbent can be successfully used to isolate planar PCB congeners having four or more chlorines in *meta* and *para* positions. Although PCB congeners with a decreasing number of *ortho* substituted chlorines were differentially retained in the column (Stalling *et al.*, 1980), all the PCBs with at least one *ortho* chlorine were eluted by the first two solvent mixtures and collected in one fraction. The mixture of 1 part of AX-21 activated carbon and 20 parts of LPS-2 silica gel was relatively easy to pack and use.

The efficiency of the column was initially checked with a mixture of PCBs, Aroclor 1254 (5,000 ng ml^{-1}), spiked with the four planar PCB congeners. These analytes were added in triplicate to the Aroclor mixture at three different concentrations (20, 50 and 100 ng ml^{-1}). Fig. 27 shows the chromatograms of spiked Aroclor 1254 (a), PCB congeners recovered in the first fraction, i.e. 50 ml of 1:4 methylene chloride and cyclohexane followed by 30 ml of 9:1 methylene chloride and toluene (b), and planar PCBs eluted in the second fraction, i.e. 40 ml of toluene (c). Recoveries of planar PCB congeners are reported in Table 5. Recoveries for the three highly toxic planar PCB congeners were above 90%, whereas that for PCB 81 was slightly lower. Recoveries in the first fraction of PCB congeners having one to four chlorines in the *ortho-ortho'* positions were, in all cases, near 100%.

To investigate the efficiency of the column with environmental samples with high lipid concentrations, dolphin blubber extracts were spiked with the same four planar PCB congeners at a concentration of 50 ng g^{-1} each. Total PCB concentration in the dolphin blubber was 3,700 ng g^{-1}. Fig. 28 shows the chromatograms of the spiked dolphin blubber sample (a) as well as the *ortho*- and non-*ortho*chlorine substituted PCB congeners recovered in the first and second fractions, b and c respectively. Also, these planar PCB congeners were isolated from other organochlorine compounds present in the blubber

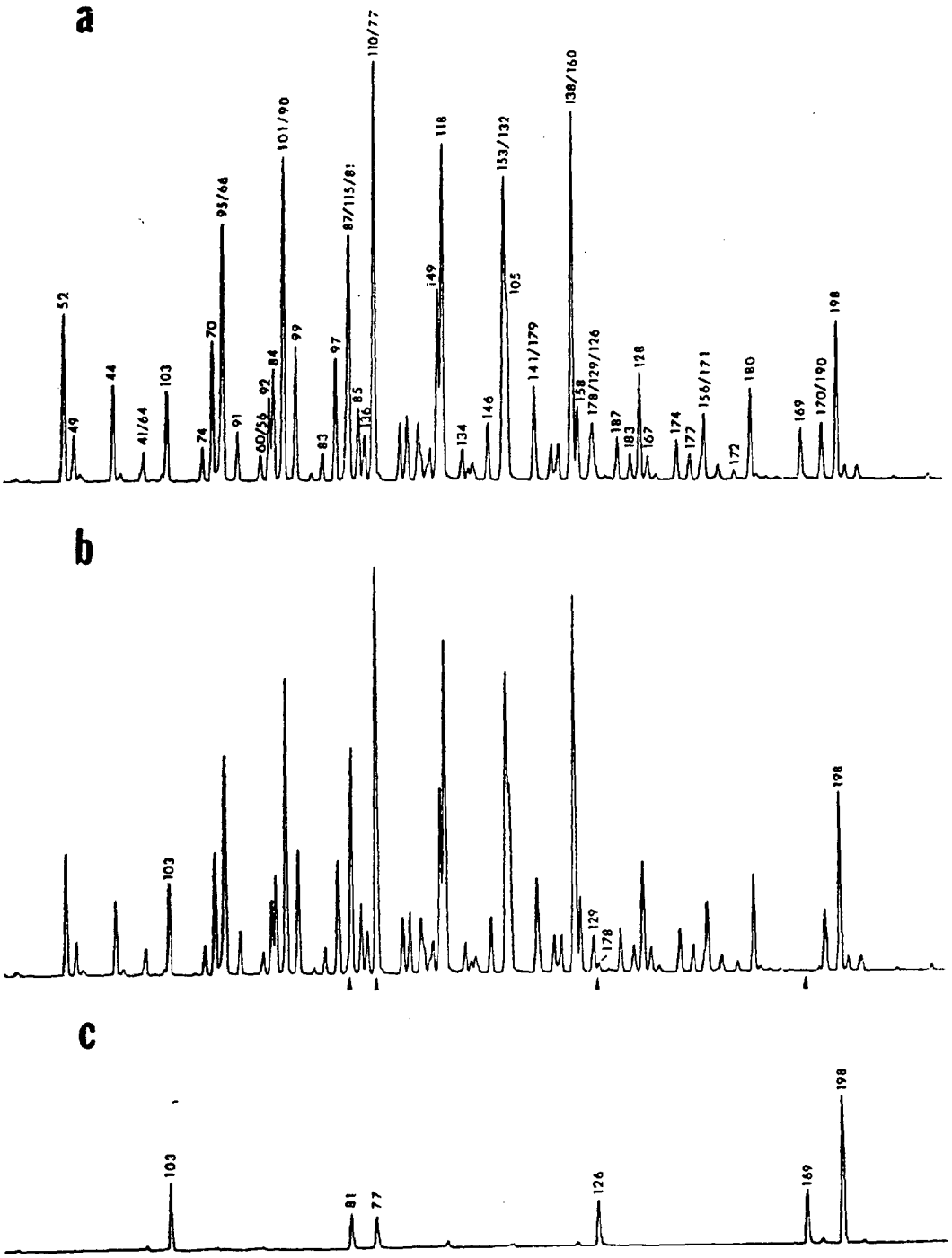


Fig. 27. High-resolution gas chromatographic analyses of (a) Aroclor 1254 spiked with planar PCB congeners (i.e., 77, 81, 126 and 169), (b) PCB congeners recovered in Fraction 1, and (c) planar PCB congeners eluted in Fraction 2. PCB congeners 103 and 198 are external standards. PCB congeners are numbered according to Ballschmitter & Zell, 1980.

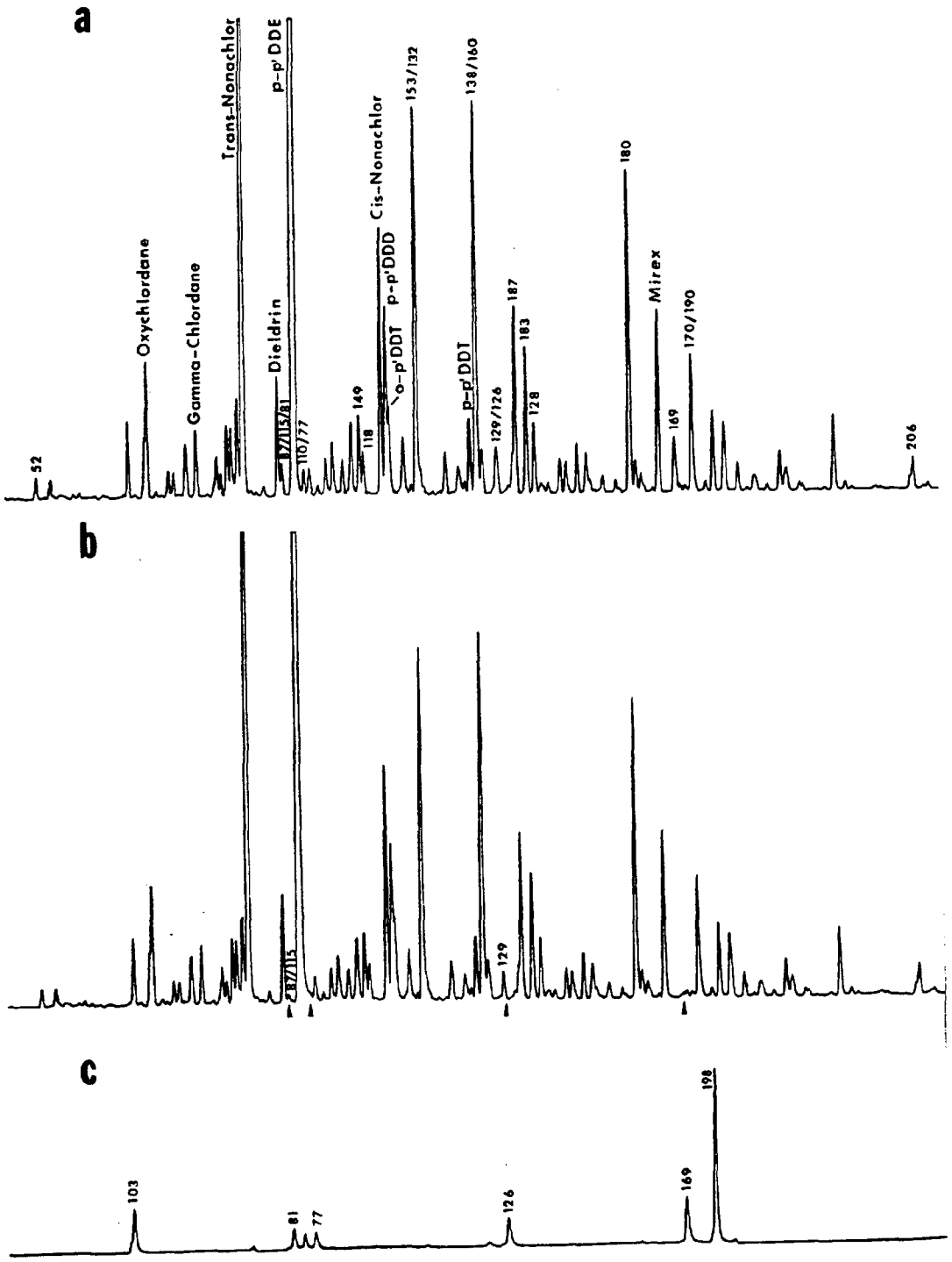


Fig. 28. High-resolution gas chromatographic analyses of (a) dolphin blubber extract spiked with planar PCB congeners 77, 81, 126 and 169, (b) chlorinated hydrocarbons recovered in Fraction 1, and (c) planar PCB congeners eluted in Fraction 2. PCB congeners are numbered according to Ballschmitter & Zell, 1980.

extract, i.e. chlordane-related compounds and DDT and its metabolites DDD and DDE. Spiked dolphin blubber samples also had excellent recoveries for these PCB congeners, comparable to those calculated for the spiked Aroclor mixture (Table 5). Recoveries for all the chlorinated hydrocarbons originally present in the dolphin blubber sample were close to 100%. Only a negligible concentration of *p-p'* DDE, detected in the original sample at a very high concentration (1,450 ng g^{-1}) was present in the final fraction (Fig. 28c).

Overall, this method yielded higher or similar recoveries for PCB congeners 77, 126 and 169 than those reported by Kamops *et al.* (1979), Huckins *et al.* (1980), Smith *et al.* (1984), Tanabe *et al.* (1987) and Hong & Bush (1990) at comparable concentrations using either florisil or carbon chromatography on pure standard solutions or spiked samples.

Uptake of Planar PCBs by Transplanted Oysters

Ship Channel, Hanna Reef, transplanted Hanna Reef-to-Ship Channel, transplanted Ship Channel-to-Hanna Reef and relocated Hanna Reef-to-Ship Channel-back to-Hanna Reef oysters are refered as SC, HR, HRSC, SCHR and HRSCHR oysters, respectively, in this and the following sections.

Concentrations of planar congeners in transplanted HRSC oysters were encountered at very low concentrations, e.g. parts per trillion (pg g^{-1}) to parts per billion (ng g^{-1}). The lowest concentrations corresponded to congener 3,3',4,4',5,5' (169), which was present at concentrations near or below the detection limits. Fig. 29 illustrates both the applicability of this technique to real environmentally contaminated samples and the difficulties involved in the analysis of planar PCBs because of their extremely low concentrations. The chromatograms correspond to an extract of indigenous SC oysters. Fig. 29a shows the *ortho* substituted PCBs eluted in the first fraction with the two

TABLE 5

Recoveries of Four Planar PCB Congeners from Spiked Aroclor 1254 and Dolphin Blubber Samples Using Activated Carbon:Silica Columns.

PCB congeners	Aroclor 1254			Blubber	Average
	Level I	Level II	Level III		
	20 ng ml^{-1}	50 ng ml^{-1}	100 ng ml^{-1}	50 ng g^{-1}	
3,4,4',5 (81)	85±1.8	82±8.6	79±4.0	82±5.4	70±2.3
3,3',4,4' (77)	100±4.8	94±9.0	90±4.1	94±7.2	87±4.0
3,3',4,4',5 (126)	90.±0.6	96±5.5	94±2.1	93±3.9	91±2.8
3,3',4,4',5,5' (169)	94±1.0	96±3.4	97±1.1	96±2.5	97±2.3

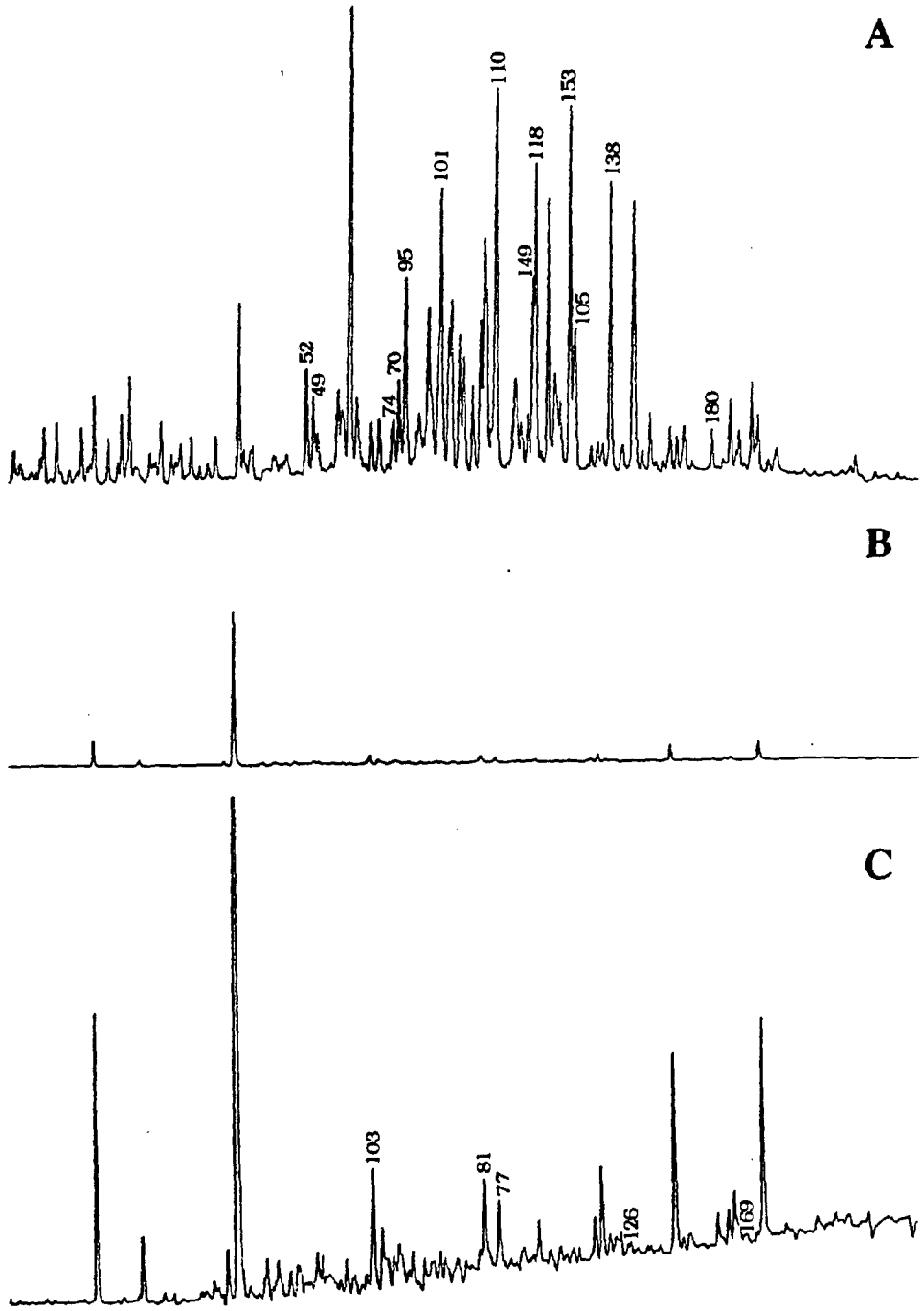


Fig. 29. Example of high-resolution gas chromatograms obtained from an extract of indigenous Ship Channel oysters. PCB congeners are numbered according to Ballschmitter & Zell, 1980.

different solvent mixtures. Fig. 29b shows, for comparison, the planar congener fraction, i.e. second fraction, at the same magnification as the chromatogram corresponding to the first fraction and Fig. 29c shows the second fraction magnified 20 times.

Both, 3,3',4,4' tetraCB (77) and 3,3',4,4',5 pentaCB (126) exhibit fairly well-defined uptake and depuration curves. Fig. 30 shows the concentrations of PCB congeners 77 and 126 versus time during the uptake and depuration periods. The concentrations of these two planar PCB congeners in HRSC oysters increased over the seven week exposure period. PCB congener 77 reached a concentration similar to that encountered in indigenous SC oysters within a month. The uptake of congener 126 was slower and only approximated the concentration of SC oysters by the end of the exposure period. Contrasting with planar PCBs 77 and 126, it was not possible to observe a clear trend in the concentration of congener 169 versus time. This is mainly because of its extremely low concentration.

The final concentrations of the accumulated congeners decreased as the number of chlorines substituted in the biphenyl rings increased. This trend was also reported in transplanted green-lipped mussels (*Perna viridis Linnaeus*) during an exposure experiment in Hong-Kong waters (Kannan *et al.*, 1989). Kannan *et al.* (1987) reported the concentrations of these planar congeners in different commercial PCB mixtures. In general, congener 77 is 1-2 and 3-5 orders of magnitude higher than congeners 126 and 169, respectively. Comparing these relative concentrations with those observed in transplanted oyster samples, it appears that congeners 126 and 169 in oyster tissues were enriched with respect to congener 77. The same observation was made by Kannan *et al.* (1989). This is not surprising since the log K_{OW} (octanol-to-water coefficient) increases with the number of chlorines substituted in the biphenyl rings (6.36, 6.89 and 7.42 for congeners 77, 126 and 169, respectively; Hawker & Connell, 1988). In general,

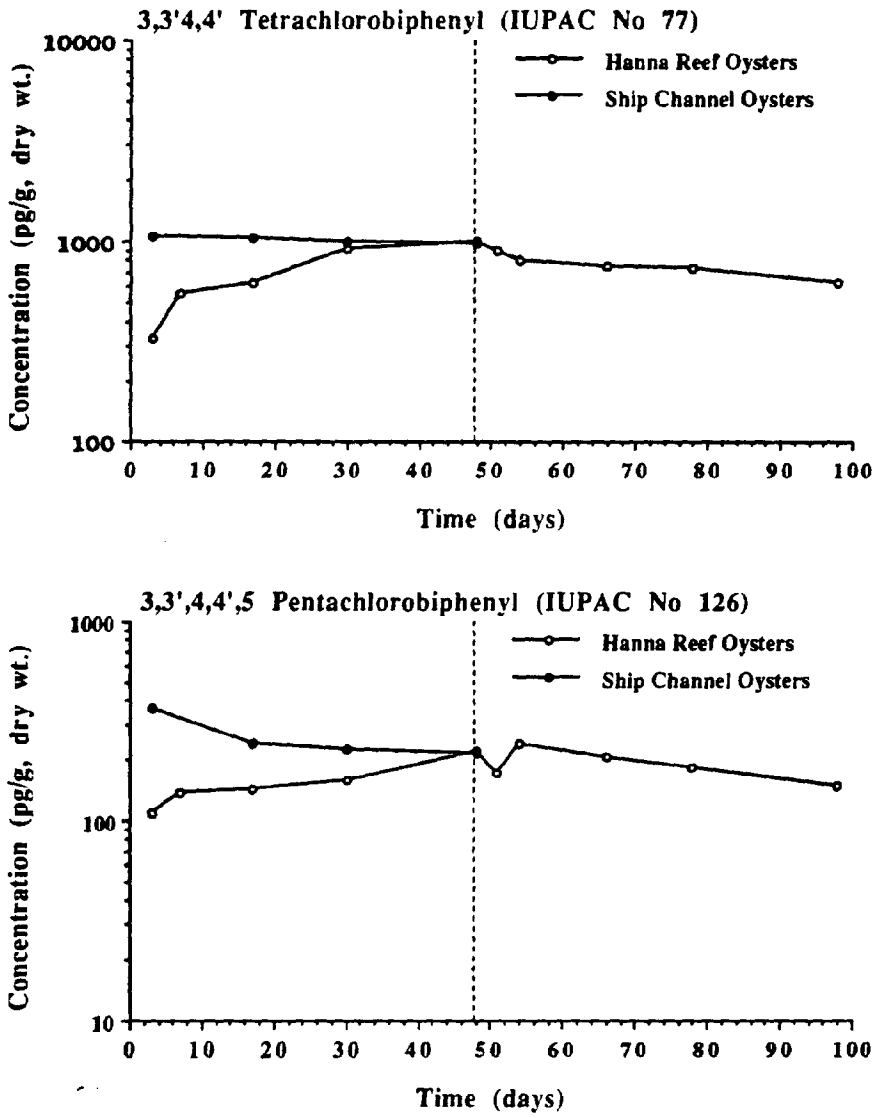


Fig. 30. Concentrations of planar polychlorinated biphenyl congeners 77 and 126 in tissues of Hanna Reef oysters during the uptake and depuration phases of the transplantation experiments at Galveston Bay.

concentrations for congener 77 in oyster tissue were 3-5 and 10-12 times higher than those measured for congeners 126 and 169, respectively.

Depuration of Planar PCBs by Newly Contaminated Oysters

When transplanted to the Hanna Reef area, exposed oysters slowly depurated the concentrated planar congeners. These PCBs were still present at high concentrations, relative to original HR oysters, 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 Linnaeus*), at the end of the exposure period (32 days), were substantially higher than those found in native individuals.

Depuration of congener 77 was comparatively faster than the clearance rate observed for congener 126. Calculation of half-lives and related kinetic parameters of these trace organic pollutants will be discussed in greater details in Chapter VI. For comparison purposes, the estimated biological half-lives of these toxic PCBs were 88 and 107 days for congeners 77 and 126, respectively. These estimated values were significantly higher than those reported by Kannan *et al.* (1989) for mussels (9 and 13 days, respectively). However, it must be noted that, as previously discussed in Chapter III, all the biological half-lives reported for different PCB congeners in that transplantation study (i.e. Tanabe *et al.*, 1987a; Kannan *et al.*, 1989) were significantly lower than the estimated half-lives during this study and previous reports involving bivalves as well as many other organisms (Table 4). The estimated biological half-lives for tetra- and pentachloro substituted PCB congeners during this study were in the 14 to 39 and 25 to 73 days ranges, respectively (Chapter VI, Table VII). It is clear that, compared to other *ortho*-substituted congeners with the same number of chlorine per molucule, planar PCBs are removed more slowly from the lipid pool of oysters. The same observation was reported

for transplanted green-lipped mussels (*Perna viridis Linnaeus*) in Hong-Kong (Kannan *et al.*, 1989).

CONCLUDING REMARKS

A simple, sensitive, precise and specific method for the isolation of planar PCB congeners, with four or more chlorines in non-*ortho* positions, from other PCBs in environmental samples was developed for this study. This method, which can easily be coupled to existing cleanup procedures in most environmental laboratories currently involved in the high-resolution gas chromatographic analysis of PCBs, yields acceptable recoveries of these PCB congeners. Compared to other methods, this methodology saves both time and materials, i.e. solvents, and is safer for the analyst and the environment.

Two of the most toxic planar PCB congeners, i.e. congeners 77 and 126, were biococentrated by transplanted oysters during the seven-week exposure period. Congener 77 attained an equilibrium concentration with the indigenous oysters in a shorter period of time than congener 126. Because of the low concentrations, it was not possible to observe a clear trend in the uptake of PCB congener 169.

When newly contaminated oysters were transplanted back to the Hanna Reef area, they depurated both 77 and 126 planar PCB congeners; however, the estimated depuration half-lives were significantly longer than those corresponding to non-planar PCBs with the same number of chlorines substituted in the biphenyl molecule. Also, the final concentrations of these planar PCB congeners in HRSCHR oysters at the end of the depuration phase of this experiment remained relatively high. Because of their toxicity and persistency, these planar PCB congeners are of importance in environmental studies. These congeners are bioconcentrated and retained by bivalves and constitute a potential health hazard for higher consumers, including human beings.

CHAPTER V

UPTAKE AND DEPURATION OF TRIBUTYLTIN BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

INTRODUCTION

Tributyltin is the active component in antifouling paints. However, this compound has been shown to be highly toxic to a wide variety of aquatic organisms rather than being specific to the target individuals. This observation has generated a growing interest in the bioaccumulation of TBT by marine organisms. In this chapter, the bioconcentration of TBT and its depuration by transplanted and chronically contaminated oysters are discussed.

TBT: A REVIEW

Background Information

Evans & Karpel (1985) defined organotin compounds as compounds in which at least one direct tin-carbon bond exists. Most of the organotin compounds have tin in the IV+ oxidation state giving four series of organotin compounds: mono-, di-, tri- and tetraorganotins. Properties of these organotin classes are different. While monoorganotins have low toxicity, for example, the triorganotin compounds have biocidal properties. Diorganotins are used as stabilizers in the plastic industry.

These organotin compounds, which were introduced commercially in U.S.A. in the 1940s (Evans & Karpel, 1985), found use as stabilizers of polyvinylchloride (PVC), industrial catalyzer in the synthesis of polyurethane foams, epoxy resins, plastic materials, wood preservative and biocide. Within the scope of the last application, butyltins are the organotin compounds most widely used. Butyltins, in the form of tributyltin (TBT)-based paints, are highly effective as antifouling agents. With a useful life between 5 and 7 years (Champ & Pugh, 1987) and an effectiveness 10 to 100 times greater than copper-based paints (Anderson & Dalley, 1986; Ludgate, 1987), the use of paints containing TBT presents important economic benefits. For example, it has been reported that a six-month accumulation of fouling organisms, e.g. barnacles, seaweeds and tubeworms, on ship bottoms increases up to 40% the normal fuel consumption (Hall & Pinkney, 1985). Associated with the economical benefits, there were environmental risks. Because of the slow mode of action of TBT, standard, short duration tests failed to indicated its toxicity (Laughlin & Linden, 1987).

Contamination of the coastal marine environment by tributyltin has been investigated since the early 1980s when French workers discovered that TBT caused malformations and reduced growth in the Pacific oyster, *Crassostrea gigas* (Alzieu *et al.*, 1980, 1982). Similar effects have been reported in England (Anonymous, 1980; Abel *et al.*, 1986) and the United States of America (Stephenson *et al.*, 1986; Salazar *et al.*. 1987, Salazar and Salazar, 1987, 1988; Valkirs *et al.*, 1987a). As a result, the use of antifouling paints containing TBT on vessels under 25 m has been banned in France (1982), England (1987), and the United States of America (1988) (Anonymous, 1980; Knipe, 1989; U.S. Environmental Protection Agency, 1987). The increased concern about the adverse effects of TBT to non-target organisms led to the decision in the U.S. to include the analysis of butyltin compounds as part of the National Oceanic and Atmospheric

Administration's National Status and Trends "Mussel Watch" (NOAA's NS&T) Program (e.g. Wade *et al.*, 1988a).

Distribution and Occurrence in Galveston Bay

Because TBT was not considered to be an environmental threat until the late 1980s, studies directed at understanding the occurrence and fate of this contaminant in Galveston Bay are recent and very limited (Table 6). Wade *et al.* (1988) reported the results of a study designed to understand its temporal and spatial variations in bivalves collected from four sites in Galveston Bay. This study indicated that the TBT concentrations were higher in samples from sites located closer to known sources of inputs, i.e. the Galveston Bay Yacht Club. A decrease in TBT concentrations is reported toward the outer part of the Bay. Similarly, the highest TBT concentrations in Galveston Bay sediment samples were reported near the Galveston Bay Yacht Club (Wade *et al.*, 1990).

Bivalve Uptake and Depuration Studies

Compared to PAHs and PCBs, the number of studies of uptake and depuration of TBT by bivalves is limited. Although contamination of the coastal environment by TBT, for example, has been investigated since early 1980s, it was not until the late 1980s that this compound was considered to be a real threat to the quality of coastal waters.

Controlled flow-through experiments have shown that mussels accumulate increasing amounts of TBT over a 60 day period, reaching a steady state concentration thereafter (Salazar *et al.*, 1987). Reported half-life for TBT in field studies with different bivalves were relatively short (*Mytilus edulis*, 14 days, Laughlin *et al.*, 1986; *Crassostrea gigas* and *Ostrea edulis*, 10 days, Waldock *et al.*, 1983). Depuration rate constants calculated from laboratory data were found to be much lower than those obtained in environmental studies (Laughlin *et al.*, 1986). For example, the longer half-life (40 days) recently

TABLE 6

TBT, DBT and MBT Concentrations (in ng Sn g^{-1} on a Dry-Weight Basis) in Oyster and Sediment Samples from the Galveston Bay Area.

Location	Sample	TBT	DBT	MBT	Total OTs	Reference
Yacht Club	oysters	660	70	10	740	Wade *et al.*, 1988a
Todd's Dump	oysters	380	30	10	420	Wade *et al.*, 1988a
Confederate Reef	oysters	380	30	10	420	Wade *et al.*, 1988a
Hanna Reef	oysters	110	10	<5	120	Wade *et al.*, 1988a
Yacht Club	sediments	13	<5	<5	13	Wade *et al.*, 1990
Todd's Dump	sediments	7	<5	<5	7	Wade *et al.*, 1990
Confederate Reef	sediments	6	<5	<5	6	Wade *et al.*, 1990
Hanna Reef	sediments	11	<5	<5	11	Wade *et al.*, 1990

reported for mussels (*Mytilus edulis*) in laboratory depuration studies (Zuolian & Jensen, 1989) might reflect the effects of bivalve manipulation.

UPTAKE AND DEPURATION OF TBT

Experimental Design, Sample Collection and Methods

The experimental design and sample collection used for the study of TBT were the same as those discussed for PAHs (Chapter II).

Extraction and sample fractionation

The analytical procedure used during this study is a modification of previously reported methods (Maguire, 1984; Unger *et al.*, 1986; Matthias *et al.*, 1986) and is discussed in detail elsewhere (Wade *et al.*, 1988a). Approximately 15 g (wet weight) of tissue sample was weighed into a 250 ml centrifuge tube. Anhydrous sodium sulfate (40 g), 0.2% tropolone in methylene chloride (100 ml) and tripropyltin chloride as an internal standard were added. The sample was extracted for 3 min with a Tekmar Tissuemizer, centrifuged, and the supernatant was decanted into a 500 ml flat-bottom flask. The extraction was repeated two more times with 0.2% tropolone in methylene chloride (100 ml). The combined extracts were concentrated in a water bath (60°C) and the methylene chloride was replaced by hexane. The sample was then purged with nitrogen and hexylmagnesium bromide (2 ml, 2 M Grignard reagent) was added. The hexylation reaction was carried out for 6 h at 50°C. HCl (5 ml, 6 M) was then added to neutralize the excess Grignard reagent. The sample was shaken vigorously and the organic and aqueous phases were allow to separate. Two more extractions were perfomed using a mixture of pentane:methylene chloride (2:1, 125 ml). The organic phase was dried with anhydrous sodium sulfate and concentrated to 2 ml in hexane. The hexylated organotin compounds

were isolated on a column containing combusted alumina (400°C, 17 g) and silica (170°C, 13.5 g). The column was eluted with pentane (60 ml). The sample was then concentrated to 500 µl. Samples were spiked with tetrapropyltin before analysis to determine recovery of the internal standard for the whole analytical procedure.

Instrumental analysis

Butyltin species were analyzed by gas chromatography on a Hewlett-Packard 5790 gas chromatograph (GC) equipped with a capillary column (DB-5, 30 m x 0.25 mm i.d. x 0.25 µm coating thickness) and a flame photometric detector (FPD). The GC temperature was programmed from 60°C to a final temperature of 300°C, at a rate of 12°C/min, with a final 10 min hold time. Injector and detector temperatures were 300 and 250°C, respectively. Helium was used as the carrier gas. The response of the FPD was selective for Sn using a 610 nm filter. The limit of detection of TBT and breakdown products, dibutyltin (DBT) and monobutyltin (MBT), was 5 ng Sn g^{-1} dry weight.

Ancillary parameters

Methodologies for the sediment grain-size analysis and extractable lipids percentage were discussed in the materials and methods section of Chapter II.

Statistical analysis

The statistical analyses performed on the TBT data were previously discussed in the materials and methods section of Chapter II.

Uptake of TBT by Transplanted Oysters

In this and the following sections, Ship Channel, Hanna Reef, transplanted Hanna Reef-to-Ship Channel, transplanted Ship Channel-to-Hanna Reef and relocated Hanna

Reef-to-Ship Channel-back to-Hanna Reef oysters are refered as SC, HR, HRSC, SCHR and HRSCHR oysters, respectively.

Average concentrations of TBT encountered in oyster and sediment samples, are reported in Table A-10 (Appendix). TBT concentrations in SC oysters during the uptake phase of this experiment were very stable (mean= 340±39 ng Sn g^{-1}, relative standard deviation = 11%, range = 330-420 ng Sn g^{-1}) suggesting that bioavailable TBT to the oysters was relatively constant. Therefore, it is assumed that the accumulation rate in HRSC transplanted oyster was not affected by changes in the concentration of bioavailable TBT.

Approximately a 10-fold increase in TBT concentrations was observed by the end of the exposure period in HRSC oysters (Fig. 31). By the end of the seven-week exposure period, the concentration of TBT in HRSC oysters was similar to the level found in SC oysters. This increase is similar to previously reported uptake data in exposed mussels after about 50 days (Laughlin, Jr., *et al.*, 1986; Zuolian & Jensen, 1989). Controlled flow-through experiments have shown that mussels reach a steady state concentration of TBT after a 60-day exposure period (Salazar *et al.*, 1987). A steady state concentration was not attained in this study; however, the continued increasing concentrations of TBT measured in transplanted oysters by the end on the seven-week uptake period seem to indicate that, given enough time, a true equilibrium concentration comparable to the levels measured in native oysters would have been reached.

DBT, the major breakdown product of TBT (Maguire, 1984; Seligman *et al.*, 1986), did not show any accumulation during the exposure period and was only detected at low concentrations in both groups of oysters. This might suggest that DBT, a more polar and soluble compound than TBT, may be quickly depurated from the oyster tissues. DBT concentrations ranged from 22 to 34 ng Sn g^{-1} and <5 to 22 ng Sn g^{-1} in SC and HRSC

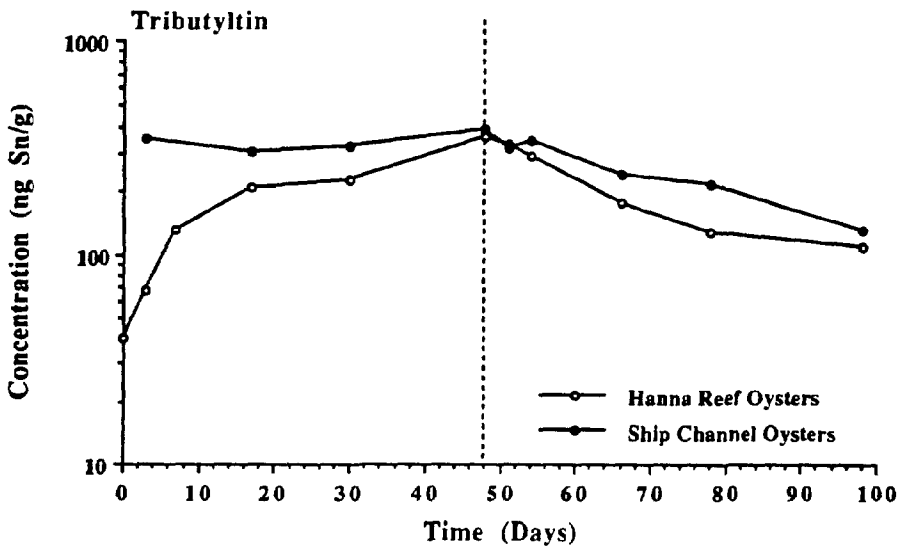


Fig. 31. Concentrations of tributyltin in tissues of Hanna Reef and Ship Channel Oysters during exposure to the Ship Channel area contaminant levels and following transplant to the Hanna Reef are.

oysters, respectively, while MBT concentrations were, in all cases, below the 5 ng Sn g^{-1} limit of detection.

Sediment samples collected from the Ship Channel and Hanna Reef locations during this study had TBT, DBT, and MBT concentrations below the detection limits.

Depuration of TBT by Newly and Chronically Contaminated Oysters

Both oyster populations showed statistically significant depuration of TBT after back-transplantation to the Hanna Reef area. The final TBT concentration encountered in HRSCHR individuals at the end of the 50- day depuration period was over 100% higher than the levels measured in the same group of oysters before the transplantation experiment to the Ship Channel area.

The calculated half-life for TBT in the originally uncontaminated Hanna Reef oysters (21 days) was higher than the values reported for mussels (*Mytilus edulis*, 14 days, Laughlin *et al.*, 1986) and the Pacific (*Crassostrea gigas*) and European (*Ostrea edulis*) oysters (10 days, Waldock *et al.*, 1983). Depuration rate constants calculated from laboratory data were found to be much lower compared to environmental studies (Laughlin *et al.*, 1986). For example, the longer half-life (40 days) recently reported for mussels (*Mytilus edulis*) in laboratory depuration studies (Zuolian & Jensen, 1989) might reflect the effects of bivalve manipulation. Comparatively, the TBT half-life in chronically exposed oysters, i.e. SCHR oyster, was 27 days.

As discussed in previous chapters, similar differences in the depuration rates, i.e. half-lives, were observed for other trace organic contaminants between newly and chronically contaminated oysters. In the specific case of TBT, a possible explanation of these different depuration rates could be the existence of ligands within the oyster body as suggested by Laughlin (1990). These ligands, which do not seem to be induced by TBT exposure, might be produced slowly by chronically exposed bivalves. Tissue molecules

with groups containing sulfur, oxygen or nitrogen are mentioned as the obvious ligand candidates. Therefore, the existence of these ligands in one group of oysters, but not in the other, might explain the difference observed in depuration rates.

CONCLUDING REMARKS

Although a steady state concentration was not reached, transplanted oysters rapidly accumulated TBT to practically reach an equilibrium with the concentrations encountered in indigenous oysters at the end of the 48-day exposure period. DBT, a more polar compound than TBT, has only been detected at low concentrations in the oyster tissues.

When relocated to the Hanna Reef area, both oyster populations significantly depurated TBT; however, the observed depuration rates were different. HRSCHR oysters depurated at a rate about 30% faster than the clearance rate observed in SCHR oysters. This is reflected in the estimated TBT half-lives for HRSCHR and SCHR oysters (21 and 27 days, respectively). The same observation was made when comparing half-lives for PAHs and PCBs in HRSCHR and SCHR oysters.

CHAPTER VI

MECHANISM OF THE UPTAKE AND RELEASE OF TRACE ORGANIC CONTAMINANTS BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

INTRODUCTION

The relationship between a pollutant concentration in organisms and their aquatic habitat was first explained as a simple partition process across external membrane surfaces (Hamelink *et al.*, 1971). Since then, the dynamic equilibrium between uptake from and depuration to water, together with the balance between ingested and excreted matter, has been widely used to explain bioaccumulation data. After the introduction of the n-octanol-water partition coefficient (K_{ow}) to assess the potential of different organic compounds to be bioaccumulated under equilibrium conditions, several studies have reported a correlation between the concentration factors of organic contaminants in tissues and the logarithms of their K_{ow} coefficients (Geyer *et al.*, 1982; Mackay, 1982; Pruell *et al.*, 1986). The K_{ow} coefficient has been found to be very useful in predicting the environmental partitioning of some lipophilic compounds.

In this chapter, the kinetics involved in the uptake and release of selected trace organic contaminants (PAHs, PCBs, including planar congeners, and TBT), as well as their concentration factors by American oyster (*Crassostrea virginica*) during transplant experiments, are reported. In the following sections, Ship Channel, Hanna Reef, transplanted Hanna Reef-to-Ship Channel, transplanted Ship Channel-to-Hanna Reef and

relocated Hanna Reef-to-Ship Channel-back to-Hanna Reef oysters are refered as SC, HR, HRSC, SCHR and HRSCHR oysters, respectively.

MECHANISM OF BIOCONCENTRATION

Kinetics

Bioconcentration is defined as the balance between uptake and depuration processes, which may proceed by first order kinetics characterized by the rate constants k_u and k_d, respectively (Shaw & Connell, 1984). The bioconcentration factor (BCF) is defined as the proportionality constant relating the concentration of a chemical in water to the concentration in an aquatic organism at steady state equilibrium.

The following characteristics describing the kinetics of bioconcentration using a single compartment model was adapted from Connell (1990). The one-compartment approach is the mathematical expression of the hydrophobicity model, which considers bioconcentration as the partitioning of a chemical between the exposure media and the lipidic pools of an organisms, and vice versa, with no physical barriers (Barron, 1990). The general first-order equation that describes the uptake and depuration of lipophilic compounds, such as PAHs and PCBs, is expressed by

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

where C_t is the concentration in the organism at time = t and C_w is the concentration in water. Since the net amount of an analyte in the water represents a large reservoir compared to the relatively lower amount taken up by organisms, C_w can be regarded as constant. By integration and rearrangement

$$C_t = (k_u/k_d)C_w(1 - e^{-k_d t}) + Ce^{-k_d t} \qquad (2)$$

where C_{to} is the initial concentration in the organism. This equation predicts that C_t will increase in concentration with time and with a declining rate of increase. Thus, at time = infinity, t_∞

$$C_{t_\infty} = k_u/k_d\ C_w \tag{3}$$

and

$$C_{t_\infty}/C_w = k_u/k_d = K_b \tag{4}$$

where K_b is the bioconcentration factor (BCF). Similarly, the BCF can be calculated from equation (1) when uptake and depuration are in equilibrium, i.e. at t = infinity

$$dC_t/dt = 0 = k_u\ C_w - k_d\ C_{t_\infty} \tag{5}$$

The theoretical time period to reach equilibrium occurs when $e^{-k_d t}$ is zero, which is when t is infinity. However, effective equilibrium can be considered to be reached at t_{eq} when C_t is 0.99 of the concentration value at infinity. Thus, from equation (2)

$$C_{t_{eq}} = (k_u/k_d)\ C_w\ (1 - e^{-k_d t_{eq}}) \tag{6}$$

$$C_{t_{eq}} = 0.99\ (k_u/k_d)\ C_w \tag{7}$$

From equations (6) and (7)

$$0.99 = 1 - e^{-k_d t_{eq}} \tag{8}$$

and

$$t_{eq} = 4.605/k_d \tag{9}$$

Because of the lenghts of time required for transplanted oysters to reach a concentration equal to 99% the equilibrium concentration, a more time-realistic approach would be to consider the time to attain 90% of the equilibrium concentration for organic contaminants. Equation (9) is then modified to

$$t_{90\%} = 2.303/k_d \qquad (10)$$

If exposure to the compound is terminated by transfer to uncontaminated water or, more realistically, to a site were environmental concentrations are negligible, then C_w can be regarded as zero and

$$dC_t/dt = -k_d C_t \qquad (11)$$

This indicates that during exposure, both uptake and depuration were operating, but now, in very low concentration or uncontaminated water, uptake can be neglected. By integration

$$C_t = C_{t_0} e^{-k_d t} \qquad (12)$$

or

$$\log C_t = \log C_{t_0} - k_d t/2.303 \qquad (13)$$

where C_{t_0} is now the initial concentration at time zero for the depuration period. This equation shows that as t increases C_t declines, but the rate of decline decreases with increasing time. Also, since C_{t_0} and k_d are constants, log C_t is linearly related to time. When half of the initial compound has been cleared, then $C_t = C_{t_0}/2$ and the half life, $t_{1/2}$, is represented by

$$t_{1/2} = \log 2\ (2.303/k_d) = 0.693/k_d \qquad (14)$$

The kinetic parameters obtained for PAHs, PCBs, planar PCBs and TBT are given in Table 7. Concentration factors for PAHs and PCBs were calculated comparing the concentrations measured in HRSC and SC oyster tissues at the end of the seven-week exposure period and the average concentrations encountered in water samples (Tables A-2, A-3, A-6 and A-7, Appendix).

Polynuclear aromatic hydrocarbons

As previously discussed in Chapter II, transplanted HRSC oysters bioconcentrated most of the PAHs to concentrations that were not significantly different from the concentrations encountered in indigenous SC oysters at the end of the uptake period. Bioconcentration factors in both groups of oysters increased with the number of aromatic rings for PAHs having two-, three- and four-rings per molecule and decreases thereafter. The maximum concentration factors in both group of oysters were for pyrene, chrysene and benzo(a)anthracene. The lowest values were for compounds with molecular weights less than or greater than pyrene.

Depuration constant values for PAHs can be divided in two groups in both oyster populations. The first one represents the two- and three-ring PAHs, which ranged from 0.0268 to 0.0297 days^{-1} in HR oysters and from 0.0166 to 0.320 days^{-1} in SC oysters. The second group includes the remaining PAHs with k_d values ranging from 0.0439 to 0.0787 days^{-1} in HR oysters and from 0.0430 to 0.0708 days^{-1} in SC oysters. In the first case, the low k_d values result in longer biological half-lives and longer time to reach a concentration within 10% the concentration at equilibrium. PAHs in the second group had considerably shorter half-lives and reached within 10% of the equilibrium concentration in a shorter period of time. Estimated PAH half-lives ranged from 9 days

TABLE 7

Estimated Days to 90% Uptake Equilibrium ($t_{90\%}$), Bioconcentration Factors (BCF), Depuration Rates (k_d) and Biological Half-Lives (BHL) for PAHs and PCB Congeners in Hanna Reef and Ship Channel Oysters During the Field Studies.

Analyte		Hanna Reef Oysters				Ship Channel Oysters			
	$t_{90\%}$ (days)	BCF[a]	k_d ($days^{-1}$)	BHL (days)	R^2[b]	BCF (days)	k_d ($days^{-1}$)	BHL (days)	R^2
PAHs									
2,3,5-Trimethynaphthalene	80	16,000	0.0287	24	0.74	23,000	0.0320	22	0.83
Anthracene	78	29,000	0.0295	24	0.67	29,000	0.0166	42	0.68
1-Methylphenanthrene	78	43,000	0.0297	23	0.97	60,000	0.0283	24	0.96
Fluoranthene	86	310,000	0.0268	26	0.90	310,000	0.0215	32	0.69
Pyrene	35	890,000	0.0663	10	0.95	880,000	0.0557	12	0.98
Benz(a)anthracene	44	450,000	0.0525	13	0.96	490,000	0.0453	15	0.99
Chrysene	41	490,000	0.0565	12	0.99	530,000	0.0439	16	0.99
Benzo(b)fluoranthene	38	290,000	0.0601	12	0.95	270,000	0.0488	14	0.98
Benzo(k)fluoranthene	34	340,000	0.0674	10	0.96	330,000	0.0561	12	0.98
Benzo(e)pyrene	38	300,000	0.0602	12	0.97	310,000	0.0430	16	0.98
Benzo(a)pyrene	29	200,000	0.0787	9	0.98	210,000	0.0708	10	0.99
Perylene	35	140,000	0.0649	11	0.94	140,000	0.0532	13	0.99
Indeno[1,2,3-c,d]pyrene	35	44,000	0.0665	10	0.96	42,000	0.0647	11	0.93
Dibenzo(a,h)anthracene	52	27,000	0.0439	16	0.93	24,000	0.0506	14	0.90
Benzo(g,h,i)perylene	38	120,000	0.0610	11	0.96	110,000	0.0574	12	0.98

TABLE 7
(continued)

Analyte	$t_{90\%}$ (days)	Hanna Reef Oysters BCF[a]	k_d ($days^{-1}$)	BHL (days)	R^{2b}	Ship Channel Oysters BCF (days)	k_d ($days^{-1}$)	BHL (days)	R^2
PCBs									
2,2',5 (18)	48	110,000	0.0480	14	0.82	110,000	0.0362	19	0.93
2,3',5 (26)	73	-	0.0314	22	0.88	-	0.0327	22	0.99
2,4,4' (28)	55	-	0.0419	17	0.96	-	0.0205	34	0.93
2,2',3,3' (40)	47	-	0.0488	14	0.87	-	0.0383	18	0.97
2,2',3,4 (41)	77	-	0.0299	23	0.83	-	0.0127	55	0.92
2,2',3,5' (44)	91	97,000	0.0253	27	0.83	83,000	0.0153	45	0.94
2,2',4,5' (49)	131	210,000	0.0176	39	0.84	220,000	0.0114	61	0.94
2,2',5,5' (52)	88	100,000	0.0261	27	0.80	100,000	0.0155	45	0.91
2,3',4',5 (70)	100	610,000	0.0235	30	0.88	740,000	0.0120	58	0.96
2,4,4',5 (74)	100	200,000	0.0231	30	0.87	220,000	0.0147	47	0.95
2,2',3,3',6 (84)	123	-	0.0187	37	0.84	-	0.0087	80	0.79
2,2',3,4,5'/2,3,4,4',6 (87/115)	181	-	0.0127	55	0.73	-	0.0038	132	0.45
2,2',3,4',6 (91)	84	370,000	0.0275	25	0.78	330,000	0.0140	50	0.89
2,2',3,5,5' (92)	99	-	0.0233	31	0.89	-	0.0108	63	0.93

TABLE 7
(continued)

Analyte	$t_{90\%}$ (days)	Hanna Reef Oysters BCF[a]	k_d ($days^{-1}$)	BHL (days)	R^{2b}	Ship Channel Oysters BCF (days)	k_d ($days^{-1}$)	BHL (days)	R^2
2,2',3',5',6 (95)	149	-	0.0155	45	0.81	-	0.0073	95	0.79
2,2',4,4',5 (99)	165	210,000	0.0140	49	0.88	330,000	0.0076	91	0.79
2,2',4,5,5'/2,2',3,4',5 (101/90)	184	160,000	0.0125	55	0.86	260,000	0.0060	116	0.78
2,3,3',4,4' (105)	211	120,000	0.0109	63	0.76	140,000	0.0058	120	0.76
2,3,3',4',5 (107)	100	-	0.0231	30	0.82	-	0.0151	46	0.75
2,3,3',4',6/3,3',4,4' (110/77)	149	250,000	0.0155	45	0.74	330,000	0.0067	103	0.67
2,3',4,4',5 (118)	242	300,000	0.0095	73	0.79	480,000	0.0023	299	0.19
2,2',3,3',4,4' (128)	253	-	0.0091	76	0.75	-	0.0030	229	0.42
2,2',3,4,4',5'/2,3,3',4,5,6 (138/160)	658	46,000	0.0035	200	0.32	79,000	0.0012	595	0.11
2,2',3,4',5,5' (146)	371	-	0.0062	111	0.60	-	0.0029	239	0.27
2,2',3,4',5',6/2',3,4,4',5 (149/123)	435	74,000	0.0053	130	0.46	150,000	0.0016	439	0.24
2,2',4,4',5,5'/2,2',3,3',4,6' (153/132)	169	87,000	0.0136	51	0.71	140,000	0.0068	102	0.90
2,2',3,3',4',5,6 (177)	171	50,000	0.0135	52	0.83	65,000	0.0048	145	0.54
2,2',3,3',5,5',6 (178)	172	-	0.0134	52	0.62	-	0.0076	91	0.83
2,2',3,4,4',5,5' (180)	167	12,000	0.0138	50	0.94	14,000	0.0049	142	0.63
2,2',3,4',5,5',6 (187)	233	61,000	0.0099	70	0.65	82,000	0.0027	258	0.56

TABLE 7
(continued)

Analyte	$t_{90\%}$ (days)	Hanna Reef Oysters BCF[a]	k_d ($days^{-1}$)	BHL (days)	R^2[b]	Ship Channel Oysters BCF (days)	k_d ($days^{-1}$)	BHL (days)	R^2
Coplanar PCBs									
3,3',4,4' (77)	291	-	0.0079	88	0.85	-	-	-	-
3,3',4,4',5 (126)	360	-	0.0064	107	0.50	-	-	-	-
Butyltin species									
Tributyltin	68	72,000[c]	0.0251	27	0.95	78,000[c]	0.0202	34	0.96

[a] Bioconcentration factor = concentration in oyster tissue at the end of the uptake period / concentration in water.
[b] R^2 = square of the correlation coefficient for the regression equation to obtain k_d.
[c] = estimated BCF (see text).

for benzo(a)pyrene to 26 days for fluoranthene and from 10 days for benzo(a)pyrene to 42 days for anthracene in HRSCHR and SCHR oysters, respectively. Most of the values were, however, between 10 and 13 days for HRSCHR oysters and between 13 and 16 days for SCHR oysters. In general, originally uncontaminated oysters depurated faster than chronically exposed individuals.

Polychlorinated biphenyls

Bioconcentration factors for PCB congeners show approximately the same general behavior discussed for PAHs. Concentration factors are higher for tetra- and pentachlorobiphenyls congeners and lower for tri-, hexa- and heptachlorobiphenyls. Octa-, nona- and decachlorobiphenyls were detected at very low concentrations.

The decreasing values of k_d and the increasing values of $t_{90\%}$ and half-lives with the higher degree of chlorination of the biphenyl molecule reflect the more rapid uptake and release of the lower chlorinated biphenyls. Previous reports indicate that the less lipophilic congeners reach an equilibrium concentration, either during uptake or depuration, at a faster rate than those compounds that are more lipophilic (e.g. Ellegehausen *et al.*, 1980; Bruggeman *et al.*, 1981; Tanabe *et al.*, 1987a).

Biological half-lives for PCB congeners in HRSCHR and SCHR oysters ranged from 14 to 200 days and from 19 to 595 days for congeners 2,2',5-trichlorobiphenyl (18) and 2,2',3,4,4',5'-hexachlorobiphenyl (138), respectively. Planar PCBs showed a slower clearance rate than other PCBs within the same homolog groups (Fig. 32). These slower depuration rates are reflected in longer half-lives and time periods to reach 90% of equilibrium concentrations. As in the case of PAHs, most of the bioconcentrated PCB congeners were eliminated faster by originally clean oysters than by chronically contaminanted bivalves.

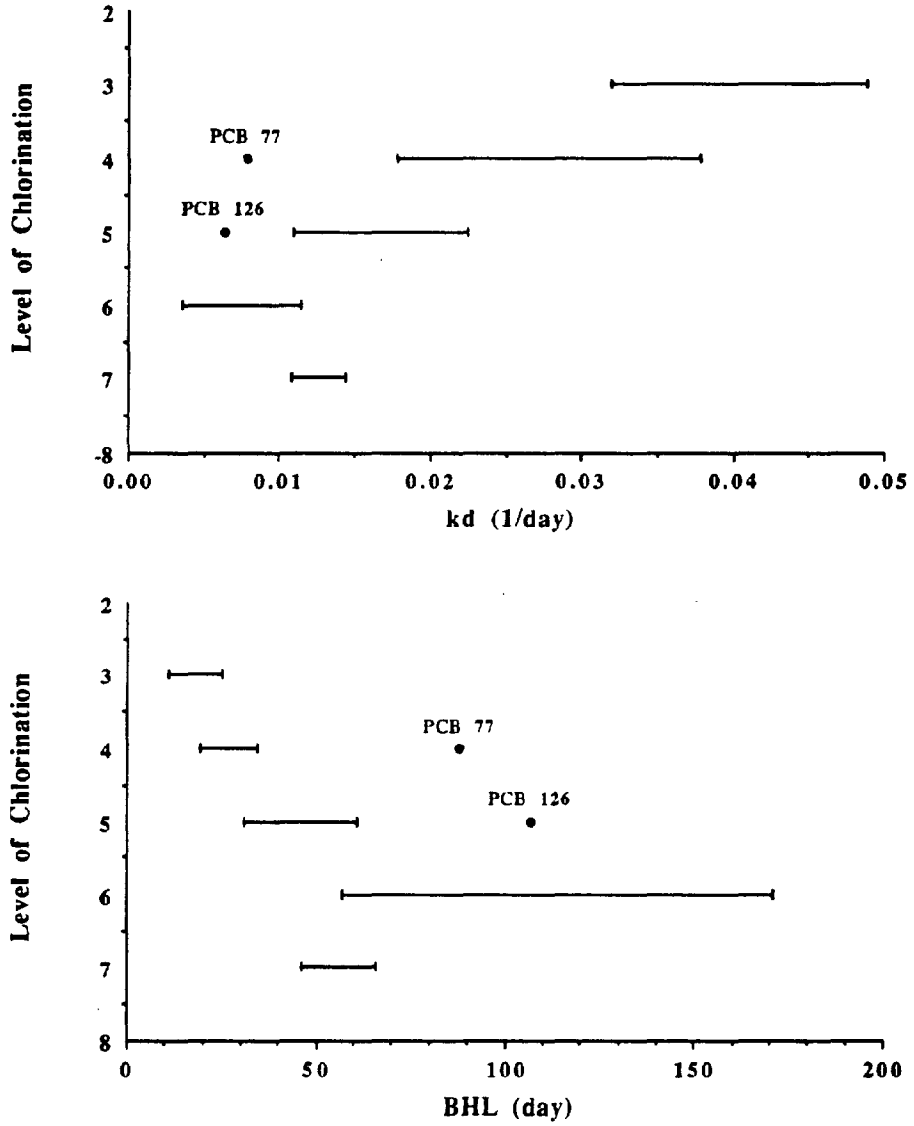
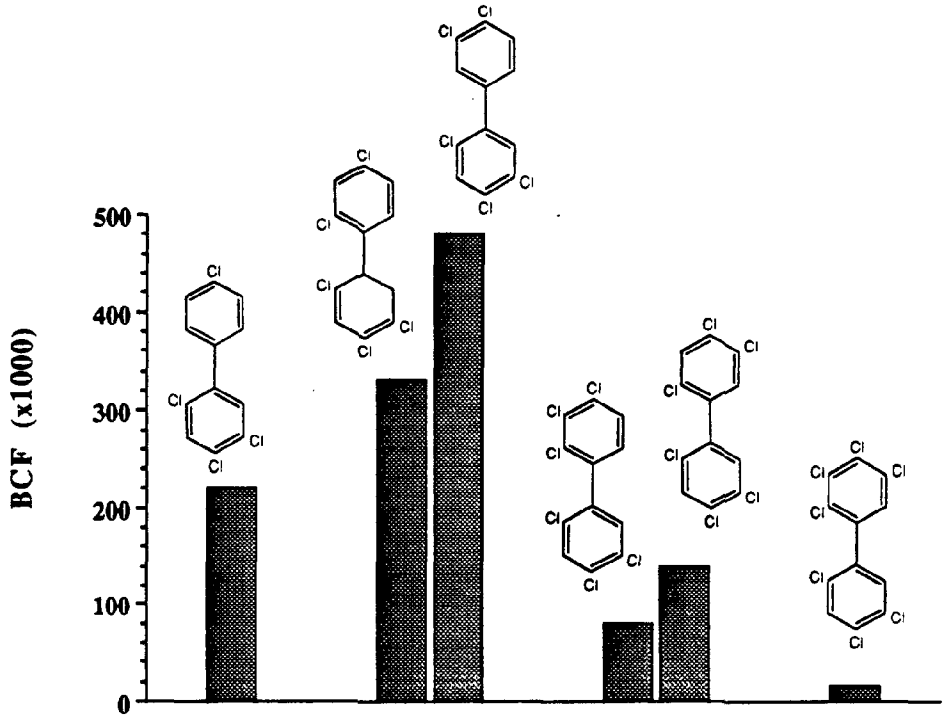


Fig. 32. Depuration constant (kd) and biological half-lives (BHL) of planar PCB congeners compared to ranges of values calculated for non-planar PCBs.

Several reports have suggested that bioaccumulation of PCBs by different organisms might be influenced by physicochemical factors (Jan & Josipovic, 1978; Tulp & Hutzinger, 1978; Matsuo, 1980; Shaw & Connell, 1980, 1982, 1984; Opperhuizen *et al.*, 1985; Samuelian & O'Connor, 1985). Several parameters have been suggested that may be suitable to measure the effect of these factors on the kinetics of bioaccumulation, e.g. molar volume, parachor, steric effect coefficients.

Competitive partition between aqueous and nonpolar phases, e.g. lipids, as well as stereochemistry appear to be significant factors influencing the uptake of these compounds. As discussed earlier, maximum PCB uptake by organisms is observed for congeners having four to six chlorine atoms. Low chlorinated congeners have higher water solubilities and, as a consequence, lower lipophilicity. In contrast, isomers in the higher homolog groups have unfavorable steric configurations (Shaw & Connell, 1984). Opperhuizen *et al.* (1985) reported that the BCFs of polychlorinated naphthalenes and biphenyls depend on molecular size, e.g. molecular volume and cross-sectional area that are directly related to chlorine substitution patterns, rather than hydrophobicity.

As an example of the antagonistic effects that lipophilicity and size of the different congeners have on their accumulation, the bioconcentration factors of six related PCB congeners are compared in Fig. 33. Log K_{ow} and total surface area (TSA x 10^{-20} m^2) values are also indicated (Hawker & Connell, 1988). These congeners have a common 2,4,5-chlorine distribution in one ring while one to four chlorines are sequentially substituted on the second ring. It is clear that the more favorable lipophilicity/size conditions for bioaccumulation are present in PCB 99. Congener 180 is the most lipophilic of the six PCBs shown and also has the largest total surface area. On the other hand, the smaller size of congener 74 for membrane transport into the tissue is countered by its higher water solubility.



PCB Congener	74	99	118	138	153	180
Log K_{ow}	6.20	6.39	6.74	6.83	6.92	7.36
TSAx10^{-20} (m^2)	246	252	262	265	267	280

Fig. 33. Bioconcentration factors of six selected PCB congeners in relation to their liphophilicity and size.

Not only the bioconcentration of PCB congeners seems to be affected by the different chlorine substitution in the biphenyl rings, but their depuration may be affected by these factors. For example, Fig. 34 shows PCB congeners at different levels of chlorination that have a fixed substitution pattern for all chlorines but one. The extra chlorine, in bold, is sequentially substituted in *para*, *meta* and *ortho* positions giving three different substition patterns. Also indicated in the figure are the estimated half-lives for these congeners in chronically contaminated Ship Channel oysters. The experimental data shows that there is a decrease in the estimated biological half-lives when the extra chlorine is substituted in the *para* > *ortho* > *meta* positions.

Tributyltin

Unfortunately, the lack of data on concentrations of TBT in seawater samples from the Ship Channel area do not permit the calculation of a bioconcentration factor. However, when the limit of detection of the analytical method for seawater is used to calculate the bioconcentration factor, the minimum value can be estimated. The detection limit for TBT in seawater is 5 ng Sn L^{-1}; this gives a bioconcentration factor for transplanted and indigenous oysters, on a dry weight basis, on the order of 72,000 and 78,000 at the end of the exposure period, respectively. On wet weight basis, these estimated values convert to 9,000 and 9,750, respectively, which compare well with previously published concentration factors in mussels (up to 5,500; Laughlin *et al.*, 1986) and oysters (up to 6,000; Waldock *et al.*, 1983).

Depuration constants for TBT in newly and chronically contaminated oysters were 0.0251 and 0.0202 $days^{-1}$, respectively. These values compare well with those encountered for the lower molecular weight PAHs and PCBs. Similarly to those organic contaminants, the estimated half-life for TBT in originally uncontaminated HR oysters (27

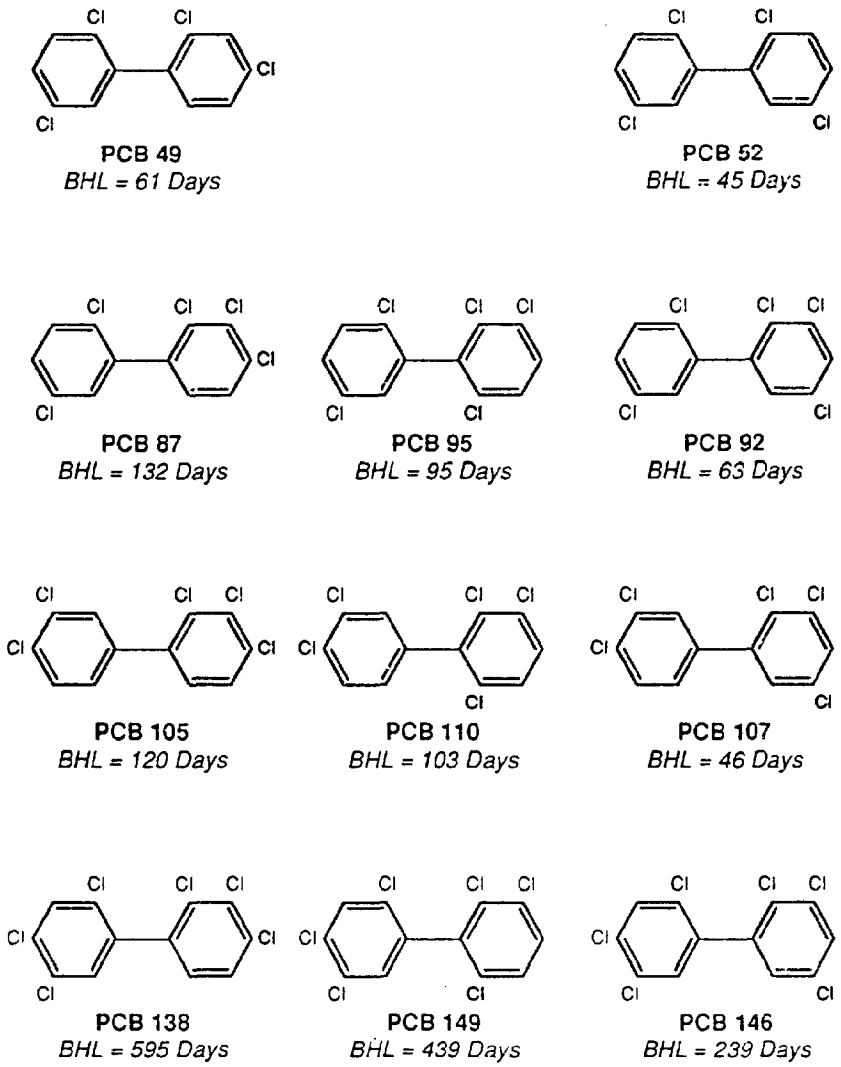


Fig. 34. Relationship between chlorine-substitution patterns in PCBs and their depuration half-lives. See text for explanation.

days) was lower than the corresponding value for chronically exposed individuals (34 days).

The Octanol-to-Water Partition Coefficient

The octanol to water partition coefficien (K_{ow}) is defined as:

$$K_{ow} = C_o/C_w \tag{15}$$

where C_o and C_w are the concentrations of the analyte in n-octanol and water, respectively. Although many organic solvents have been used for this purpose, n-octanol is considered to be the best surrogate for organism lipids. Since the introduction of the K_{ow} partition coefficient in early 1960s (Hansch & Fujita, 1964), it has been used in numerous studies to explain the concentration of different organic compounds in biological tissues. The observed bioconcentration factor, or more commonly its log value, is related to log K_{ow} by the following equation

$$\log K_b = a\, K_{ow} + b \tag{16}$$

If n-octanol behaves as a perfect surrogate for organism lipids, the constant (a) should be equal to 1. A deviation from unity indicates how much octanol differs from biological lipids.

Fig. 35 shows the log of the calculated bioconcentration factor of individual PAHs or selected PCB congeners in both oysters populations plotted against the log of their octanol-to-water coefficients, respectively. Values of the log of the PAH and PCB octanol-to-water partition coefficients are from Isnard and Lambert (1989) and Patil (1991), respectively. The plots of the log of calculated concentration factors versus log K_{ow} have bell shaped curves. In general, an increase of the bioconcentration factors is

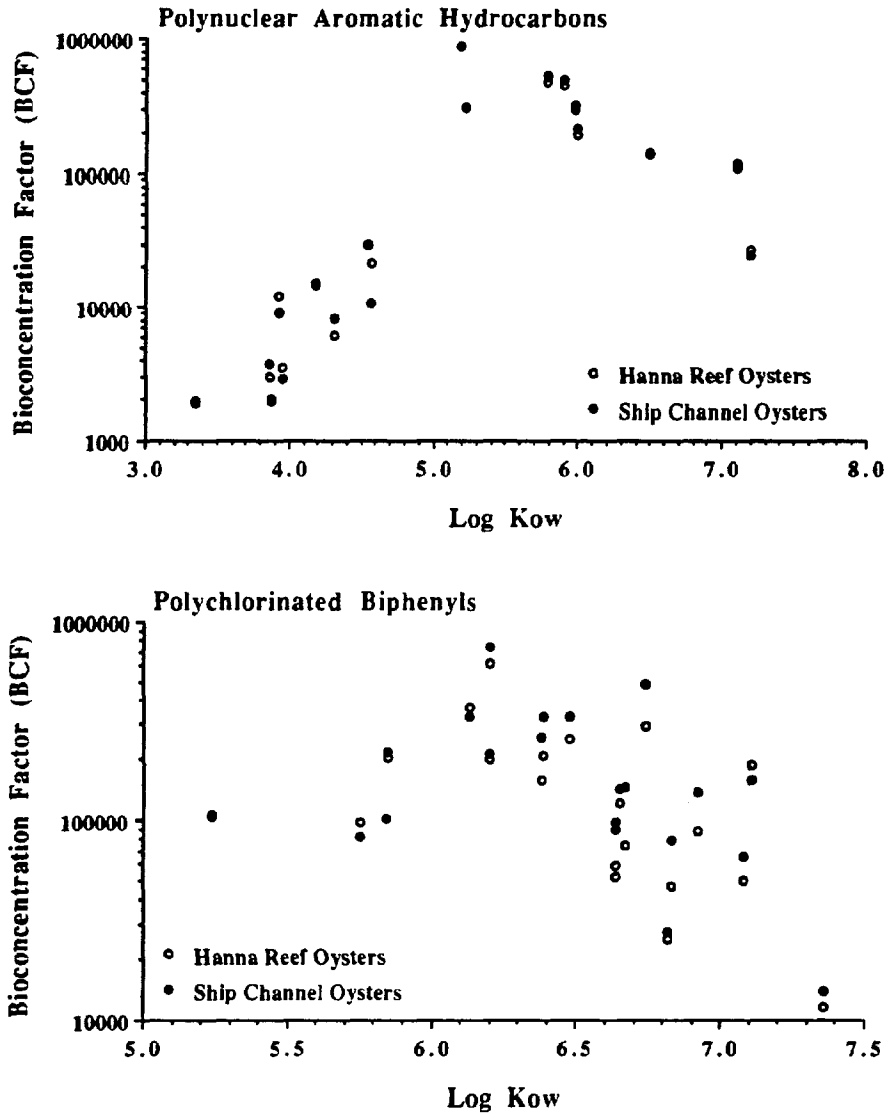


Fig. 35. Bioconcentration factors of polynuclear aromatic hydrocarbons and polychlorinated biphenyls calculated for transplanted Hanna Reef and indigenous Ship Channel oysters during the exposure period versus log octanol to water partition coefficients (K_{OW}).

observed until log K_{ow} reaches about 6, then there is a decline for the more lipophilic compounds with high K_{ow}. Dobbs & Williams (1983) indicated that compounds with K_{ow} greater than 6 exhibit a decrease in their lipid solubility. These compounds, often referred as "superlipophilic" were redefined by Connell (1990) as "superhydrophobic."

A departure from the predictive relationship (16) has been observed in other studies (e.g. Oliver, 1984, 1987). Hawker & Connell (1985) have indicated that the little attention devoted to the time required by highly lipophilic chemicals to reach equilibrium might result in underestimated k_d values which cause the observed change of the slope in the plot. They estimated that the necessary time for "superhydrophobic" analytes to attain an equilibrium concentration in exposed organisms range from a minimum 0.5 years, for log K_{ow} = 6, up to 12 years, for log K_{ow} = 8. That study, however, was done by exposing uncontaminated organisms in the laboratory to different organic compounds.

Besides the fact that extrapolations of laboratory produced data to real world situations are not always possible, and in most cases inaccurate, this extrapolation is further complicated if the uptake of these xenobiotics by an uncontaminated adult organisms is compared to the uptake by organisms that are developing in a chronically contaminated area. It seems obvious that tissues and lipid pools being formed by juvenile organisms in chronically contaminated environments will have a better chance to truly incorporate xenobiotic compounds than have tissues and lipid pools already formed in uncontaminated adult individuals later exposed to the same xenobiotic compounds.

In the present study, Ship Channel oysters have been chronically exposed to the high xenobiotic concentrations present in the Ship Channel area since the earliest stages of their lives. With average lengths between 7 and 9 cm, the oysters used in this study were adult organisms at the time of sampling and the analyte concentrations in their bodies can be assumed to represent the equilibrium concentration at infinity (t_∞). Because of the similarities in the shapes of the curves obtained for SC and HRSC oysters, it is clear that

less than two months are needed for newly exposed oysters to reach equilibrium concentrations that are, in general, comparable to those encountered in chronically exposed individuals.

Table 8 compares the relationships between log K_d and log K_{ow} (16), obtained for this study with previuosly reported works. Since the linear relationship between log K_b and log K_{ow} does not exist for log K_{ow} values higher than 6, values for constants a and b are commonly calculated considering only log K_{ow} values up to 6. Connel & Hawker (1988) have suggested that octanol is not a good surrogate for "superhydrophobic" compounds (i.e. those with log $K_{ow} > 6$) and, therefore, the concentration of these compounds by organisms can not be accurately predicted from their log K_{ow} values.

The Two-Compartment Model Approach

In depuration studies, it is also important to understand the effects that partitioning among different body compartments might have on the elimination of accumulated organic contaminants. The observed differences in depuration rates and, consequently, in the half-life estimations between HRSCHR and SCHR oyster populations seems to indicate that at least a two compartment model rather than the single compartment approach would be more accurate in describing the depuration kinetics in exposed oysters. The two compartment model, with the first compartment representing a peripheral system and the second a central system, was described by Moriarty (1975). Briefly, the initial rapid exchange across external membranes is followed by a slower but more persistent accumulation in fatty tissues through the circulatory system. Chronic, or long term, exposure would result in accumulation of organic xenobiotics in deeper deposits of lipids stored as energy reserves. In this study, for example, xenobiotic chemicals may be better partitioned between both compartments in chronically contaminated SC oysters than in newly exposed HRSC oysters. If the accumulation of organic xenobiotics in bivalves is

TABLE 8

Characteristics of the Relationships Between log K_b and log K_{ow} for Bioconcentration of Trace Organic Contaminants in Different Organisms.

Analytes	n	a	b	Organism	Reference
PAHs	22	0.78	-0.35	fish	Schüürmann & Klein (1988)
PAHs	30	0.95	-1.06	fish	Connell & Schüürmann(1988)
PAHs	6	0.97	-1.40	mussels	Pruell *et al.* (1986)
PAHs	13	1.17	-0.88	oysters (SC)	This study
PAHs	13	1.15	-0.77	oysters (HR)	This study
Crude oil	14	0.49	1.03	oysters	Ogata *et al.* (1984)
PCBs	4	0.59	1.73	mussels	Pruell *et al.* (1986)
PCBs	7	0.69	1.22	oysters (SC)	This study
PCBs	7	0.65	1.46	oysters (HR)	This study
Pesticides	8	0.70	-0.26	alga	Ellegehausen *et al.* (1980)
Pesticides	8	0.83	-1.71	fish	Ellegehausen *et al.* (1980)
Organics	16	0.86	-0.81	mussels	Geyer *et al.* (1982)

the result of a simple partition between tissues and seawater, then the elimination of the source of contamination should reverse the process. Within the scope of the two compartment model, tissues with low lipid contents, i.e. muscle and mantle, are generally reported to have relatively lower tissue burdens than those with higher lipid levels, i.e. gonads and gills (e.g. Laughlin *et al.*, 1986). Clearly, with two different compartments capable of accumulation of organic xenobiotics, k_d values in exposed oysters can no longer represent the simple partition between oyster and ambient seawater but a more complicated and longer process involving different equilibrium constants among the compartments and with the environment.

Depuration versus Degradation

Biotransformation of organic compounds into more polar and, therefore, more soluble metabolites decreases the equilibrium level of the accumulated chemicals by increasing the rate of depuration. Thus, the observed k_d constants for PAHs and TBT might be a combination of at least two constants, the physical partition rate between the oyster tissues and ambient seawater and the biological breakdown rates to more polar compounds. This will result in an increase of the clearance rate beyond that due solely to the physical process described in the preceding sections.

Although early reports indicated that bivalves could not metabolize petroleum hydrocarbons (e.g. Lee *et al.*, 1972; Payne & Penrose, 1975; Vandermeulen & Penrose, 1978), later studies have shown that bivalves are able to metabolize PAHs (e.g. Anderson, 1978a, 1978b, 1979; Payne & May, 1979; Stegeman, 1980). However, the metabolic rates are relatively lower than those observed in other marine animals. For example, Anderson (1978a) detected comparatively low concentrations of aryl hydrocarbon hydroxylase, an enzyme inducible by exposure to benzo(a)-pyrene, in the digestive gland of oysters. Similarly, metabolism of TBT has been shown by Lee (1985,

1986). Lee (1985) reported that after three days exposure 10% of the radioactivity of the applied [^{14}C]TBT was found in the digestive gland of oysters in the form of DBT and other more polar metabolites. Other biodegradation studies have shown that DBT, a more polar compound than TBT, was the major breakdown product of TBT, whereas MBT was detected at very low concentrations (Maguire, 1984; Seligman *et al.*, 1986). In the case of PCBs, there is no report that indicates that bivalves are able to metabolize these compounds.

CONCLUDING REMARKS

Bioconcentration factors (BCF) for PAHs and PCBs increased with the increasing octanol-to-water partition coefficient up to a value of K_{ow} around 6 and decreased thereafter. Maximum BCF were observed for four-ring PAHs (e.g. pyrene, chrysene and benzo(a)anthracene) and for PCB congeners having four and five substituted chlorines.

In general, depuration of PAHs was faster than the clearance rates observed for PCB congeners. Bioconcentration and release of different PCB congeners seemed to be more affected by physicochemical factors such as molecular size and chlorine substitution patterns than by hydrophobicity. Thus, the magnitude of the accumulation of hydrophobic organic compounds by oysters is not exclusively determined by the contents of lipids in the organisms as previously speculated.

The most commonly reported distribution profile of PCB congeners in different organisms is similar to that encountered in the commercial PCB mixture Aroclor 1254 or in mixtures of Aroclors 1254 and 1248 or 1260. This might point to these mixtures as the most probable sources of the PCB congeners in different organisms. However, as a consequence of physicochemical factors that discriminate against the uptake of different congeners, the organisms may present a distribution of PCB congeners similar to that

found in Aroclor 1254 or in mixtures of Aroclors 1254 and 1248 or 1260 even if the organisms are exposed to a more complete suite of PCB congeners in the environment. The influence of these physicochemical factors in bioaccumulation is particularly evident in the case of planar PCB congeners. Compared to other PCBs within the same level of chlorination, planar congeners take a longer time to equilibrate into the lipid pools of the oysters ($t_{90\%}$). Similarly, the time needed for depuration of these from the oyster tissues was longer and this is paralleled by their significantly longer biological half-lives.

In general, TBT showed a biological half-life slightly longer than those observed for most PAHs, comparable to the values calculated for low molecular weight PCB congeners and significantly shorter than the half-lives estimated for most high molecular weight PCB congeners.

PAHs, PCBs and TBT were, in most cases, depurated faster by newly contaminated oysters than by chronically exposed individuals. A two-compartment model seems to be more appropriate to explain this phenomenon than a single one.

CHAPTER VII

SIMULTANEOUS UPTAKE AND DEPURATION OF PAHs AND PCBs BY THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

INTRODUCTION

PCBs and PAHs are known to be highly toxic to marine organisms (Hargis *et al.*, 1984; Malins *et al.*, 1984, 1987) and interactions between the two groups of contaminants are known to occur. PCBs, for example, can affect the toxicity of PAHs. Hawkes (1979) observed a more severe intestinal sloughing in marine chinook salmon when simultaneously exposed to PCBs and petroleum incorporated in food relative to separate exposures at similar concentrations. PCB exposure induces *in vitro* hepatic metabolism and DNA binding of benzo(a)pyrene in rainbow trout (Egaas & Varanasi, 1982).

Not only is the toxicity of these xenobiotics affected by PAH-PCB interactions but also their biological fate. The availability of PCBs to benthic organisms, for example, is reduced by the presence of oil in the substrate (Meier & Rediske, 1984). A prior exposure of Coho salmon to Aroclor 1254 substantially altered the biological disposition of [^{14}C]-labeled dimethylnaphthalene in the fish by increasing the levels of dimethylnaphthalene metabolites (Collier *et al.*, 1985). Bioaccumulation of [^{14}C]-naphthalene by oysters decreased with the simultaneous exposure to [^{14}C]-labeled PCBs and ^{3}H-benzo(a)pyrene (Fortner & Sick, 1985). The same study indicated that accumulation of a [^{14}C]-labeled PCB mixture by oysters was not always antagonistically

affected by simultaneous exposure to the three contaminants. Tissue accumulation of [^{3}H]-benzo(a)pyrene was not significantly affected. It has also been shown that simultaneous exposure of English sole to sediment-associated [^{3}H]-benzo(a)pyrene (BaP) and [^{14}C]-PCBs significantly increased the concentrations of BaP-derived radioactivity and decreased the concentrations of PCB-derived radioactivity in some tissues (Stein *et al.*, 1984). Several other studies have demonstrated PCB induction of many of the enzymes responsible for the metabolism of PAHs in different aquatic species (Moore *et al.*, 1980; Spies *et al.*, 1982; Anderson, 1985; Livingston, 1985).

In this chapter the uptake and depuration of selected PAHs and PCBs by the American oyster (*Crassostrea virginica*), exposed in the laboratory to particle-associated PAHs, PCBs and PAHs plus PCBs, are discused.

SIMULTANEOUS EXPOSURE TO PAHs AND PCBs: A LABORATORY STUDY

Aquarium Exposure

The aquarium exposures were conducted simultaneously with the transplant experiments in Galveston Bay (Chapters II and III). Oysters collected by dredge from the Hanna Reef area were transfered as soon as possible to 40 l glass aquariums and adapted to laboratory conditions for 7 days prior to the experiments. One hundred and twenty oysters were exposed in three aquariums, forty organisms per aquarium, to particles containing PCBs, PAHs and both PCBs and PAHs. Kaolin ($Al_2H_2Si_2O_8.H_2O$), which was found to have beiiefical effects on oystes growth (Langdon & Siegfried, 1984), was used as the adsorbant for PCBs and PAHs in this study. A fourth aquarium was used as a control. Uptake experiments were performed at one dosing level. The aquarium set-ups were as follows:

Aquarium A. Oysters were exposed to uncontaminated suspended particles at a nominal concentration of 10 mg l^{-1}.

Aquarium B. Oysters exposed to particle-associated PCBs. Nominal concentrations of suspended particles and total exposure PCBs were 10 mg l^{-1} and 10 μg g^{-1}, respectively. Nominal aquarium total PCB concentration was 0.10 μg l^{-1} (approximately 0.1 ppb).

Aquarium C. Oysters exposed to particle-associated PAHs. Nominal concentrations of suspended particles and total exposure PAHs were 10 mg l^{-1} and 240 μg g^{-1}, respectively. Nominal aquarium total PAH concentration was 2.4 μg l^{-1} (approximately 2.4 ppb).

Aquarium D. Oysters exposed to particle-associated PCBs and PAHs. Nominal concentrations of suspended particles and total exposure PCBs and PAHs were 10 mg l^{-1}, 10 μg g^{-1} and 240 μg g^{-1}, respectively. Nominal aquarium total PCB and PAH concentrations were 0.10 μg l^{-1} (0.1 ppb) and 2.4 μg l^{-1} (2.4 ppb), respectively.

The general experimental set up is shown in Fig. 36a. Each aquarium was designated to use recirculated seawater simulating a flow-through system (Fig. 36b). Briefly, seawater was recirculated by gravity through an activated charcoal-glass wool-polyurethane foam filter and air pumped back into the aquarium. Activated charcoal and polyurethane foam plugs act as solid adsorbant retaining dissolved PCBs and PAHs as well as gases and metabolic products from the test organisms (Gesser *et al.*, 1971; Basu & Saxena, 1978; Afghan *et al.*, 1984). Activated charcoal and foam plugs were changed daily. Foam plugs were washed with water, extracted three times with acetone and air dried prior to their use in the filtering systems. PCBs, PAHs and PCBs plus PAHs, adsorbed onto particles at environmental realistic concentrations, were pumped with a peristaltic pump (Fig. 36c) and mixed with the seawater entering the aquariums after

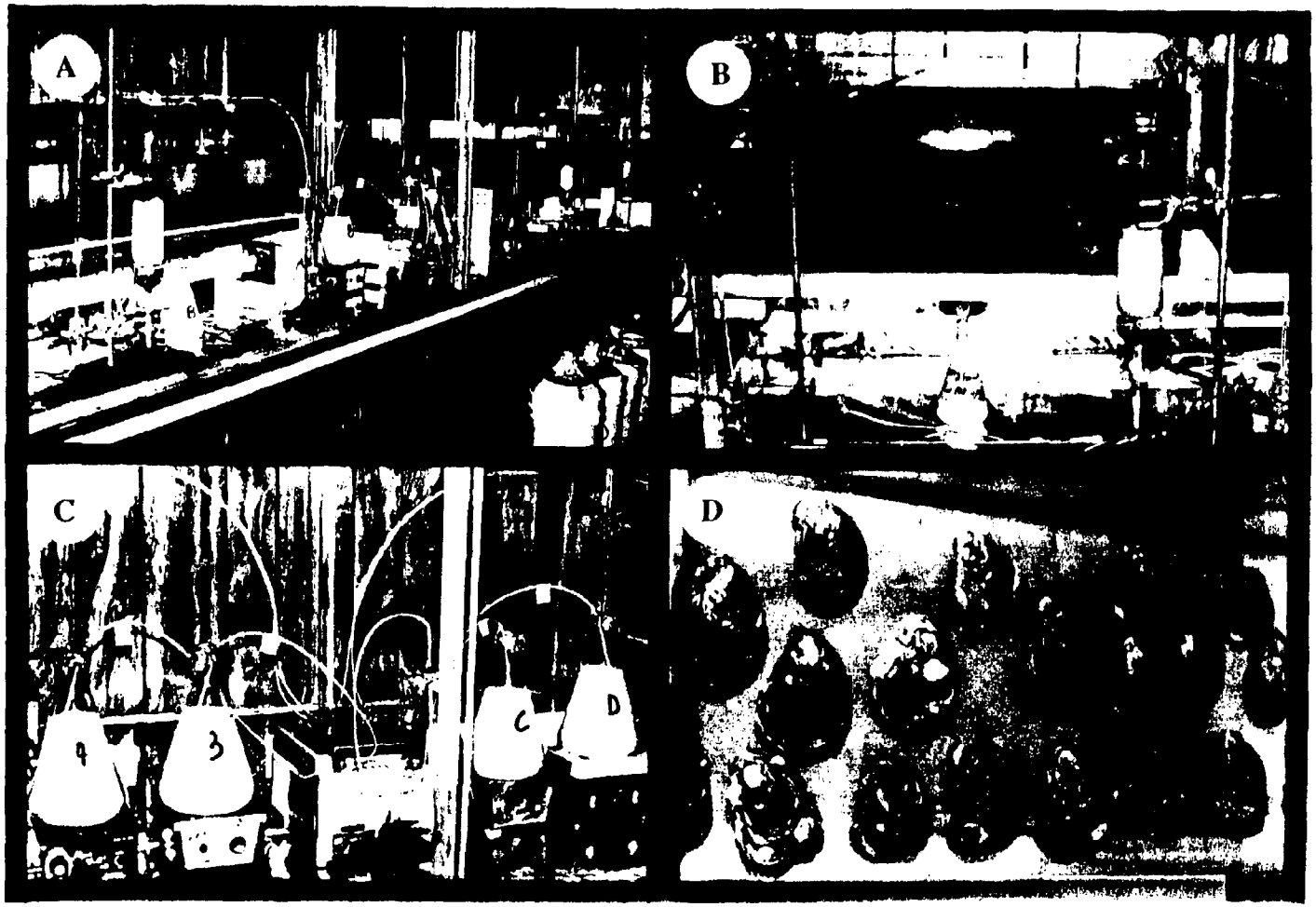


Fig. 36. General laboratory set-up (a), details of an aquarium and water recirculation system (b), dosing and feeding system (c), and oysters used during the experiment (d).

aeration to prevent losses of the most volatile contaminants during this process. A different line out of the peristaltic pump was used to feed the oysters. The bivalves were continuously fed with a mixture of two algae, *Thalassiosira fluviatilis* and *Isochrysis galbana*, raised on an f/2 algae food mixture. Temperature, pH, salinity, suspended particles and recirculation flow for each aquarium were monitored daily. Uptake studies lasted one month. Groups of five oysters, water and suspended particle samples were collected from each aquarium during the 3rd, 7th, 15th, and 30th days after the experiments started. A total of 20 oysters per aquarium were sampled during uptake experiments. Fig. 36d shows the sizes of the laboratory exposed oysters.

For depuration studies, groups of five oysters were sampled from each aquarium during the 3rd, 7th, 15th, and 30th days after the contaminant inputs were discontinued and the organisms were transferred to clean seawater. A total of 20 oysters per aquarium were sampled during the depuration period. Water samples from each aquarium were also collected.

Temperature, pH, salinity and recirculation flow for each aquarium were checked daily. With an average flow of approximately 5 l h^{-1} and a volume of water in the aquariums of 40 l, 95% of the water in the aquariums was filtered every 24 h (Spague, 1969). When necessary, the salinity of the aquarium was adjusted with HPLC water to the starting value of 18‰. Particle concentrations in the aquariums were in the range of 6.4 to 11 mg l^{-1}, a concentration range commonly reported for coastal marine environments (see, for example, Cadee, 1982; Colijn, 1982). Mortality of the exposed oysters was minimal throughout the experiment (one oyster in Aquarium D). Control oysters showed little change in analyte concentrations during the 60-day exposure and depuration experiments. The reported concentrations correspond to five pooled oysters.

Extraction, fractionation and instrumental analyses of PAHs and PCBs

The extraction and fractionation, as well as instrumental analyses of PAHs and PCBs were discussed in Chapters II and III, respectively.

Polynuclear Aromatic Hydrocarbons

PAH concentrations measured in oysters collected from Aquariums A (control), C (PAHs), and D (PAHs plus PCBs) during the uptake and depuration experiments are plotted in Fig. 37. In general, exposed oysters rapidly accumulated four- and five- and some three-ring compounds. In this molecular range, some PAHs reached an apparent steady state concentration 10 days after the start of the experiments (e.g., 1-methylphenanthrene and pyrene). Most of the analytes, however, had not reached a concentration plateau after 30 days (e.g., benz(a)anthracene, chrysene, benzo(e)pyrene and perylene). Two- and most of the three-ring PAHs were detected at low concentrations in both groups of oysters.

The PAHs accumulated in highest concentration were the same in organisms exposed to PAHs alone or simultaneously to PAHs plus PCBs (Fig. 38). However, concentrations of individual PAHs in oysters exposed solely to PAHs were, at the end of the 30-day exposure period, lower than the concentrations encountered in oysters exposed to the mixture PAHs plus PCBs. The PAHs accumulated to the highest concentration after 30 days in oysters exposed only to PAHs were: benzo(b)fluoranthene, benzo(e)pyrene, benz(a)anthracene, chrysene and indeno-[1,2,3-c,d]pyrene whereas the accumulation order found in second group of oysters was: benzo(b)fluoranthene, benz(a)anthracene, benzo(e)pyrene, chrysene and indeno[1,2,3-c,d]pyrene. Most of these PAHs were also preferentially accumulated by HRSC and SC oysters under field conditions. Therefore, the laboratory uptake confirms the environmental findings. When exposed to a wide molecular weight range of PAHs, i.e. two- to six-ring compounds,

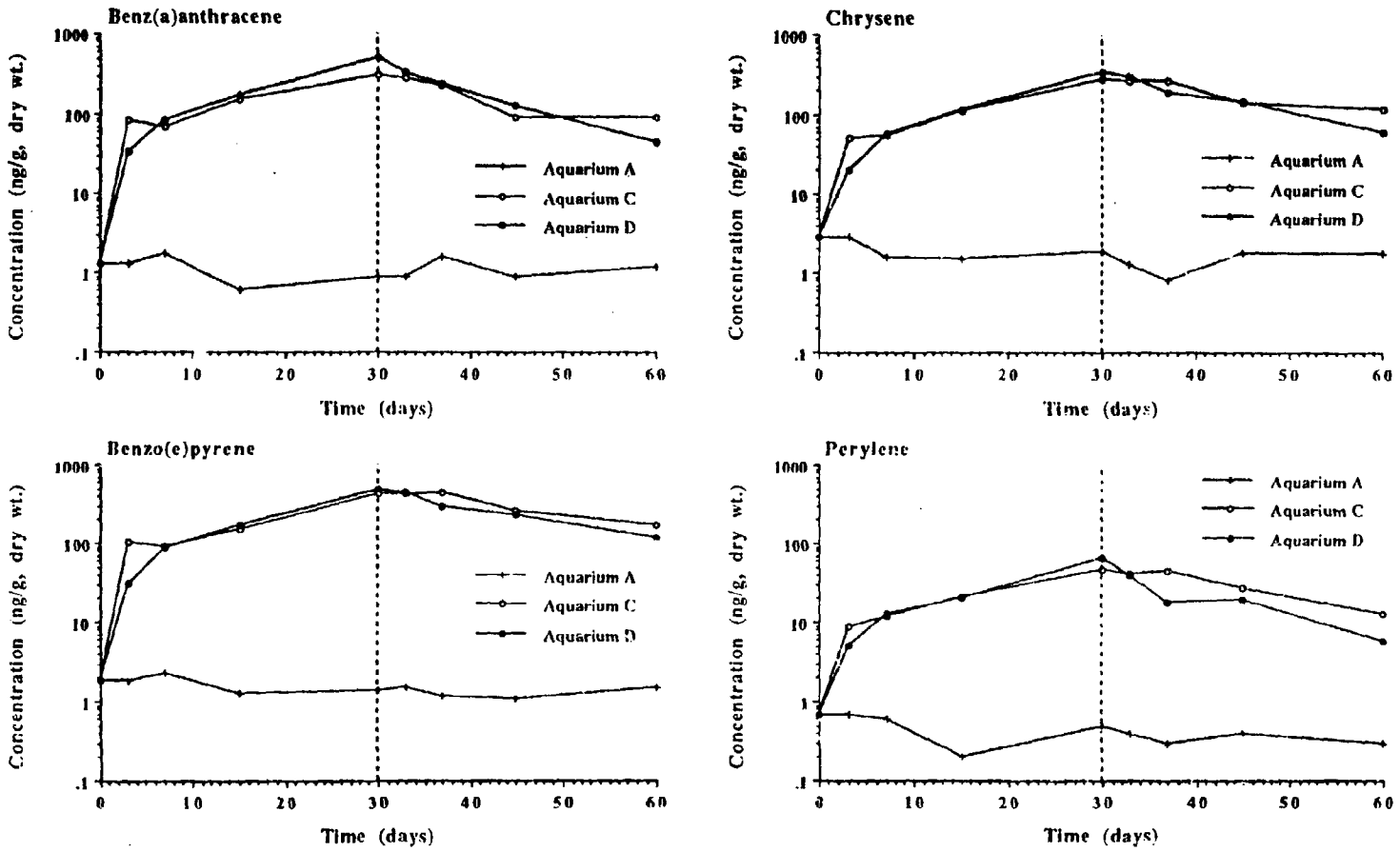


Fig. 37. Concentrations of selected polynuclear aromatic hydrocarbons in tissues of oysters during exposure to particle-associated PAHs alone (Aquarium C) and PAHs + PCBs (Aquarium D) and following transplant to contaminant-free aquariums. Aquarium A was used as control.

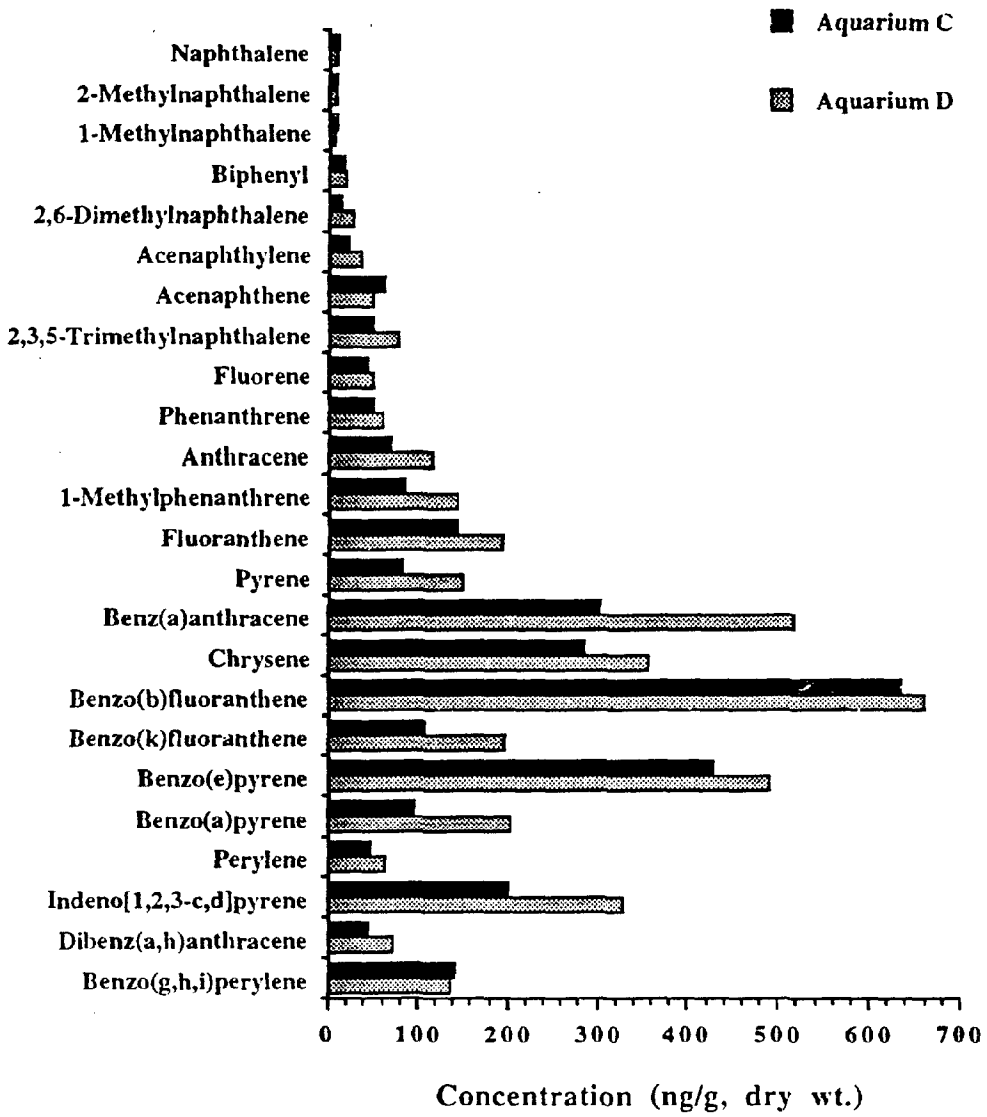


Fig. 38. Concentrations of individual polynuclear aromatic hydrocarbons in tissues of laboratory exposed oysters after the 30-day exposure period to particle-associated PAHs (Aquarium C) and PAHs + PCBs (Aquarium D).

oyster preferentially bioconcentrate those analytes having four and five rings. This preferential uptake is unrelated to the presence or absence of PCBs.

When the input of contaminants was stopped, both oyster groups showed statistically significant depuration of most of the PAHs accumulated during the first phase of these experiments. However, as previously discussed for environmentally contaminated oysters, they were unable to reach the low concentrations encountered for some of these analytes before the exposure. Fig. 39 compares the final concentrations of PAHs at the end of the 30-day depuration period in oysters exposed to particle-associated PAHs and PAHs plus PCBs. At the end of the 30-day depuration period, the total PAH loads in both groups of exposed oysters were dominated by heavier molecular weight PAHs, i.e. four- and five-ring compounds.

Oysters that had been exposed simultaneously to PAHs and PCBs depurated PAHs at a faster rate than oysters exposed only to PAHs. Calculated half-lives for both groups of oysters are shown in Table 9. In PAH exposed oysters, the estimated half-lives ranged from 9 (fluoranthene and pyrene) to 25 (benzo(b)fluoranthene/benzo(k)fluoranthene) days. Comparatively, PAHs plus PCBs exposed oysters yielded PAH half-lives ranging from 6 (pyrene) to 15 (benzo(e)pyrene) days. Most of the values were, however, in the range 15 to 17 days and 8 to 10 days, for the first and second groups of oysters, respectively.

In general, the estimated half-lives are in good agreement with previously published values and with the calculated clearance rates for HRSCHR and SCHR oysters presented in Chapters II and VI; however, some differences exist. First, it is evident that, except for 2,3,5-trimethylnaphthalene, anthracene, 1-methylphenanthrene and fluoranthene, the half-lives calculated for oysters exposed simultaneously to a mixture of PAHs and PCBs compare better to the environmental half-life values estimated for HRSCHR oysters than the values obtained from the oysters exposed only to PAHs. This is consistent with the

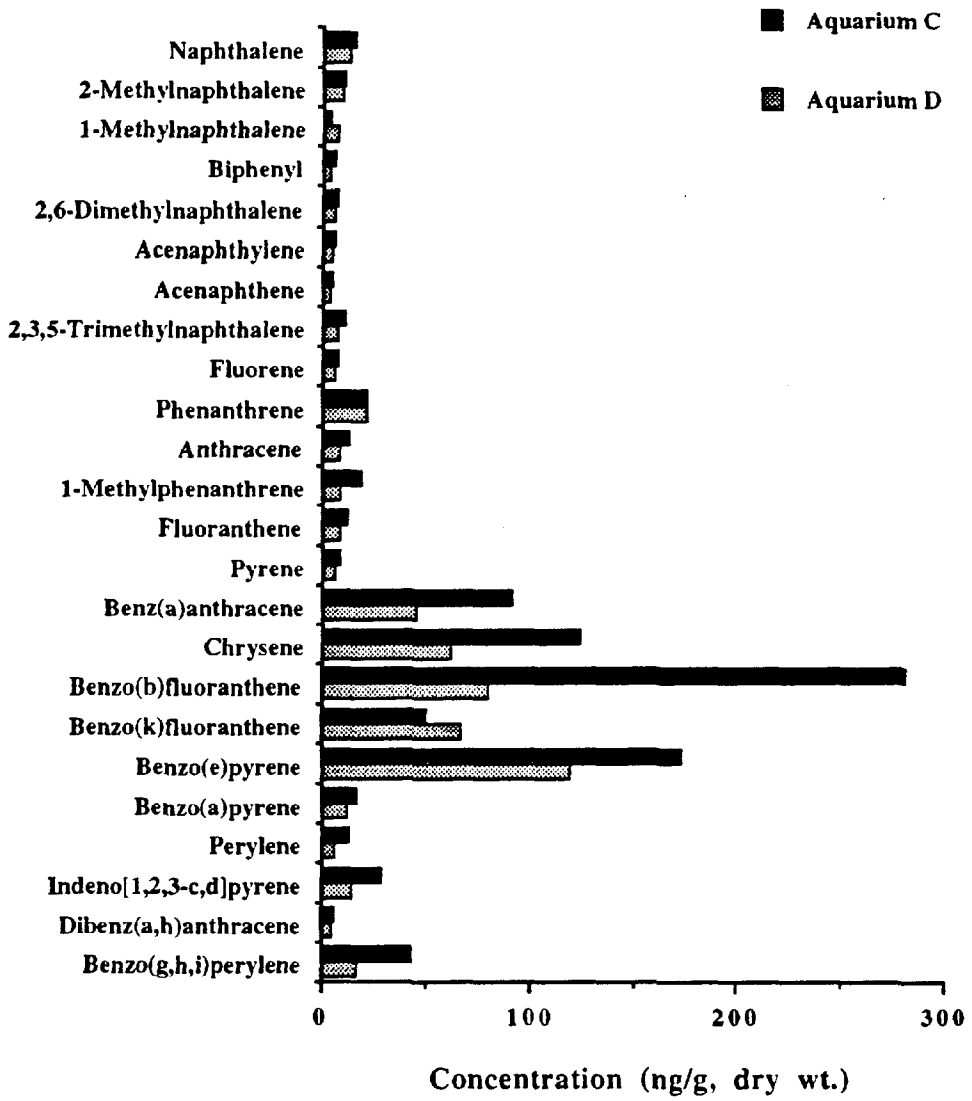


Fig. 39. Concentrations of individual polynuclear aromatic hydrocarbons in tissues of oysters previously exposed in the laboratory to particle-associated PAHs (Aquarium C) and PAHs + PCBs (Aquarium D) after the 30-day depuration period in contaminant-free aquariums.

TABLE 9

Biological Half-Lives of PAHs in *Crassostrea virginica* Oysters Exposed in the Laboratory to Particle-Associated PAHs Alone (Aquarium C) and PAHs + PCBs (Aquarium D).

Analyte	Biological Half-Lives (R^2)[a]	
	Aquarium C	Aquarium D
2,3,5-Trimethylnaphthalene	16 (0.75)	10 (0.91)
Anthracene	16 (0.68)	8 (0.89)
1-Methylphenanthrene	16 (0.72)	7 (0.88)
Fluoranthene	9 (0.78)	7 (0.85)
Pyrene	9 (0.75)	6 (0.89)
Benz(a)anthracene	16 (0.81)	9 (0.99)
Chrysene	22 (0.86)	12 (0.98)
Benzo(b)fluoranthene/ Benzo(k)fluoranthene	25 (0.94)	13 (0.95)
Benzo(e)pyrene	21 (0.93)	15 (0.98)
Benzo(a)pyrene	12 (0.87)	9 (0.78)
Perylene	15 (0.96)	10 (0.89)
Indeno[1,2,3-c,d]pyrene	10 (1.00)	8 (0.78)
Dibenz(a,h)anthracene	11 (0.97)	10 (0.69)
Benzo(g,h,i)perylene	16 (0.96)	11 (0.92)

[a] R^2 = square of the correlation coefficient for the regression equation.

When combined, these congeners accounted for 43.9 and 36.7% of the total PCBs in oysters and sediments, respectively.

ΣPCB Congeners/Total PCB Relationship

Several methods have been used to quantitate PCBs in environmental samples. In the past, for example, PCB concentrations have been expressed as the equivalent Aroclor mixtures (e.g. Bopp *et al.*, 1981; Pugsley *et al.*, 1985; Brownawell & Farrington, 1986) or as their similar foreign technical formulations, e.g. Clophen (Eder *et al.*, 1981) or Phenochlor (Elder *et al.*, 1979). An accurate determination of total PCBs in environmental samples would have to be carried out with the use of each individual congener as reference material (Duinker *et al.*, 1980). With the introduction of capilary columns and the availability of almost every individual PCB congener as a standard, several reserchers have attempted to report total PCB concentrations as the sum of all the measurable individual congeners. However, some of these congeners are not always separated from other congeners on a single GC capillary column (Duinker *et al.*, 1988a). Duinker *et al.* (1988b) suggested a number of different congeners, in addition to those recommended by ICES, that could be accurately analyzed in environmental samples. These congeners cover all the levels of chlorination and satisfy the condition of good GC separation on an SE-54 or similar capillary column. A variant of this approach was initially used in reporting the total PCB concentrations in oyster and sediment samples collected from the Gulf of Mexico as part of the NS&T "Mussel Watch" Program.

During 1986 and 1987, 18 different congeners (i.e. PCB 8, 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206 and 209) were supplied by NIST, formerly NBS, for making quantitation standards. These congeners, which are some of the major congeners found in commercial Aroclor mixtures, are among those commonly reported in environmental samples. Nine of these congeners (i.e. PCB 8, 28, 52, 101,

153, 170, 195, 206 and 209), specified by NOAA, were used as reference congeners, representative of a given degree of chlorination from Cl_2 to Cl_{10}, to determine other congener concentrations at each level of chlorination. Results were reported as the sum of congeners within each level of chlorination and total PCB as the sum of these amounts. One obvious problem with this method of quantitation is the different relative response factor for each congener. Thus, a congener that does not have a standard to be directly compared to might be underestimated or overestimated because of the difference between its relative response factor and that of the corresponding representative congener.

Discussions among the different participating laboratories in this program directed to improve the PCB reporting led to the adoption of an equation that relates the sum of the 18 individual congener concentrations in the samples with the total PCB loads. Fig. 48 shows, for example, the correlation encountered in oyster samples collected in the Gulf of Mexico during 1986. Therefore, starting in 1988, total PCB concentrations in oyster and sediment samples from the Gulf of Mexico, Atlantic and Pacific coasts, including Hawaii, were estimated and reported using this new approach.

During the first year of the NS&T program total, PCBs ranged from 10 to 4,020 ng g^{-1} (Sericano *et al.*, 1990a). Nearly 95% of the samples had a total PCB load below 500 ng g^{-1}. Therefore, the correlation between the sums of the 18 individual PCB congeners and the total PCB concentrations in oyster samples is likely to be affected by a small percentage of samples collected from heavily contaminated sites. Table 11 shows correlations for the same data set when ranges are chosen to eliminate the bias introduced by the highly contaminated samples. For example, just eliminating the highest PCB concentration measured in a sample collected near the Yacht Club, in Galveston Bay, reduces the near-perfect correlation of 0.99 to 0.97. Considering samples with total PCB concentrations of 500 ng g^{-1} or lower (i.e. 95% of the samples) decreases the correlation to 0.92 (Fig. 48b). Further reductions of the data set to maximum concentrations lower

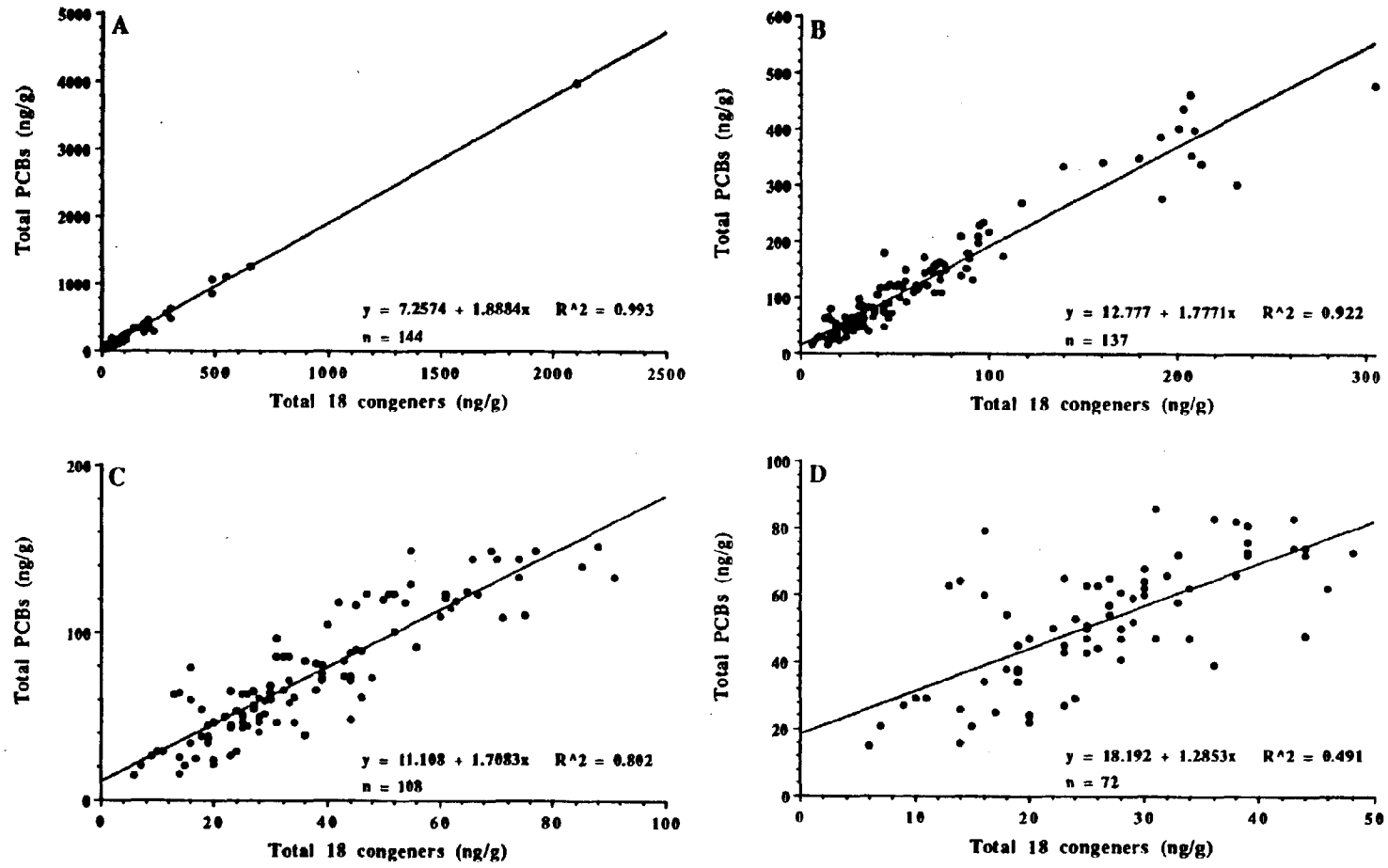


Fig. 48. Relationships between the sum of 18 selected PCB congeners and the total PCB load encountered in Gulf of Mexico oysters for the first year of NOAA's National Status and Trends Program. See text for discussion.

TABLE 11

PCB Congeners/Total PCB Relationships in Gulf of Mexico Oyster Samples.

n	Total PCB[a] ng g^{-1}	Fraccion %	Regression equation	R^2
			1986	
144	all data	100	$\Sigma PCB=7.25+1.89\Sigma Cong$[b]	0.99
143	≤2000	99	$\Sigma PCB=6.52+1.90\Sigma Cong$	0.97
140	≤1000	97	$\Sigma PCB=12.29+1.79\Sigma Cong$	0.95
137	≤500	95	$\Sigma PCB=12.78+1.78\Sigma Cong$	0.92
108	≤157	75	$\Sigma PCB=11.11+1.71\Sigma Cong$	0.80
66	≤86	50	$\Sigma PCB=18.19+1.29\Sigma Cong$	0.49
			1987[c]	
149	all data[d]	99	$\Sigma PCB=0.81+2.30\Sigma Cong$	0.96
140	≤300	95	$\Sigma PCB=13.8+1.89\Sigma Cong$	0.81
129	≤200	87	$\Sigma PCB=12.5+1.83\Sigma Cong$	0.75

[a]upper limit of the data range corresponding to the total PCBs calculated from level of chlorination; [b]sum of 18 individual PCB congeners; [c]Brooks *et al.* (1988); [d]two outliers eliminated from regression analysis.

than or equal to 157 ng g^{-1} (75% of the samples) or to 86 ng g^{-1} (50% of the samples) will reduce the correlation coefficient to 0.80 and 0.52, respectively (Fig. 48c and d, respectively). Similar correlations were reported for oyster and sediment samples collected during 1987 (Brooks *et al.*, 1988; Table 11). Joiris & Overloop (1991) showed the correlations between the sum of nine of the most "classical" congeners (i.e. PCB congeners 28, 52, 101, 118, 138, 153, 170, 180 and 194) and total PCBs expressed as Aroclor 1254 as well as correlations between individual congeners and total PCBs in particulate matter (mainly phytoplankton) and netplankton (mainly zooplankton with some phytoplankton) samples collected in the Indian sector of the Southern Ocean. Although no correlation factors are given in the report, coefficients of regressions (R^2) between the sum of the nine congeners and total PCB concentrations, estimated from new plots made from their figures, were about 0.85 and 0.56 for particulate matter and netplankton samples, respectively. Total PCB concentrations in particulate matter and netplankton samples ranged from about 200 to 2900 ng g^{-1} and from 70 to 510 ng g^{-1} on a dry weight basis, respectively. This data analysis indicates that the correlation between the sums of individual PCB congeners and the total PCB concentrations in environmental samples appears to be dependent on the total PCB load.

Although it has been shown that after exposure to a wide range of molecular weight PCBs oysters will preferentially uptake four-, five-, and six-chlorine substituted congeners (see Chapter VII), there might be differences in the residual PCB profiles among oyster samples collected from different geographical areas as a result of a variety of local sources. The Σcongeners/total PCB ratios calculated from the data reported by Schulz *et al.* (1989) encountered in Aroclor mixtures 1016, 1242, 1254 and 1260 were 2.61, 2.86, 2.63 and 2.55, respectively. These ratios can be modified in the environment as a consequence of the differential physico-chemical and biological properties of individual congeners controlling their water transport, bioaccumulation, etc. Different

residual PCB compositions in oysters will obviously produce different results. For example, total PCB concentrations, calculated as the sum of all measurable PCB congeners, in oyster samples collected near the Houston Ship Channel area in Galveston Bay over a seven-week period (see Chapter III for more details) yielded concentrations that constantly were between 30 to 35% higher than the concentrations estimated with the above correlation (Fig. 49). Another way to illustrate this assertion is to consider the PCB profiles encountered in uncontaminated Hanna Reef oysters when transplanted to the upper Galveston Bay area near the Houston Ship Channel (Fig. 17, Chapter III). During this experiment, low molecular weight PCBs were bioaccumulated at a faster rate than congeners with higher level of chlorination. By the end of the 48-day exposure period, the amount of total PCB estimated by the equation was up to 60% lower than the total PCB load measured as the sum of all individual congeners (Fig. 49).

Although the transplantation experiment can be compared to an extreme case of a rapid environmental PCB contamination, similar disequilibrium between PCB concentrations in oysters and environmental leves might also be a consequence of natural processes related to the bivalves themselves such as spawning. It has been suggested that high variability in xenobiotic concentrations in bivalves from a given location might be more related to the stage of the reproductive cycle and its associated biochemical modifications than to environmental changes (Jovanovich & Marion, 1987). Most organisms have a marked increase in their lipid contents during gametogenesis, which is followed by a drastic loss of lipidic material with the gametes at spawning (Phillips, 1986). Since most hydrophobic trace organic contaminants will tend to concentrate in lipid-rich tissues, such as eggs, it is evident that their concentration will vary with the sexual cycle. Release of hydrocarbons and pesticides during spawning has been reported for *Mytilus edulis* and *Crassostrea virginica*, respectively (Lowe *et al.*, 1971; Fossato & Canzonier, 1976).

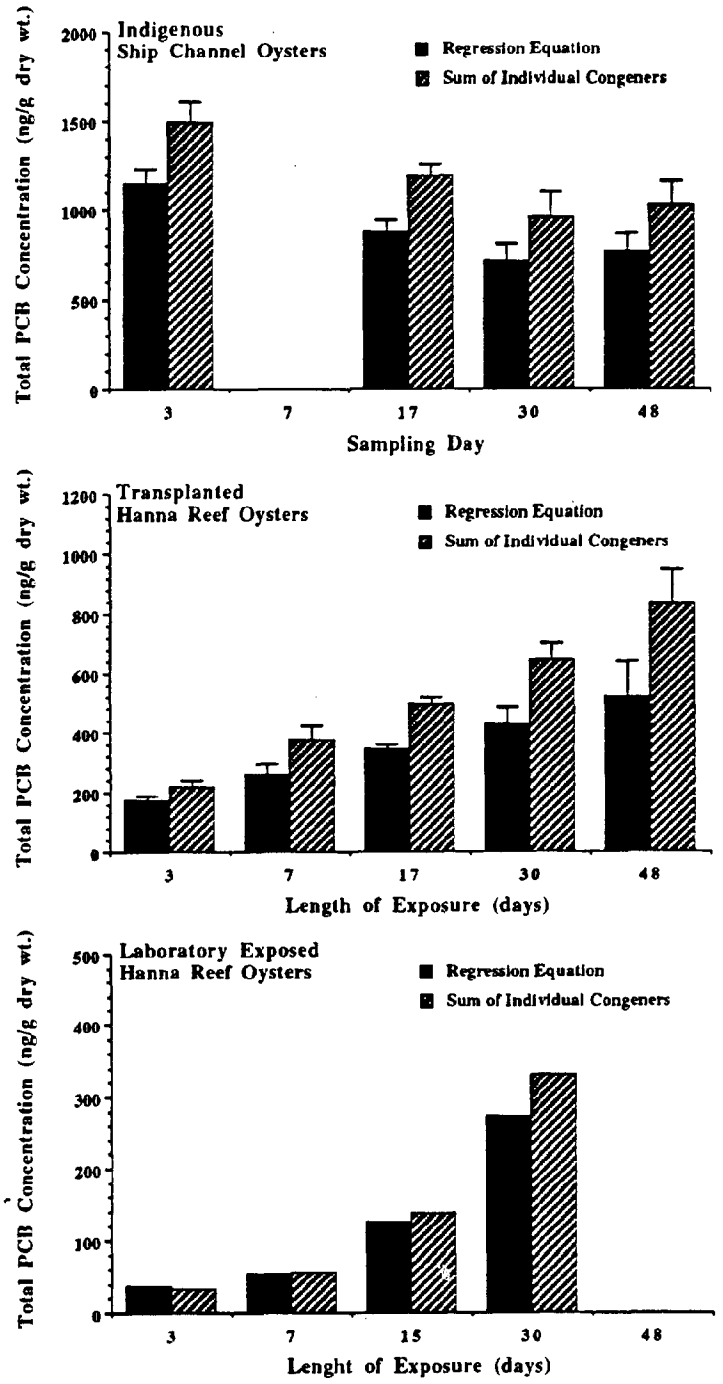


Fig. 49. Three different examples of the bias introduced in the report of total PCB concentrations by using the regression equation (see text) compared to the total PCB load calculated as the sum of all measurable individual congeners.

It may be concluded that even though there is a reasonable correlation between the sums of 18 individual PCB congeners and the total PCB concentrations in oyster samples and it might provide an estimation of the total PCB load, the preceeding discussion indicates that it must be applied with caution when reporting and interpreting environmental data. The greatest disadvantage of this procedure is that much of the information is lost when complete congener characterization of PCB residues in environmental samples is not reported. This is emphasized by the fact that PCB composition changes drastically as they move from one environmental compartment to another.

Planar PCB Congeners

PCB congeners have been widely reported in oyster samples collected as part of this program in the Gulf of Mexico (Sericano *et al*, 1990a); however, the occurence of toxic planar PCB congeners, i.e. 77, 126 and 169, have not until recently been reported (Sericano *et al.*, 1992).

The concentrations of planar congeners, 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. 50), collected during winter 1990-1991 (year 4 of the NS&T program), are summarized in Table 12. 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 shows high values near population centers. The same correlation between urban centers and concentrations of planar PCBs can be

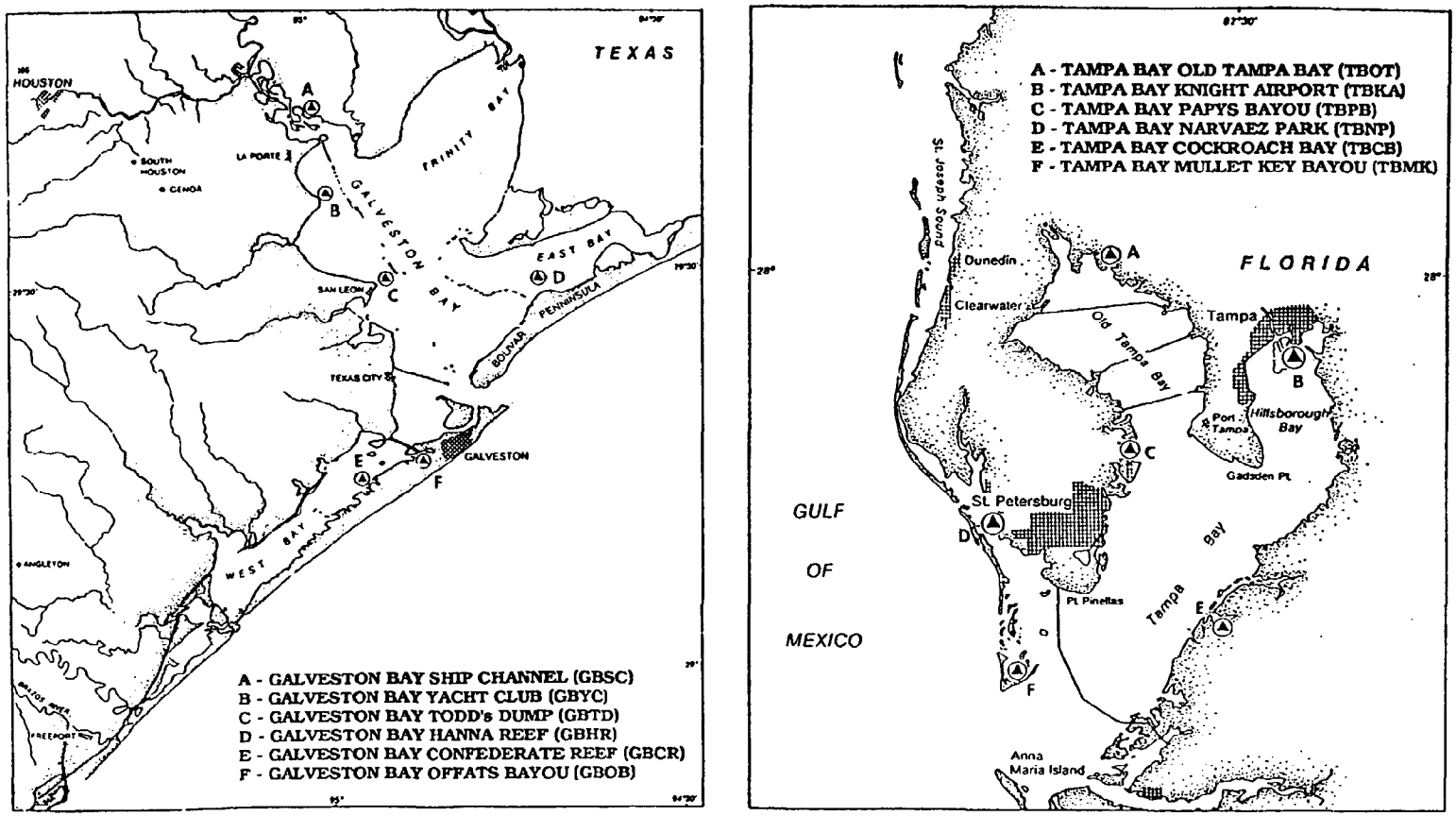


Fig. 50. NOAA's National Status and Trends sampling locations in Galveston and Tampa Bays.

TABLE 12

Planar and Total 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	2,000	2,200	790	1,100±120
GBYC	330	210	190	210±14
GBTD	140	120	54	110±18
GBHR	89	110	89	50±7.0
GBCR	100	94	51	77±9.6
GBOB	500	400	93	160±44
Tampa Bay				
TBOT	170	320	280	55±8.5
TBKA	1,500	330	84	580±230
TBPB	85	100	51	75±27
TBNP	260	140	150	120±31
TBCB	200	290	100	49±20
TBMK	ND	ND	ND	38±14

ND = not detected

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; Schulz *et al.*, 1989), these congeners were detected at much lower concentrations than other mono- and di-*ortho* substituted PCB congeners. In commercial PCB mixtures, the concentration of congener 77 is one to two and three to five orders of magnitude higher than concentrations of congeners 126 and 169, respectively (Kannan *et al.*, 1987). Therefore, it appears that congeners 126 and 169 are enriched with respect to congener 77 in oyster samples from Galveston and Tampa Bays. This is not surprising since the log K_{ow} (octanol-to-water coefficient) increases with the number of chlorines substituted in the biphenyl rings (6.36, 6.89 and 7.42 for congeners 77, 126 and 169, respectively; Hawker & Connell, 1988). 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 review, Safe (1990) discussed the environmental and mechanistic considerations behind the development of the Toxic Equivalent Factor (TEF) concept. Safe proposed provisional TEF values of 0.01, 0.1 and 0.05 for planar congeners 77, 126 and 169, respectively. Recently, the validation and limitations of these factors have been reported (Safe, 1992). Calculated 2,3,7,8-TCDD equivalents, in pg g^{-1}, in oyster tissues collected from Galveston and Tampa Bay, as well as their averages, are listed in Table 13. In Tampa and Galveston Bays, the total 2,3,7,8-TCDD equivalents ranged from 14 to 52 pg g^{-1} and from 13 to 280 pg g^{-1}, respectively. 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

TABLE 13

2,3,7,8-TCDD Equivalents (pg g^{-1}) Corresponding to Non-*Ortho* Substituted PCB in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays.

Sample	Congener			Total
	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	2.0	29	5.0	36
TBMK	-	-	-	-

or sport oystering due to bacteria concentrations; therefore, the high PCB levels are not a human health threat.

As discussed earlier, congeners 77, 126 and 169 are present at trace concentrations in commercial PCB mixtures and at very low concentrations in environmental samples; however, their mono-*ortho* derivatives (e.g. congeners 105, 118, 156 and 189) may be more important in terms of both TCDD-like activity and occurrence (Safe, 1984). Certain di-*ortho* derivatives of the *m,m' p,p'* sustitution pattern (e.g. congeners 128, 138, 153 and 170) are significant components of PCB residues (Duinker *et al.*, 1988a; Schulz *et al.*, 1989; Schwartz *et al.*, submitted). Congeners 128, 138 and 170 have reduced TCDD-like activity compared to their parent planar congeners whereas PCB 153 lacks of TCDD-like responses (Hansen, 1987). Safe (1990) proposed provisional TEF values of 0.001 and 0.00002 for mono- and di-*ortho* chlorine substituted PCB congeners, respectively.

The concentrations of PCBs 105, 118, 128 and 138 as well as total PCBs in oyster samples from sites in Galveston and Tampa Bays are summarized in Table 14. These congeners are derivatives of planar PCB 77. Individually, the concentrations of these mono- and di-*ortho* congeners were, as expected, one to two orders of magnitude higher than planar PCB concentrations. In order to assess the environmental significance of these congeners in terms of TCDD-like effects in oyster samples from Galveston and Tampa Bay, the calculated 2,3,7,8-TCDD equivalents (Table 15) are compared to those corresponding to planar congeners. In spite of the relatively lower toxic effect of congeners 105 and 118 compared to planar PCBs, these congeners might have a significant toxic impact in the environment. Most of the relative toxicity in oyster, however, are due to the presence of planar PCBs (53.8 to 94.3%; Fig. 51). Contribution of congeners 105 plus 118 to the total 2,3,7,8-TCDD equivalents was as high as 45.4%; in contrast, the contribution of di-*ortho* congeners is negligible (<1.0%). The lesser

TABLE 14

Selected Mono- and Di-*Ortho* Substituted PCB and Total PCB Average Concentrations (ng g^{-1}) in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays.

Sample	Congener				Total PCBs
	105	118	128	138	
Galveston Bay					
GBSC	39±4.1	48±5.8	4.4±0.6	50±6.7	1,100±120
GBYC	4.1±1.7	9.0±0.3	1.5±0.2	13±3.2	210±14
GBTD	1.3±0.2	5.2±1.0	0.6±0.2	5.7±1.1	110±18
GBHR	0.6±0.5	1.2±0.3	0.6±0.2	4.3±0.8	50±7.0
GBCR	0.7±0.6	2.8±0.2	0.7±0.3	5.0±1.4	77±9.6
GBOB	3.2±1.8	10±2.7	1.0±0.3	8.7±3.4	160±44
Tampa Bay					
TBOT	0.4±0.2	2.4±1.6	0.2±0.2	4.0±0.8	55±8.5
TBKA	7.6±3.7	36±15	2.0±1.0	30±13	580±230
TBPB	0.4±0.1	3.0±0.7	0.3±0.2	6.1±2.6	75±27
TBNP	1.3±0.2	7.3±1.8	0.6±0.2	8.9±3.1	120±31
TBCB	0.4±0.2	3.0±1.1	0.2±0.2	2.8±1.2	49±20
TBMK	0.3±0.2	1.6±0.3	0.2±0.1	3.3±2.0	38±14

TABLE 15

Average 2,3,7,8-TCDD Equivalents (pg g^{-1}) Corresponding to Selected Mono- and Di-*Ortho* Substituted PCBs in Oysters (*Crassostrea virginica*) from Galveston and Tampa Bays.

Sample	Congener				Total	Total[a]
	105	118	128	138		
Galveston Bay						
GBSC	39	48	0.1	1.0	89	379
GBYC	4.1	9.0	<0.1	0.3	13	47
GBTD	1.3	5.2	0.1	0.1	6.7	23
GBHR	0.6	1.2	<0.1	0.1	1.9	18
GBCR	0.7	2.8	<0.1	0.1	3.6	17
GBOB	3.2	10	<0.1	0.2	14	64
Tampa Bay						
TBOT	0.4	2.4	<0.1	0.1	2.9	51
TBKA	7.6	37	<0.1	0.6	45	97
TBPB	0.4	3.0	<0.1	0.1	3.6	18
TBNP	1.3	7.3	<0.1	0.2	8.8	33
TBCB	0.4	3.0	<0.1	0.1	3.5	40
TBMK	0.3	1.6	<0.1	0.1	2.0	2.0

[a]Includes PCB congeners 77, 126, 169, 105, 118, 128 and 138.

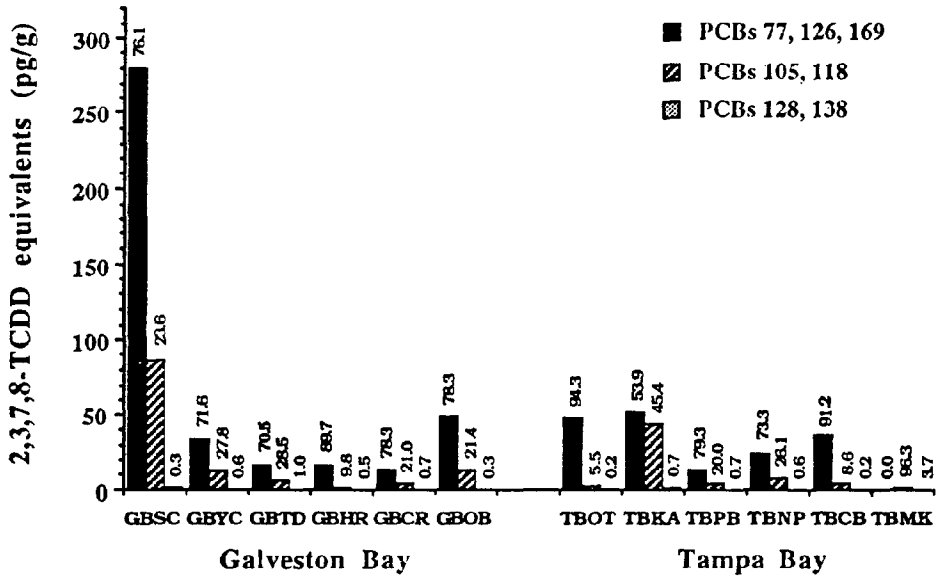


Fig. 51. Toxic equivalents corresponding to three planar PCBs and selected mono- and di-*ortho* chlorine-substituted congeners in oyster samples collected from six different locations in Galveston and Tampa Bays.

toxicity of the di-*ortho* congeners is a consequence of their much lower TCDD-like activity rather than lower concentrations. As shown in Fig. 52, most of the toxicity corresponding to planar PCBs is contributed by congener 126 while that corresponding to mono-*ortho* derivatives is due to congener 118.

Although none of the other PCB congeners considered to be inducers of hepatic aryl hydrocarbon hydroxylase (AHH) activity, i.e. congeners 123, 114, 158, 166, 167, 156, 157, 170 and 189), have been quantitated in Galveston and Tampa Bay oyster samples, the concentration of most of them in commercial Aroclor mixtures are very low (Schulz *et al.*, 1989). With the exemption of congeners 123, 158 and 170, which range from the minimum reporting level (<0.05%) to 0.81, 1.55 and 3.91%, respectively, in different Aroclor mixtures, the contributions of the rest of the individual AHH-active congeners are below 0.30%. Therefore, the contribution of these mono- and di-*ortho* AHH-active PCBs to the total toxicity of environmental samples is expected to be negligible. For example, congeners 77, 126, 169, 105 and 118 accounted for nearly 99% of the total toxicity, calculated as the sum of the toxic equivalents of each individual AHH-active congener, encountered in eggs (Smith *et al.*, 1990). Thus, it can be speculated that the total toxic equivalents reported for oyster samples collected in Galveston and Tampa Bays (Table 15) would not increase by more than 10% of the total TEF values if all the AHH active PCBs had been analyzed.

BUTYLTIN SPECIES

The decision to include butyltin compounds as part of NOAA's NS&T Program was a consequence of the increasing concern about the adverse effects of TBT to non-target organisms. Thus, butyltin compounds have been monitored in Gulf of Mexico oyster samples since 1986.

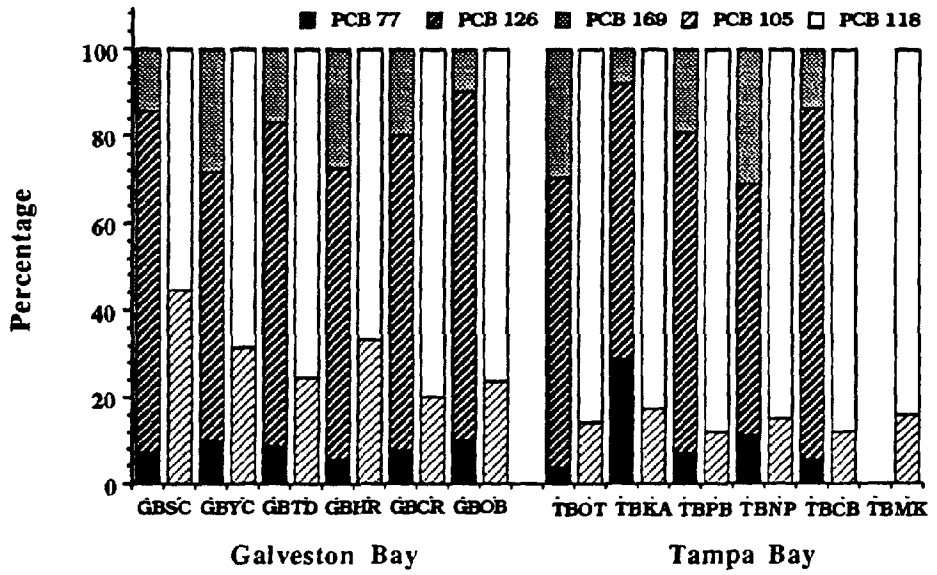


Fig. 52. Contribution of planar and selected mono-ortho chlorine substituted PCB congeners to the total toxicity in oyster.

The use of TBT in antifouling paints in the U.S., on vessels under 25 m, was banned in 1988 (U.S. EPA., 1987). In that year, the reported average concentration of TBT in bivalves for U.S. coastal sites was 366 ng Sn g^{-1} (Wade *et al.*, 1988b). In general, the body burden of the butyltin species was TBT>DBT>MBT. With a half-life of 34 $days^{-1}$ in chronically contaminated oyster (see Chapter V), it would have taken about 240 days (0.6 years) for the average concentrations encountered in Gulf of Mexico oysters of TBT to be below the present detection limit (5 ng Sn g^{-1}) if all the inputs were stopped at that time. Obviously, this is not a realistic estimation because the use of TBT was not completely banned and because there might be a number of boats in use that had been painted just before the ban. Also, TBT present in sediments, with a reported half-life of more than 20 weeks, may be a long term source of TBT to the environment (Harris & Cleary, 1987; Johnson *et al.*, 1987; Maguire, 1986; Stang & Seligman, 1987; Unger *et al.*,1987; Valkirs *et al.*, 1986, 1987b).

Some changes can be seen, however, at some areas that have been followed since the beginning of the Status and Trends Program. As mentioned before, it coincides with the ban on the use of TBT-containing paints in U.S. waters. For example, Naples Bay, Florida, has a very heavy recreational boating activity. At this site, a decreasing trend in the total concentration of butyltins has been observed since 1988 (Fig. 53). A similar decrease has been detected at Biloxi Bay, Mississippi. Under the actual input/degradation conditions, it seems that a decrease of 50% in environmental TBT concentrations in these and other areas similar to Naples and Biloxi Bays takes about 2-3 years. This is about an order of magnitude larger than the time needed for oysters to depurate when transplanted to a clean environment. At this rate, and assuming no environmental redistribution of TBT, its concentrations in oysters from sites like Naples and Biloxi Bays should be below the present detection limits (i.e. 5 ng Sn g^{-1}) in 8 to 12 years. The much slower decreases at sites with extensive recreational boating suggests that in spite of the

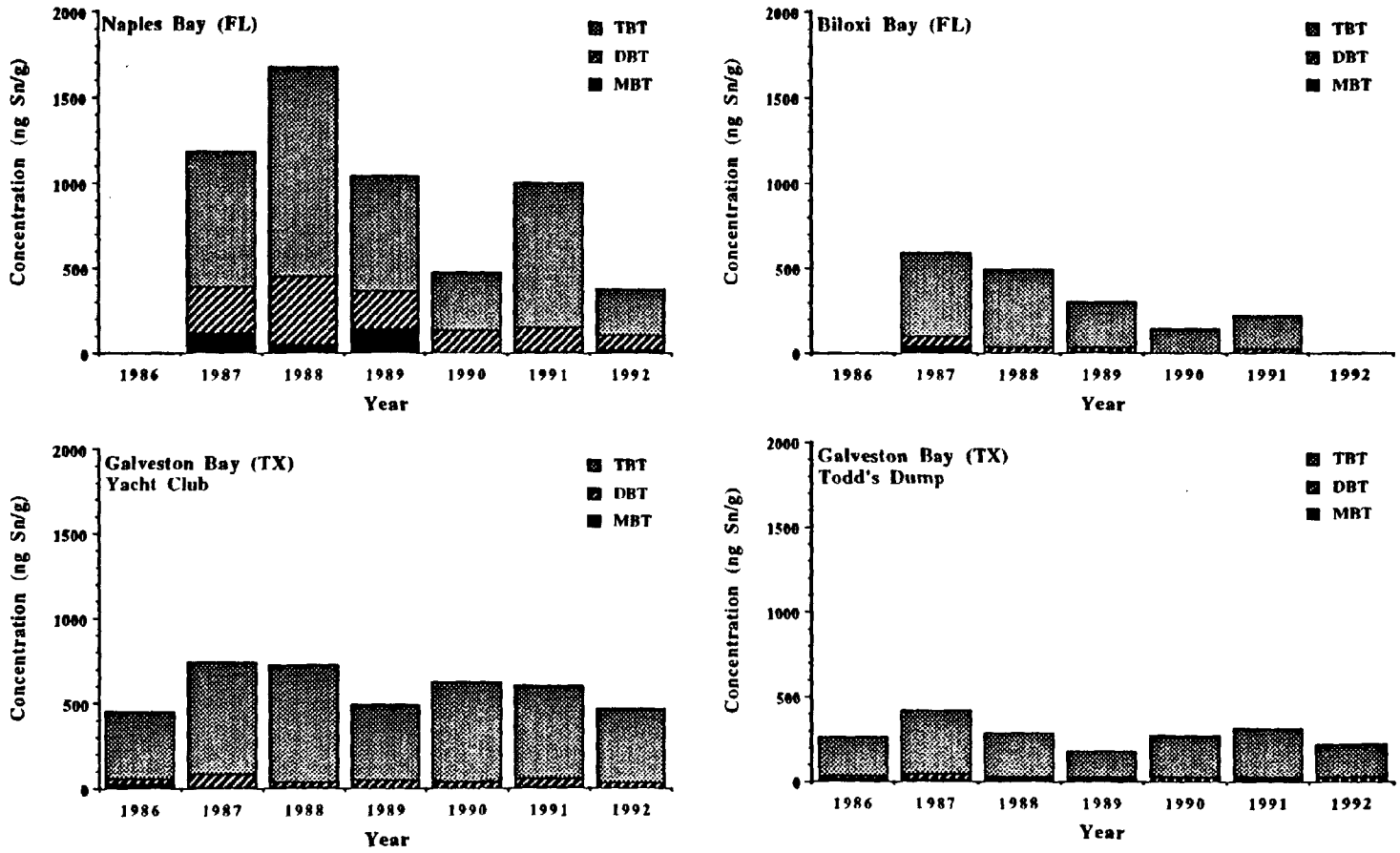


Fig. 53. Total butiltin concentrations at selected sites in the Gulf of Mexico sampled between 1986 and 1992 as part of NOAA's National Status and Trends Program.

restrictions applied in 1988 to the use of paints containing TBT a decreasing but still significant amount of TBT is being introduced into the coastal marine environment. These inputs may be from boats painted before 1988, TBT in sediments and/or TBT usage on larger vessels. Other areas with important recreational boating activities but also heavy maritime usages, like Galveston Bay, Texas, did not show any decrease and, even with the actual restrictions to the TBT usage, no decreases in the near future may be found.

CHAPTER IX

SUMMARY AND PROSPECTIVES

Polynuclear aromatic hydrocarbons (PAHs), low molecular weight polychlorinated biphenyls (PCBs), i.e. di-, tri- and tetrachlorobiphenyls, and tributyltin (TBT) were rapidly bioaccumulated by oysters under environmental conditions. Apparent steady state concentrations for these analytes were reached after 20 to 30 days of exposure. In contrast, high molecular weight PCBs did not reached an equilibrium plateau at the end of the seven week exposure period to relatively high PCB concentrations. However, the still increasing concentrations encountered for these PCBs by the end of the exposure period suggest that, given enough time, the equilibrium concentrations would eventually be reached. When back-transplanted to their former location near Hanna Reef, originally uncontaminated oysters depurated PAHs, low molecular weight PCB congeners and TBT at similar rates while the heavier molecular weight PCB congeners were depurated at considerably slower rates. In neither case, however, the original background concentrations were reached after the 50-day depuration period.

Chronically contaminated Ship Channel oysters were also transplanted to the Hanna Reef area during the second phase of the field experiment in Galveston Bay to allow for a direct comparison with newly contaminated Hanna Reef individuals. In general, the observed clearance rates in Ship Channel oysters were slower than those exhibited by for Hanna Reef bivalves. The differences might be explained as a consequence of different distributions of PAH, PCB and TBT in the various body compartments in chronically

exposed oysters compared to recently contaminated individuals or a more effective clearance response by originally uncontaminated oysters. A combination of both of these processes should not be disregarded.

The present study presents evidence to substantiate the theory that the rates of uptake and depuration of PCB congeners by the oyster *Crassostrea virginica* decreases as the number of substituted chlorines in the two phenyl rings increases. However, in spite of their lower uptake rates compared to low molecular weight congeners, the pentachlorobiphenyls were the congeners bioaccumulated to the highest concentrations. It was also observed that although heavier molecular weight congeners, i.e. heptachlorinated biphenyls or higher, are more liphophilic, they have less favorable steric configurations, which antagonistically affected their bioaccumulation and latter depuration by oysters. Thus, bioconcentration and clearance of different PCB congeners appear to be more affected by molecular size, e.g. molecular volume and cross-sectional area, which are directly related to the number of chlorines substituted in the two phenyl rings and their substitution patterns, rather than by hydrophobicity.

The influence of the chlorine substitution patterns in the bioaccumulation of PCBs by oysters is particularly evident in the case of the highly toxic planar congeners, i.e. PCBs 77 and 126. Compared to other PCBs within the same level of chlorination, these planar congeners take a longer time to equilibrate into and out of the organism's lipid pools. Because of this, the importance of lipid content in oysters in determining potential environmental hydrophobic organic accumulation might not be as significant as usually speculated. Furthermore, the tendency for larger organic molecules to be less concentrated in the lipidic pools of the organisms as a consequence of unfavorable steric configurations suggests that these large molecules may also partition less easily into the cells. Because of this low diffusivity among the different compartments in the organism, it may take longer for the larger molecules to reach toxic concentrations.

The identification of the source of PCB congeners can also be confounded by the differential PCB congeners uptake by oysters. Oysters exposed in the laboratory to a wide molecular range of PCB congeners (1:1:1:1 mixture of Aroclor 1242, 1248, 1254 and 1260), preferentially bioaccumulated congeners with four, five and six chlorines per molecule resulting in a PCB profile similar to the distribution of homologs that would be encountered in an approximately 2:1 mixture of commercial Aroclors 1248 and 1254. Similar distribution of homologs has been observed in transplanted Hanna Reef oysters during the field study near the Houston Ship Channel in Galveston Bay. Comparatively, the profile of PCB homologs in indigenous Ship Channel oysters, exposed longer to the the local levels, had a distribution profile with a slighly larger contribution of Aroclor 1254 (approximately 1:1 Aroclor 1248 and 1254). Although it can be speculated that the profile distributions encountered in chronically contaminated and newly exposed oysters are the result of exposure to Aroclors 1248 and 1254 sources, it seems very probable that the observed profiles are a consequence of the congener uptake discrimination from a more complex mixture. However, it could also be that, even with a more complex mixture of different Aroclors, there was a natural fractionation of the low, middle and high molecular weight congeners. It is well known that a PCB mixture can not be considered as a simple chemical contaminant but as a theoretical mixture of 209 congeners with distinctive physico-chemical properties that can be environmentally fractionated. The loss of the lowest and the highest molecular weight PCB congeners from a more complex Aroclor mixture by evaporation/dissolution and adsorption/deposition, respectively, after input can result in a profile distribution similar to that of Aroclor 1254 or a mixture of Aroclors 1254 with 1248 or 1260. Therefore, it seems that, independently of the composition of the original PCB mixture, the environmental fractionation together with preferential uptake will indicate Aroclor 1254 as the most probable contaminant source. Incidentally, Aroclor 1254, which is one of the most commonly reported PCB mixtures as

the source in environmental pollution studies, is not the one that was produced in the largest quantities. The most popular blend in the U.S. was Aroclor 1242, which comprised over 50% of the total domestic production between 1957 and 1970 (Cairns *et al.*, 1986).

Similarly to what was observed for PCB congeners, oysters exposed in the laboratory to a wide molecular range of PAHs showed the preferential uptake of four- and five-ring PAHs. This observation was confirmed by the results obtained from the field experiments in Galveston Bay. If oysters preferentially bioaccumulate combustion-derived PAHs, i.e. four- and five-ring compounds, compared to petroleum-derived PAHs, i.e. two- and three-ring compounds, then how accurate do they represent the contamination at a site where most of the PAHs are petroleum-derived? This particular area requires further attention.

Other areas requiring more investigation are the effect that simultaneous exposure to PCBs and PAHs have on the concentrations encountered in environmentally contaminated oysters and how well these concentrations correlate with local environmental levels. During this study, it has been shown that oysters exposed in the laboratory to a mixture of PCBs and PAHs, depurated PAHs at a faster rate when the contaminants input was stopped than oysters that were not simultaneously exposed to PCBs. The half-lives for individual PAHs encountered in oysters exposed in the laboratory to a mixture of PCBs and PAHs compared more closely to those found during the field experiment in Galveston Bay.

It can be concluded that indigenous oysters can be valuable bioindicators of environmental contamination by trace organic compounds, particularly PAHs, PCBs and TBT, <u>only</u> if their limitations are fully understood. Within these limitations, transplanted oysters can be succesfully used to monitor environmental contamination by PAHs and TBTs in areas lacking indigenous bivalves if deployed *in-situ* for a period of time of at

least 30 days; for PCBs, however, much longer time period, i.e over 6 months, may be required.

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APPENDIX

TABLE A-1

Biological Ancillary Parameters in Transplanted and Indigenous Oysters.

Sample	Days after transplants	Shell length (cm)	Wet weight (g)	Lipids (%)
Hanna Reef-to-Ship Channel (HRSC)				
HRSC	3	7.6+0.9	7.6+2.4	10+2.2
HRSC	7	7.5+0.8	7.9+3.1	10+3.4
HRSC	17	7.8+1.2	10+3.7	9.7+1.7
HRSC	30	7.3+0.5	8.3+1.9	11+4.0
HRSC	48	8.1+1.2	11+3.7	11+2.8
Hanna Reef-Ship Channel-Hanna Reef (HRSCHR)				
HRSCHR	51	7.2+0.5	7.9+1.3	13+2.3
HRSCHR	54	7.9+1.1	10+3.8	11+0.6
HRSCHR	66	7.6+1.2	9.3+3.5	12+2.4
HRSCHR	78	7.4+0.8	9.6+3.6	11+0.8
HRSCHR	98	7.7+1.0	13+3.9	12+2.5
Ship Channel (SC)				
SC	3	7.4+1.3	7.3+1.6	14+3.6
SC	7		Sample was not collected	
SC	17	10+1.1	17+4.4	14+0.6
SC	30	8.9+1.1	11+2.3	15+1.3
SC	48	9.3+1.1	11+3.0	15+0.3
Ship Channel-to-Hanna Reef (SCHR)				
SCHR	51	10+1.5	16+3.4	13+1.0
SCHR	54	8.7+1.1	13+3.0	13+1.4
SCHR	66	8.7+1.5	12+6.0	12+0.9
SCHR	78	8.4+1.2	11+4.1	12+1.3
SCHR	98	7.3+1.7	14+1.6	13+0.8

TABLE A-2

Average PAH Concentrations (± 1 S.D.) in Seawater, Sediment and Indigenous Ship Channel (SC) oyster Samples During the Uptake Phase of the Experiment at the Ship Channel Site and Estimated Bioconcentration Factors (BCF).

Analyte	Water	Sediment	Uptake phase (days)						BCF[a]
			0	3	7	17	30	48	
	ng l^{-1}	ng g^{-1}	ng g^{-1}						
Naphthalene	3.8±1.0	10±2.7	-	25±2.9	-	12±0.4	7.8±0.8	7.2±1.8	1,900
2-Methylnaphthalene	2.5±0.7	15±4.6	-	25±4.5	-	20±6.7	19±2.9	9.1±0.6	3,600
1-Methylnaphthalene	1.9±0.6	7.8±1.4	-	13±3.1	-	13±4.0	9.1±2.1	3.8±0.8	2,000
Biphenyl	1.2±0.3	3.6±0.2	-	11±2.1	-	6.2±1.1	7.1±0.4	3.6±0.2	3,000
2,6-Dimethylnaphthalene	1.6±0.5	12±4.8	-	22±2.2	-	17±0.6	49±3.3	13±3.5	8,100
Acenaphthylene	0.7±0.1	23±7.2	-	14±3.3	-	7.9±0.3	6.8±0.2	6.9±0.2	9,900
Acenaphthene	0.6±0.1	9.5±3.0	-	21±2.6	-	13±1.0	31±1.3	5.8±0.4	9,700
2,3,5-Trimethylnaphthalene	2.4±0.6	32±5.3	-	53±9.1	-	83±14	120±6.3	56±6.2	23,000
Fluorene	1.0±0.2	15±7.2	-	23±1.8	-	22±2.2	34±4.1	15±1.7	15,000
Phenanthrene	2.2±0.7	51±5.8	-	45±3.6	-	39±2.7	110±12	23±2.9	10,000
Anthracene	1.0±0.3	54±11	-	31±9.4	-	29±3.8	46±1.4	30±3.5	30,000
1-Methylphenanthrene	1.5±0.6	37±15	-	63±12	-	110±2.8	88±13	89±11	59,000
Fluoranthene	1.6±1.2	140±58	-	390±45	-	560±95	790±83	490±51	310,000
Pyrene	2.1±1.6	190±38	-	1,200±130	-	1,400±270	1,400±59	1,900±80	900,000
Benz(a)anthracene	0.8±0.1	110±42	-	130±25	-	160±18	220±13	280±13	350,000
Chrysene	0.9±0.3	150±38	-	400±51	-	310±26	380±25	490±21	540,000
Benzo(b)fluoranthene	0.8±0.2	170±41	-	170±30	-	130±14	170±14	210±24	260,000

TABLE A-2
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
Benzo(k)fluoranthene	0.3±0.1	140±38	-	52±15	-	48±2.8	66±7.2	82±14	270,000
Benzo(e)pyrene	1.1±0.3	160±30	-	310±49	-	200±24	240±20	340±5.8	310,000
Benzo(a)pyrene	0.8±0.1	142±45	-	57±16	-	73±7.1	97±11	160±6.3	200,000
Perylene	1.1±0.3	110±18	-	110±25	-	110±11	140±16	160±4.1	150,000
Indeno[1,2,3-c,d]pyrene	0.5±0.1	86±15	-	16±3.4	-	11±1.4	16±2.3	22±0.4	44,000
Dibenzo(a,h)anthracene	0.6±0.1	37±4.3	-	16±3.2	-	9.9±0.6	10±0.7	15±3.2	25,000
Benzo(g,h,i)perylene	0.6±0.1	65±20	-	54±8.5	-	33±2.9	45±3.7	68±0.8	110,000
Total 2-Rings	13±3.1	80±7.4	-	150±18	-	150±23	210±14	93±7.5	7,200
Total 3-Rings	7.1±1.9	190±24	-	200±31	-	220±12	310±26	170±6.5	24,000
Total 4-Rings	5.2±2.1	580±170	-	2,100±250	-	2,400±390	2,800±68	3,100±120	600,000
Total 5-Rings	4.6±0.8	800±170	-	720±140	-	570±57	710±67	970±42	210,000
Total 6-Rings	1.1±0.2	150±26	-	70±11	-	44±4.2	61±6.0	90±1.2	82,000
Total PAHs	32±7.0	1,800±380	-	3,200±420	-	3,400±470	4,100±170	4,400±146	140,000

[a] Bioconcentration factor = concentration in transplanted oyster tissue at the end of the uptake period/concentration in water.

TABLE A-3

Average PAH Concentrations (± 1 S.D.) in Seawater, Sediment and Hanna Reef-to-Ship Channel (HRSC) Transplanted Oyster Samples During the Uptake Phase of the Experiment at the Ship Channel Site and Estimated Bioconcentration Factors (BCF).

Analyte	Water	Sediment	Uptake phase (days)						
			0	3	7	17	30	48	BCF[a]
	ng l^{-1}	ng g^{-1}	ng g^{-1}						
Naphthalene	3.8±1.0	10±2.7	22±7.5	12±1.9	12±3.0	14±3.6	8.3±2.2	7.5±1.1	2,000
2-Methylnaphthalene	2.5±0.7	15±4.6	16±7.8	11±2.5	6.7±1.5	14±1.9	12±3.8	7.5±1.2	3,000
1-Methylnaphthalene	1.9±0.6	7.8±1.4	9.0±3.6	5.7±0.9	3.9±1.0	9.9±1.6	5.6±1.7	3.9±0.6	2,100
Biphenyl	1.2±0.3	3.6±0.2	8.3±3.2	7.2±1.8	5.6±1.6	6.8±2.2	6.8±1.6	4.3±0.5	3,600
2,6-Dimethylnaphthalene	1.6±0.5	12±4.8	15±3.2	9.8±0.9	6.8±1.1	12±1.3	39±6.5	10±1.5	6,300
Acenaphthylene	0.7±0.1	23±7.2	7.3±2.1	7.4±1.5	6.5±1.8	6.7±1.5	6.1±1.2	6.5±1.7	9,300
Acenaphthene	0.6±0.1	9.5±3.0	4.0±1.7	9.2±0.8	9.5±1.1	9.1±1.6	25±6.6	7.6±1.3	13,000
2,3,5-Trimethylnaphthalene	2.4±0.6	32±5.3	26±5.9	22±4.9	14±3.3	50±11	90±20	38±11	16,000
Fluorene	1.0±0.2	15±7.2	9.3±1.5	11±1.2	11±1.4	16±2.3	26±5.9	16±3.1	16,000
Phenanthrene	2.2±0.7	51±5.8	14±4.2	20±1.8	21±1.6	28±5.4	87±19	48±21	22,000
Anthracene	1.0±0.3	54±11	16±1.6	11±1.6	12±3.1	20±4.1	36±11	30±6.7	30,000
1-Methylphenanthrene	1.5±0.6	37±15	62±31	23±4.2	20±5.2	65±16	96±19	64±27	43,000
Fluoranthene	1.6±1.2	140±58	11±2.5	110±18	130±19	410±45	620±110	490±76	310,000
Pyrene	2.1±1.6	190±38	16±3.5	300±44	340±50	1,000±100	1,300±190	1,900±190	900,000
Benz(a)anthracene	0.8±0.1	110±42	8.3±5.0	22±3.3	30±4.9	140±17	180±26	260±27	330,000
Chrysene	0.9±0.3	150±38	7.7±3.5	71±9.5	110±16	250±20	320±38	450±49	500,000
Benzo(b)fluoranthene	0.8±0.2	170±41	3.0±1.0	28±4.9	39±3.7	100±2.7	140±14	220±20	280,000

TABLE A-3
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
Benzo(k)fluoranthene	0.3±0.1	140±38	3.3±0.6	11±1.1	14±0.9	41±4.3	50±9.1	86±11	290,000
Benzo(e)pyrene	1.1±0.3	160±30	4.0±1.0	49±3.2	76±9.1	150±4.2	210±14	330±24	300,000
Benzo(a)pyrene	0.8±0.1	142±45	6.3±5.0	15±3.0	16±0.9	64±6.6	74±9.1	150±31	190,000
Perylene	1.1±0.3	110±18	3.0±0.1	23±2.9	27±2.4	100±9.6	100±7.7	150±27	140,000
Indeno[1,2,3-c,d]pyrene	0.5±0.1	86±15	10±3.5	7.9±2.0	7.1±0.9	12±0.5	14±1.1	23±3.3	46,000
Dibenzo(a,h)anthracene	0.6±0.1	37±4.3	3.0±2.6	6.7±1.9	5.6±0.5	9.7±1.6	11±3.5	17±2.7	28,000
Benzo(g,h,i)perylene	0.6±0.1	65±20	7.0±4.0	15±3.9	20±2.4	31±2.1	43±3.9	73±9.9	120,000
Total 2-Rings	13±3.1	80±7.4	97±16	68±10	49±3.8	110±15	160±33	71±12	5,500
Total 3-Rings	7.1±1.9	190±24	110±27	81±9.5	80±14	150±23	280±62	170±43	24,000
Total 4-Rings	5.2±2.1	580±170	43±12	500±69	610±87	1,800±180	2,400±340	3,100±290	600,000
Total 5-Rings	4.6±0.8	800±170	30±9.0	130±16	180±17	470±18	590±46	950±110	210,000
Total 6-Rings	1.1±0.2	150±26	17±7.5	23±5.8	27±2.9	44±2.4	58±4.7	96±13	87,000
Total PAHs	32±7.0	1,800±380	290±40	810±83	950±110	2,600±220	3,500±390	4,400±330	138,000

[a] Bioconcentration factor = concentration in transplanted oyster tissue at the end of the uptake period/concentration in water.

TABLE A-4

Average PAH Concentrations (± 1 S.D.) in Sediment and Ship Channel-to-Hanna Reef (SCHR) Transplanted Oyster Samples During the Depuration Phase of the Experiment at the Hanna Reef Site and Estimated Biological Half-Lives (BHL).

Analyte	Sediment	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
	ng g^{-1}	ng g^{-1}						
Naphthalene	3.9±0.3	7.2±1.8	8.5±1.5	14±6.4	9.1±0.6	9.0±1.2	12±2.8	
2-Methylnaphthalene	5.6±0.3	9.1±0.6	11±1.9	9.7±1.2	6.1±1.3	6.5±1.5	11±2.4	
1-Methylnaphthalene	4.1±0.4	3.8±0.8	5.5±0.8	6.0±1.4	3.9±0.7	5.3±1.6	7.8±1.0	
Biphenyl	4.3±0.9	3.6±0.2	6.0±1.1	5.9±0.7	5.6±1.3	5.7±0.6	7.1±1.5	
2,6-Dimethylnaphthalene	6.1±2.0	13±3.5	17±4.9	10±1.2	3.8±1.0	7.9±1.0	6.2±2.4	
Acenaphthylene	4.8±0.4	6.9±0.2	5.1±2.6	3.1±3.3	3.7±2.7	3.8±2.0	3.1±1.2	
Acenaphthene	2.9±0.4	5.8±0.4	4.2±0.9	3.1±0.9	2.2±0.7	14±2.1	3.3±1.0	
2,3,5-Trimethylnaphthalene	5.6±0.9	56±6.2	44±10	23±6.8	17±5.4	16±2.8	9.1±2.6	22 (0.83)
Fluorene	4.9±0.8	15±1.7	14±1.4	11±2.5	5.7±1.0	15±2.1	7.5±2.8	
Phenanthrene	12±3.4	23±2.9	24±3.9	18±1.1	15±2.9	46±4.6	29±8.8	
Anthracene	7.6±1.0	30±3.5	19±7.0	16±4.2	13±3.6	16±4.4	9.5±2.8	42 (0.68)
1-Methylphenanthrene	4.8±2.4	89±11	66±16	54±17	47±15	36±6.5	18±5.4	24 (0.96)
Fluoranthene	29±8.4	490±51	350±89	250±45	260±110	320±18	110±29	32 (0.69)
Pyrene	29±7.7	1,900±80	1,300±290	870±230	500±140	300±37	95±28	12 (0.98)
Benz(a)anthracene	18±4.2	280±13	200±48	170±44	110±32	59±9.4	26±7.2	15 (0.99)
Chrysene	15±2.8	490±21	380±53	340±48	230±52	110±18	54±10	16 (0.99)
Benzo(b)fluoranthene	17±3.0	210±24	200±34	200±9.5	140±16	59±11	18±5.6	

TABLE A-4
(continued)

Analyte	Sediment	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
	ng g^{-1}	ng g^{-1}						
Benzo(k)fluoranthene	13±1.7	82±14	71±12	72±8.8	35±2.4	17±3.9	5.5±2.7	
Benzo(e)pyrene	17±2.8	340±5.8	310±16	300±6.9	210±20	89±21	44±8.7	16 (0.98)
Benzo(a)pyrene	15±1.2	160±6.3	110±22	79±17	38±8.7	14±2.2	4.4±1.3	10 (0.99)
Perylene	74±6.3	160±4.1	130±18	120±23	71±18	28±6.6	11±3.8	13 (0.99)
Indeno[1,2,3-c,d]pyrene	15±3.8	22±0.4	23±7.2	22±1.2	14±6.2	2.3±1.3	1.2±0.2	11 (0.93)
Dibenzo(a,h)anthracene	4.9±0.9	15±3.2	17±7.5	18±4.3	15±6.1	3.3±0.8	1.7±0.6	14 (0.90)
Benzo(g,h,i)perylene	14±3.5	68±0.8	70±18	73±6.1	37±7.2	13±0.4	4.8±1.7	12 (0.98)
Total 2-Rings	30±3.8	93±7.5	92±18	68±4.9	45±3.8	50±4.4	53±13	
Total 3-Rings	37±3.6	170±6.5	130±31	110±26	87±23	130±16	70±22	
Total 4-Rings	90±22	3,100±120	2,200±450	1,600±360	1,100±310	780±76	290±74	
Total 5-Rings	140±9.4	970±42	850±48	790±56	510±43	210±40	85±23	
Total 6-Rings	30±6.9	90±1.2	93±25	95±7.2	50±13	15±1.0	6.0±1.9	
Total PAHs	330±32	4,400±150	3,400±440	2,700±430	1,800±330	1200±120	500±130	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-5

Average PAH Concentrations (± 1 S.D.) in Sediment and Hanna Reef-Ship Channel-Hanna Reef (HRSCHR) Transplanted Oyster Samples During the Depuration Phase of the Experiment at the Hanna Reef Site and Estimated Biological Half-Lives (BHL).

Analyte	Sediment	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
	ng g^{-1}	ng g^{-1}						
Naphthalene	3.9±0.3	7.5±1.1	7.5±0.5	9.3±1.4	6.6±0.8	8.3±1.1	13±5.6	
2-Methylnaphthalene	5.6±0.3	7.5±1.2	12±1.9	11±3.1	5.3±0.8	6.0±0.3	11±0.6	
1-Methylnaphthalene	4.1±0.4	3.9±0.6	6.0±0.7	5.6±2.1	2.8±0.4	4.0±0.6	6.8±1.2	
Biphenyl	4.3±0.9	4.3±0.5	5.0±0.3	4.7±1.0	4.8±1.0	6.4±1.1	7.7±1.9	
2,6-Dimethylnaphthalene	6.1±2.0	10±1.5	19±0.8	7.3±0.6	3.6±1.1	7.9±1.8	6.4±2.0	
Acenaphthylene	4.8±0.4	6.5±1.7	6.3±1.7	3.9±1.2	3.2±1.2	3.5±1.5	3.6±1.4	
Acenaphthene	2.9±0.4	7.6±1.3	5.0±0.4	2.7±0.4	2.0±0.3	13±3.0	3.4±0.7	
2,3,5-Trimethylnaphthalene	5.6±0.9	38±11	41±9.2	15±4.2	16±6.5	12±0.9	8.2±0.6	24 (0.74)
Fluorene	4.9±0.8	16±3.1	15±1.4	9.0±1.7	5.1±0.5	13±2.0	8.1±1.9	
Phenanthrene	12±3.4	48±21	33±2.8	15±2.2	12±1.6	44±11	24±2.8	
Anthracene	7.6±1.0	30±6.7	26±4.8	15±2.8	17±4.9	11±2.2	5.8±2.4	24 (0.90)
1-Methylphenanthrene	4.8±2.4	64±27	46±8.4	47±4.5	29±10	26±4.0	12±2.4	23 (0.97)
Fluoranthene	29±8.4	490±76	350±7.4	160±42	190±35	220±43	80±20	26 (0.67)
Pyrene	29±7.7	1,900±190	1,400±86	570±170	350±100	170±41	56±10	10 (0.95)
Benz(a)anthracene	18±4.2	260±27	250±22	150±47	70±25	39±5.4	20±1.7	13 (0.96)
Chrysene	15±2.8	450±49	420±15	280±69	150±35	58±11	30±4.7	12 (0.99)
Benzo(b)fluoranthene	17±3.0	220±20	200±9.5	210±16	76±6.3	25±8.7	15±4.2	12 (0.95)

TABLE A-5
(continued)

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
Benzo(k)fluoranthene	13±1.7	86±11	76±8.1	78±5.4	19±4.2	6.8±2.0	4.4±1.6	10 (0.96)
Benzo(e)pyrene	17±2.8	330±24	310±21	280±19	110±26	39±12	21±4.7	12 (0.97)
Benzo(a)pyrene	15±1.2	150±31	130±25	81±19	32±3.8	8.6±2.7	3.5±1.9	9 (0.98)
Perylene	74±6.3	150±27	140±17	100±19	28±8.1	13±2.1	7.5±1.1	11 (0.94)
Indeno[1,2,3-c,d]pyrene	15±3.8	23±3.3	18±3.1	16±2.6	8.4±1.8	1.8±0.9	1.0±1.1	10 (0.96)
Dibenzo(a,h)anthracene	4.9±0.9	17±2.7	15±1.9	13±2.8	7.9±2.8	2.3±1.2	2.3±1.7	16 (0.93)
Benzo(g,h,i)perylene	14±3.5	73±9.9	61±1.2	51±3.3	17±3.6	7.7±1.1	4.1±1.6	11 (0.96)
Total 2-Rings	30±3.8	71±12	90±12	53±4.8	39±6.1	45±0.4	52±7.4	
Total 3-Rings	37±3.6	170±43	130±15	92±6.7	68±16	110±9.4	57±6.6	
Total 4-Rings	90±22	3,100±290	2,400±96	1,200±320	750±180	490±97	190±20	
Total 5-Rings	140±9.4	950±110	870±73	750±22	270±17	95±24	54±13	
Total 6-Rings	30±6.9	93±13	79±4.0	67±3.3	26±4.2	9.5±1.4	5.1±2.4	
Total PAHs	330±32	4,400±330	3,600±170	2,100±320	1,200±190	750±120	360±18	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-6

Average PCB Congener Concentrations (± 1 S.D.) in Seawater, Sediment and Ship Channel (SC) Indigenous Oyster Samples During the Uptake Phase of the Experiment at the Ship Channel Site and Estimated Bioconcentration Factors (BCF).

Analyte	Water	Sediment	Uptake phase (days)						
			0	3	7	17	30	48	BCF[a]
	ng l^{-1}	ng g^{-1}	ng g^{-1}						
18	0.05±0.03			3.0±0.7		4.7±0.3	6.8±2.1	6.1±0.6	120,000
15/17	0.03±0.02	0.16±0.11		3.1±0.9		4.2±0.3	5.6±1.8	4.8±0.5	160,000
26				35±5.3		34±2.0	30±5.9	36±8.0	
50/31		0.15±0.07		2.5±0.4		3.4±0.2	4.3±1.4	5.2±0.8	
28				6.5±1.1		7.1±0.6	6.7±1.4	8.0±1.4	
52	0.50±0.29	1.58±0.17		62±7.0		55±6.1	47±11	52±4.0	100,000
49	0.14±0.09	0.65±0.17		36±2.2		31±4.5	29±3.8	31±3.2	220,000
47/48/75				25±1.1		20±0.9	16±5.7	19±2.0	
44	0.20±0.22	0.56±0.19		27±1.1		21±1.6	19±3.9	20±3.2	100,000
37/42/59	0.09±0.09	0.21±0.06		26±0.8		28±3.0	21±3.6	33±5.3	370,000
41		0.44±0.09		21±4.6		17±4.7	15±2.3	16±2.8	
40		0.14±0.02		9.7±0.4		10±0.3	10±1.2	13±0.5	
74	0.07±0.03	0.23±0.07		17±0.9		13±3.2	12±0.9	15±0.9	210,000
70	0.05±0.03	0.87±0.41		46±2.5		34±0.9	32±0.4	38±2.0	760,000
95				95±9.0		71±0.8	56±12	63±8.2	
91	0.07±0.04	0.40±0.14		27±2.6		20±2.7	15±4.9	22±1.0	310,000
60/56				10±0.8		8.2±1.1	8.9±1.5	9.5±1.4	
92		0.45±0.21		31±7.2		32±5.8	18±5.7	26±6.6	

TABLE A-6
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
84		0.71±0.23		46±0.8		35±4.9	27±7.9	29±7.3	
101/90	0.27±0.12	2.19±0.65		110±13		85±2.2	67±8.1	71±8.5	260,000
99	0.14±0.08	1.44±0.09		62±5.5		52±1.1	41±4.9	45±4.6	320,000
97		0.68±0.26		33±3.0		26±1.4	20±2.1	20±3.7	
87/115		1.33±0.40		53±4.9		40±1.4	30±3.6	32±3.1	
110/77	0.24±0.08	3.84±1.00		130±12		110±6.8	76±15	80±12	330,000
82		0.30±0.06		14±1.3		9.2±0.8	7.7±1.0	6.3±2.3	
151	0.08±0.05	0.22±0.04		16±1.1		13±0.8	9.1±2.1	10±2.2	130,000
135	0.10±0.02	0.16±0.02		14±5.0		10±1.8	7.4±1.9	8.7±2.1	87,000
107				15±1.9		13±2.8	12±0.2	15±3.1	
149/123	0.19±0.12	0.90±0.23		44±4.5		36±2.4	31±1.9	36±3.5	190,000
118	0.12±0.04	1.35±0.37		88±12		68±5.6	51±8.3	56±6.3	470,000
146		0.13±0.05		13±3.4		10±0.9	8.0±1.1	7.8±2.1	
153/132	0.59±0.32	2.15±0.54		170±11		110±19	93±10	100±10	170,000
105	0.12±0.06	0.67±0.16		30±7.8		23±5.2	15±3.5	17±6.1	140,000
141/179	0.12±0.11	0.34±0.03		7.3±1.7		4.5±0.5	3.7±1.0	5.2±0.9	43,000
138/160	0.59±0.17	2.52±0.12		77±12		60±5.5	44±7.3	47±10	80,000
187	0.14±0.09	0.11±0.04		18±6.9		15±2.4	13±1.3	15±2.5	110,000

TABLE A-6
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
128	0.08±0.06	0.42±0.09		10±0.6		7.7±1.3	6.3±0.1	5.9±1.0	
177	0.05±0.04	0.18±0.06		5.3±0.2		4.3±0.9	3.7±0.2	4.6±0.1	92,000
180	0.33±0.18	0.40±0.06		6.5±0.6		5.5±0.9	4.1±0.5	4.6±0.2	14,000
Total dichlorobiphenyls							1.5		
Total trichlorobiphenyls	0.13±0.08	0.31±0.17		59		64	64	71	540,000
Total tetrachlorobiphenyls	1.00±0.56	6.16±1.58		270		240	210	250	250,000
Total pentachlorobiphenyls	0.99±0.38	13.5±3.49		740		580	440	490	490,000
Total hexachlorobiphenyls	1.64±0.81	6.98±1.13		360		250	210	230	140,000
Total heptachlorobiphenyls	0.78±0.50	1.14±0.12		57		46	40	52	67,000
Total octachlorobiphenyls	0.09±0.05	0.25±0.03		1.6		1.3	0.5	1.9	21,000
Total nonachlorobiphenyls				1.0		0.7	0.5	0.8	
Total decachlorobiphenyl				0.2		0.6	0.4	0.4	
Total PCBs	4.62±2.15	28.4±6.41		1500		1200	960	1100	240,000

[a] Bioconcentration factor = concentration in transplanted oyster tissue at the end of the uptake period/concentration in water.

TABLE A-7

Average PCB Congener Concentrations (± 1 S.D.) in Seawater, Sediment and Hanna Reef-to-Ship Channel (HRSC) Transplanted Oyster Samples During the Uptake Phase of the Experiment at the Ship Channel Site and Estimated Bioconcentration Factors (BCF).

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
18	0.05±0.03			1.6±0.4	2.2±0.3	3.7±1.0	4.4±0.7	6.0±1.0	120,000
15/17	0.03±0.02	0.16±0.11		1.2±0.5	11±2.7	2.9±0.8	3.0±1.2	4.5±1.3	150,000
26				6.5±1.2	11±2.8	19±2.5	23±2.1	30±9.3	
50/31		0.15±0.07		1.0±0.3	3.8±2.1	2.7±0.9	5.2±2.8	9.8±4.6	
28				2.4±0.2	3.8±1.1	5.6±0.7	7.9±1.3	9.9±1.4	
52	0.50±0.29	1.58±0.17		17±2.9	28±2.1	32±2.3	37±3.7	51±5.4	100,000
49	0.14±0.09	0.65±0.17		6.6±0.7	13±1.6	16±1.0	22±1.5	29±1.5	210,000
47/48/75				5.6±1.6	9.6±1.1	11±1.3	14±1.3	18±2.2	
44	0.20±0.22	0.56±0.19		6.9±0.7	14±2.0	14±1.3	17±1.9	22±2.3	110,000
37/42/	0.09±0.09	0.21±0.06		12±0.7	17±3.3	19±1.9	22±1.6	42±8.3	470,000
41		0.44±0.09		4.3±0.8	8.0±0.1	8.9±3.3	12±2.1	20±1.4	
40		0.14±0.02		1.4±0.5	4.6±1.4	6.7±0.7	9.4±0.9	14±2.5	
74	0.07±0.03	0.23±0.07		2.9±0.5	5.2±0.5	7.3±0.9	9.4±2.1	13±2.7	190,000
70	0.05±0.03	0.87±0.41		4.7±1.2	9.7±1.9	15±1.6	23±3.5	31±2.8	620,000
95				11±1.2	22±2.1	29±1.2	41±5.5	55±5.3	
91	0.07±0.04	0.40±0.14		4.2±0.5	9.1±1.1	11±0.9	14±2.0	24±8.6	340,000
60/56				2.2±0.5	3.5±0.6	5.4±0.8	7.3±1.4	10±1.9	
92		0.45±0.21		4.8±1.3	6.5±1.1	11±2.5	15±1.0	22±8.0	

TABLE A-7
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
84		0.71±0.23		5.9±0.5	13±2.2	17±1.5	24±3.8	29±0.9	
101/90	0.27±0.12	2.19±0.65		11±1.6	20±4.1	27±1.1	37±4.3	43±5.2	160,000
99	0.14±0.08	1.44±0.09		8.7±1.0	15±1.6	18±3.3	27±2.9	29±1.3	210,000
97		0.68±0.26		3.9±1.6	6.8±1.1	8.9±1.7	11±1.8	14±3.2	
87/115		1.33±0.40		4.7±0.6	8.6±1.3	13±0.6	16±2.0	20±1.4	
110/77	0.24±0.08	3.84±1.00		13±1.8	28±4.2	37±1.7	50±5.2	61±4.2	250,000
82		0.30±0.06		1.8±0.5	3.2±0.4	3.3±0.7	4.4±0.1	5.9±1.0	
151	0.08±0.05	0.22±0.04		1.7±0.5	2.6±0.9	4.0±0.8	4.9±0.6	6.0±1.0	75,000
135	0.10±0.02	0.16±0.02		2.6±1.4	2.2±0.7	3.6±0.9	4.0±0.6	5.0±0.5	50,000
107				1.3±0.3	2.4±0.7	3.6±0.6	5.3±1.1	6.6±1.0	
149/123	0.19±0.12	0.90±0.23		4.7±0.4	7.8±1.1	11±0.4	14±1.5	18±1.6	95,000
118	0.12±0.04	1.35±0.37		7.6±1.4	14±1.6	21±1.4	29±2.0	34±2.5	280,000
146		0.13±0.05		3.4±0.5	4.2±1.9	3.6±0.6	4.0±0.6	4.1±1.1	
153/132	0.59±0.32	2.15±0.54		16±4.9	18±6.3	28±4.8	38±7.5	64±12	110,000
105	0.12±0.06	0.67±0.16		7.6±2.7	11±1.1	13±2.0	13±1.4	14±3.0	120,000
141/179	0.12±0.11	0.34±0.03		1.7±0.6	1.6±0.5	2.3±1.0	3.3±0.7	4.7±0.5	39,000
138/160	0.59±0.17	2.52±0.12		12±2.0	17±2.7	20±0.7	27±5.2	27±4.7	46,000
187	0.14±0.09	0.11±0.04		4.3±0.4	3.4±1.3	5.9±1.7	6.4±1.1	11±2.2	78,000

TABLE A-7
(continued)

Analyte	Water ng l^{-1}	Sediment ng g^{-1}	Uptake phase (days) 0	3	7	17	30	48	BCF[a]
			ng g^{-1}						
128		0.42±0.09		1.7±0.3	2.5±0.3	2.9±0.4	3.8±0.6	3.9±0.6	
177	0.05±0.04	0.18±0.06		1.0±0.2	1.3±0.5	1.6±0.8	1.7±0.1	3.5±1.6	70,000
180	0.33±0.18	0.40±0.06		1.5±0.3	2.4±0.5	2.3±0.3	3.4±0.7	3.8±1.7	12,000
Total dichlorobiphenyls				0.1±0.1			0.3±0.6	0.1±0.2	
Total trichlorobiphenyls	0.130.08	0.310.17		16±2.5	31±5.5	46±6.5	50±6.2	66±9.3	500,000
Total tetrachlorobiphenyls	1.000.56	6.161.58		58±13	110±9.2	130±7.6	170±19	240±24	240,000
Total pentachlorobiphenyls	0.990.38	13.53.49		84±7.6	160±17	210±9.2	290±15	360±26	360,000
Total hexachlorobiphenyls	1.640.81	6.981.13		44±1.9	58±12	77±6.5	100±17	120±42	74,000
Total heptachlorobiphenyls	0.780.50	1.140.12		17±3.8	20±7.6	27±10	31±9.9	45±25	58,000
Total octachlorobiphenyls	0.090.05	0.250.03		0.9±0.9	1.5±1.2	1.1±0.5	1.1±0.9	1.3±0.9	14,000
Total nonachlorobiphenyls				0.3±0.1	0.4±0.1	0.6±0.2	0.5±0.2	1.0±0.3	
Total decachlorobiphenyl				0.3±0.2	0.2±0.1	0.3±0.2	0.3±0.2	0.9±1.0	
Total PCBs	4.622.15	28.46.41		220±20	380±49	500±25	650±58	830±110	180,000

[a] Bioconcentration factor = concentration in transplanted oyster tissue at the end of the uptake period/concentration in water.

TABLE A-8

Average PCB Congener Concentrations (± 1 S.D.) in Sediment and Hanna Reef-Ship Channel-Hanna Reef (HRSCHR) Transplanted Oyster Samples During the Depuration Phase of the Experiment at the Hanna Reef Site and Estimated Biological Half-Lives (BHL).

Analyte	Sediment	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
	ng g^{-1}	ng g^{-1}						
18		6.0±1.0	6.5±1.9	3.2±1.3	1.3±0.3	0.7±0.3	0.7±0.2	14 (0.82)
15/17		4.5±1.3	4.0±1.2	2.3±0.2	1.8±1.0	0.5±0.3	0.4±0.1	
26		30±9.3	33±10	16±1.0	15±4.6	13±8.5	5.5±2.5	22 (0.88)
50/31		9.8±4.6	4.9±0.9	3.4±1.0	2.1±1.1	3.1±1.3	3.4±1.2	
28		10±1.4	9.3±1.2	7.2±1.7	3.4±0.7	2.3±0.3	1.3±0.3	17 (0.96)
52		51±5.4	45±3.4	33±8.4	22±3.2	13±1.9	15±3.4	27 (0.80)
49		29±1.5	30±2.8	23±4.6	18±3.5	13±1.1	13±2.8	39 (0.84)
47/48/75		18±2.2	18±1.8	14±2.2	9.4±2.1	7.1±1.7	6.6±1.8	
44		22±2.3	22±2.2	16±3.2	9.7±2.3	7.2±1.0	6.7±2.2	27 (0.83)
37/42/59		42±8.3	25±8.1	13±4.3	5.5±1.9	5.1±0.2	5.4±1.9	
41		20±1.4	20±4.2	13±4.7	7.3±1.9	5.3±1.4	5.1±0.7	23 (0.83)
40		14±2.5	13±0.8	9.0±2.5	2.3±1.5	2.5±2.1	1.3±0.8	14 (0.87)
74		13±2.7	14±2.2	10±3.3	6.9±1.4	5.0±0.8	4.7±1.1	30 (0.87)
70		31±2.8	34±3.9	27±4.3	18±2.7	12±0.7	11±3.3	30 (0.88)
95		55±5.3	49±4.4	45±6.9	31±5.2	25±0.4	26±5.8	45 (0.81)
91		24±8.6	18±1.4	13±4.7	7.6±1.7	5.8±0.1	5.9±1.6	25 (0.78)
60/56		10±1.9	11±0.8	8.3±2.0	5.2±0.9	3.7±1.0	3.3±1.0	
92		22±8.0	22±2.4	17±2.0	11±2.1	9.4±1.2	7.8±2.5	31 (0.89)

TABLE A-8
(continued)

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
84		29±0.9	24±7.3	20±5.2	14±3.0	11±0.8	11±3.0	37 (0.84)
101/90		43±5.2	47±7.6	43±8.0	34±2.3	26±5.6	26±3.0	55 (0.86)
99		29±1.3	32±5.4	28±5.9	21±2.5	17±4.3	16±1.5	49 (0.88)
97		14±3.2	16±2.2	14±3.6	10±1.3	9.4±0.8	10±2.2	
87/115		20±1.4	21±2.6	18±3.9	12±1.8	11±1.4	12±2.0	55 (0.73)
110/77		61±4.2	61±6.9	50±11	32±8.4	30±0.9	31±8.3	45 (0.74)
82		5.9±1.0	6.5±1.2	5.1±2.1	6.2±2.4	4.0±0.1	4.6±0.8	
151		6.0±1.0	7.5±0.6	6.8±2.3	5.0±1.0	5.0±1.0	4.9±0.5	
135		5.0±0.5	5.7±0.7	4.9±1.2	3.3±0.7	3.8±0.4	3.7±0.5	
107		6.6±1.0	8.3±3.1	5.7±1.8	3.5±1.1	2.7±0.8	2.6±0.5	30 (0.82)
149/123		18±1.7	20±2.9	19±3.1	14±1.6	14±1.0	16±1.7	130 (0.46)
118		34±2.5	35±4.5	32±7.9	25±2.5	32±18	23±1.5	73 (0.79)
146		4.1±1.1	5.0±1.3	5.2±1.1	4.3±0.9	4.2±1.7	3.4±0.5	111 (0.60)
153/132		64±12	59±16	48±13	37±9.4	33±7.6	34±3.5	51 (0.71)
105		14±3.0	14±4.4	14±5.2	9.6±1.2	8.8±1.0	9.0±1.9	63 (0.76)
141/179		4.7±0.5	4.1±1.3	3.0±0.9	2.1±0.7	2.1±0.4	2.0±0.3	
138/160		28±4.7	30±3.8	33±6.0	25±4.2	24±3.8	26±1.9	200 (0.32)
187		11±2.2	11±3.5	8.1±1.6	6.7±2.2	6.5±1.8	6.6±0.6	70 (0.65)

TABLE A-8
(continued)

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
128		3.9±0.6	4.1±0.9	4.1±1.0	2.9±0.5	2.7±0.2	2.8±0.5	76 (0.75)
177		3.5±1.6	3.8±0.8	3.0±1.4	1.9±0.5	1.1±0.9	1.9±0.1	52 (0.83)
180		3.8±1.7	3.9±0.6	3.7±0.5	2.7±0.5	2.9±0.6	1.9±0.4	50 (0.94)
Total dichlorobiphenyls		0.1±0.2						
Total trichlorobiphenyls		66±9.3	73±13	29±10	22±10	20±9.4	13±2.9	
Total tetrachlorobiphenyls		240±24	230±17	170±38	100±17	75±5.0	74±17	
Total pentachlorobiphenyls		360±26	360±28	310±76	220±25	200±32	190±30	
Total hexachlorobiphenyls		120±42	140±25	130±19	97±19	91±14	95±4.7	
Total heptachlorobiphenyls		45±25	41±13	32±9.7	19±8.1	16±2.8	16±1.6	
Total octachlorobiphenyls		1.3±0.9	1.0±0.2	2.5±1.7	1.2±1.5	1.7±2.7	0.4±0.5	
Total nonachlorobiphenyls		1.0±0.3	0.7±0.2	0.9±1.2	0.4±0.5	0.2±0.2	0.4±0.1	
Total decachlorobiphenyl		0.9±1.0	0.5±0.4	0.5±0.9		0.1±0.1	0.2±0.1	
Total PCBs		830±110	850±82	670±120	470±76	400±59	380±50	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-9

Average PCB Congener Concentrations (± 1 S.D.) in Sediment and Ship Channel-to-Hanna Reef (SCHR) Transplanted Oyster Samples During the Depuration Phase of the Experiment at the Hanna Reef Site and Estimated Biological Half-Lives (BHL).

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
18		6.1±0.6	6.5±2.0	3.8±1.0	2.3±0.6	1.7±1.0	1.1±0.2	19 (0.93)
15/17		4.8±0.5	4.8±0.7	4.0±0.6	2.4±1.6	1.2±0.4	0.9±0.2	
26		36±8.0	37±4.6	28±11	23±10	14±10	7.1±1.5	22 (0.99)
50/31		5.2±0.8	7.9±1.3	3.2±0.4	6.7±3.1	7.6±1.7	5.5±1.1	
28		8.0±1.4	6.5±0.9	8.5±0.6	5.3±0.6	4.2±1.0	2.9±0.3	34 (0.93)
52		52±4.0	46±7.0	50±6.1	32±1.6	30±9.7	24±3.5	45 (0.91)
49		31±3.2	29±4.6	32±5.4	27±2.4	24±7.6	17±1.0	61 (0.94)
47/48/75		19±2.0	17±1.9	19±1.8	14±1.4	13±3.9	12±2.0	
44		20±3.2	20±2.2	22±2.5	15±0.9	14±5.0	9.7±0.8	45 (0.94)
37/42/59		33±5.3	23±6.4	18±3.0	9.5±0.9	8.1±2.8	6.6±0.5	
41		16±2.8	16±3.2	16±3.8	11±1.2	11±2.9	9.2±1.5	55 (0.92)
40		13±0.5	11±1.6	10±2.3	5.7±1.4	3.2±0.9	2.1±0.2	18 (0.97)
74		15±0.9	14±1.3	15±0.9	11±0.7	8.5±2.2	7.4±0.7	47 (0.95)
70		38±2.0	33±4.6	36±3.2	28±2.9	26±7.6	20±2.4	58 (0.96)
95		63±8.2	59±8.4	70±8.9	54±4.8	55±18	44±0.3	95 (0.79)
91		22±1.0	18±2.7	20±2.4	13±1.7	13±3.9	11±0.8	50 (0.89)
60/56		9.5±1.4	10±1.4	11±1.4	7.4±0.5	7.0±2.6	5.1±0.3	
92		26±6.6	26±4.8	25±5.5	20±2.1	20±5.9	15±0.7	63 (0.93)

TABLE A-9
(continued)

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
84		29±7.3	31±3.6	33±2.7	23±3.1	23±8.1	21±1.4	80 (0.79)
101/90		71±8.5	69±7.4	80±7.1	64±3.8	62±12	54±3.0	91 (0.79)
99		45±4.6	44±4.7	52±0.6	39±4.1	39±3.3	32±2.2	
97		20±3.7	20±2.3	25±1.9	21±2.3	21±6.4	21±2.7	
87/115		32±3.1	28±3.9	34±3.5	27±2.6	27±7.6	26±2.6	132 (0.45)
110/77		80±12	76±9.5	88±10	67±8.2	58±8.8	62±4.7	103 (0.67)
82		6.3±2.3	8.5±0.6	12±1.1	9.8±1.7	7.6±2.1	8.2±2.0	
151		10±2.2	10±1.0	13±1.5	11±0.6	11±1.7	10±1.3	
135		8.7±2.1	7.3±1.1	8.9±1.7	7.5±0.7	7.4±1.4	7.1±1.0	
107		15±3.1	11±4.1	10±1.1	7.3±1.0	6.6±0.7	6.3±1.4	46 (0.75)
149/123		36±3.5	31±3.5	36±5.1	32±2.7	33±6.9	31±2.0	439 (0.24)
118		56±6.3	55±5.8	68±7.4	65±18	55±5.8	52±5.3	299 (0.19)
146		7.8±2.1	8.0±1.3	10±1.9	8.2±1.2	7.9±1.9	7.3±0.9	239 (0.27)
153/132		100±10	95±24	98±27	80±10	77±10	72±5.5	102 (0.90)
105		17±6.1	17±4.7	23±2.1	20±2.8	19±6.4	17±2.5	120 (0.76)
141/179		5.2±0.9	5.8±1.1	5.4±1.2	4.7±0.9	4.6±0.5	4.6±0.9	
138/160		47±10	48±4.0	56±6.9	49±4.1	48±9.7	47±1.8	595 (0.11)
187		15±2.5	14±1.3	13±3.2	12±2.6	13±1.8	13±2.1	258 (0.56)

TABLE A-9
(continued)

Analyte	Sediment ng g^{-1}	Depuration phase (days) 0	3	6	18	30	50	BHL(R^2)[a]
		ng g^{-1}						
128		5.9±1.0	5.9±0.4	6.9±0.8	1.2±0.2	5.5±2.0	6.4±1.3	229 (0.42)
177		4.6±0.1	3.6±0.3	4.4±0.8	3.6±0.2	3.7±0.6	3.4±0.4	145 (0.54)
180		4.6±0.2	5.2±0.8	5.1±0.7	4.4±1.4	3.8±0.5	4.1±0.9	142 (0.63)
Total dichlorobiphenyls		0.0	0.6±1.1	0.0	0.4±0.7	0.3±0.3	0.0	
Total trichlorobiphenyls		71±12	67±10	53±5.1	40±12	31±12	19±3.6	
Total tetrachlorobiphenyls		250±21	220±29	230±26	160±9.4	150±43	120±11	
Total pentachlorobiphenyls		490±64	470±42	550±38	430±41	390±59	370±30	
Total hexachlorobiphenyls		230±31	220±32	240±45	200±16	200±30	190±16	
Total heptachlorobiphenyls		52±5.9	47±1.5	46±6.3	33±4.4	31±6.3	27±3.0	
Total octachlorobiphenyls		1.9±0.2	1.3±0.9	2.8±2.0	0.9±0.9	0.2±0.3	0.3±0.3	
Total nonachlorobiphenyls		0.8±0.3	1.4±1.0	1.6±0.7	0.7±0.4	0.5±0.2	0.6±0.2	
Total decachlorobiphenyl		0.4±0.3	0.9±0.7	1.2±0.3	0.3±0.3	0.1±0.1	0.1±0.1	
Total PCBs		1100±130	1000±110	1100±110	870±64	800±140	730±65	

[a] R2 = square of correlation coefficient for regression equation.

TABLE A-10

TBT, DBT and MBT Concentrations in Indigenous Ship Channel and Transplanted Hanna Reef Oysters.

Sample	Days after transplant	TBT	DBT (ng Sn g^{-1})	MBT
Hanna Reef-to-Ship Channel (HRSC)				
HRSC	0	40	13	9
HRSC	3	68	<5	<5
HRSC	7	130	10	<5
HRSC	17	210	<5	<5
HRSC	30	230	6	<5
HRSC	48	360	22	<5
Hanna Reef-Ship Channel-Hanna Reef (HRSCHR)				
HRSCHR	51	330	<5	<5
HRSCHR	54	290	21	<5
HRSCHR	66	180	<5	<5
HRSCHR	78	130	6	<5
HRSCHR	98	110	<5	<5
Ship Channel (SC)				
SC	3	350	24	<5
SC	7		Sample was not collected	
SC	17	310	22	<5
SC	30	320	32	<5
SC	48	390	34	<5
Ship Channel-to-Hanna Reef (SCHR)				
SCHR	51	320	31	<5
SCHR	54	340	62	<5
SCHR	66	240	24	<5
SCHR	78	220	16	<5
SCHR	98	130	10	<5

TABLE A-11

PCB Congener Concentrations in Oysters During Exposure in the Laboratory to a 1:1:1:1 Mixture of Aroclors 1242, 1248, 1254 and 1260 and Following Depuration in Contaminant-Free Aquariums.

	Uptake phase (days)					Depuration phase (days)				
Analyte	0	3	7	15	30	3	7	15	30	BHL (R^2)[a]
			ng g^{-1}				ng g^{-1}			
18		1.36	1.93	3.58	6.46	7.78	6.42	5.77	2.50	28 (0.87)
15/17		0.21	0.54	0.72	1.55	1.61	1.44	1.43	0.66	
50/31		1.02	1.70	3.03	5.54	6.04	4.44	5.34	2.80	
28		1.60	2.93	3.90	8.78	10.6	8.49	9.04	3.90	33 (0.78)
52		2.16	3.22	5.56	12.5	12.8	11.4	10.3	5.17	28 (0.96)
49		1.38	2.42	3.39	8.63	9.03	7.72	7.91	4.04	38 (0.86)
47/48/75		0.90	1.23	2.85	7.93					
44		1.26	2.03	3.33	8.27	9.10	7.66	7.56	3.65	34 (0.87)
37/42/59		0.87	2.05	3.07	5.64					
41		1.28	2.52	4.46	15.1	14.7	15.4	12.5	4.74	25 (0.88)
40		0.48	0.62	1.09	2.72	3.26	2.59	2.45	1.05	28 (0.86)
74		1.40	1.96	3.82	10.3	10.3	9.29	9.10	6.18	57 (0.93)
70		3.17	4.60	8.29	20.4	21.4	19.5	18.8	12.6	58 (0.90)
91		0.18	0.21	0.51	1.31	1.43	1.51	1.15	1.01	83 (0.73)
60/56		0.97	1.68	2.71	6.93	7.51	7.56	6.06	4.40	
92		0.17	0.16	0.43	1.35	1.53	1.87	1.11	1.18	99 (0.31)

TABLE A-11
(continued)

Analyte	Uptake phase (days) 0	3	7	15	30	Depuration phase (days) 3	7	15	30	BHL (R^2)[a]
		ng g^{-1}				ng g^{-1}				
84		0.45	0.62	0.97	2.55	2.81	3.04	2.25	1.85	66 (0.75)
101/90		1.92	2.29	4.41	11.2	11.4	11.5	9.10	8.55	90 (0.83)
99		1.46	1.42	2.08	5.15	4.61	4.26	4.01	3.73	101 (0.83)
97		0.70	0.87	1.86	4.31	4.19	3.90	3.86	3.08	
87/115		1.40	1.74	3.19	8.74	8.04	7.39	7.18	5.21	
110/77		1.92	2.79	4.85	16.0	13.7	12.6	13.6	10.2	80 (0.78)
82		1.38	1.33	1.84	2.61	2.44	2.65	1.86	2.25	
151		0.41	0.45	1.05	3.00	2.59	2.68	2.38	1.96	
135		0.34	0.40	0.73	2.27	1.76	1.78	1.54	1.60	
149/123		1.45	1.88	3.14	7.49	7.11	7.20	7.90	7.05	
118		2.21	1.86	4.35	10.1	9.74	11.5	10.1	7.71	103 (0.58)
146		0.41	0.50	0.78	1.39	1.55	1.34	1.58		
153/132		2.61	3.23	5.55	17.0	13.5	11.9	11.9	11.1	90 (0.59)
105		0.42	0.46	1.56	3.45	3.51	4.02	2.81	3.38	
141/179		0.47	0.76	0.74	1.82	1.89	1.51	1.33	1.42	
138/160		2.90	4.08	7.24	16.2	14.6	13.6	11.0	10.1	63 (0.86)

TABLE A-11
(continued)

Analyte	Uptake phase (days) 0	3	7	15	30	Depuration phase (days) 3	7	15	30	BHL (R^2)[a]
		ng g^{-1}				ng g^{-1}				
187		1.05	1.48	2.20	5.75	4.59	4.20	4.37	4.34	
128		0.30	0.45	0.85	2.01	1.45	1.24	1.05	0.98	48 (0.72)
177		0.35	0.50	0.87	2.36	1.81	1.77	1.62	1.80	
180		0.37	0.40	0.35	0.40	0.18	0.10	0.16	0.08	
Total dichlorobiphenyls										
Total trichlorobiphenyls		4.93	8.21	12.7	24.8	29.7	24.9	24.2	11.7	
Total tetrachlorobiphenyls		14.9	24.4	41.6	105	95.5	87.7	82.5	47.6	
Total pentachlorobiphenyls		13.1	14.8	27.6	67.6	64.6	64.7	61.0	49.4	
Total hexachlorobiphenyls		9.05	12.0	20.5	51.9	44.8	41.6	36.8	34.6	
Total heptachlorobiphenyls		2.15	2.82	3.91	9.74	7.46	6.58	6.62	6.62	
Total octachlorobiphenyls		0.09	0.12	0.18	0.20	0.32	0.24	0.15	0.35	
Total nonachlorobiphenyls										
Total decachlorobiphenyl										
Total PCBs		44.1	62.3	106	259	242	226	211	150	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-12

PCB Congener Concentrations in Oysters During Exposure in the Laboratory to a Mixture of PCBs Plus PAHs and Following Depuration in Contaminant-Free Aquariums.

Analyte	Uptake phase (days)					Depuration phase (days)				BHL (R^2)[a]
	0	3	7	15	30	3	7	15	30	
	ng g^{-1}					ng g^{-1}				
18		2.25	2.10	6.79	10.5					45 (0.82)
15/17		0.16	0.52	1.23	2.16	1.90	2.35	1.78	1.21	
50/31		0.66	1.53	3.49	7.35	6.32	7.75	5.53	4.22	
28		1.27	2.15	5.57	13.5	11.3	12.0	10.2	7.41	52 (0.94)
52		2.44	2.88	7.13	17.2	16.9	15.8	14.6	11.9	78 (1.00)
49		1.05	2.14	4.87	12.7	10.3	10.1	9.57	8.59	93 (0.77)
44		1.02	1.80	4.65	11.6	10.4	10.6	9.04	6.96	59 (0.98)
41		3.18	2.96	7.95	23.0	21.8	18.6	15.6	11.9	44 (0.98)
40		0.64	0.55	1.34	3.73	3.70	3.44	2.85	2.17	51 (0.99)
74		2.12	2.65	5.99	16.1	13.5	13.8	12.2	9.50	61 (0.94)
70		2.14	4.47	11.4	29.1	26.1	25.6	22.9	19.1	74 (0.97)
91		0.34	0.43	0.77	1.75	1.70	1.65	1.51	1.52	143 (0.77)
60/56		0.83	1.72	4.06	10.4	11.3	9.70	8.66	7.21	
92		0.20	0.45	0.97	2.25	2.01	1.98	2.01	1.67	119 (0.84)
84		0.37	0.59	1.49	3.78	3.76		3.36	2.54	72 (0.96)
101/90		1.30	2.60	6.37	14.4	16.5	13.2	13.7	12.5	155 (0.50)

TABLE A-12
(continued)

Analyte	Uptake phase (days) 0	3	7	15	30	Depuration phase (days) 3	7	15	30	BHL (R^2)[a]
		ng g^{-1}				ng g^{-1}				
99		0.48	1.00	2.18	6.57	5.43	4.80	4.90	4.74	120 (0.50)
97		0.33	0.76	2.12	5.88	4.51	4.27	4.37	3.94	
87/115		0.57	1.47	3.88	11.2	9.61	8.77	7.51	7.22	72 (0.79)
110/77		0.61	2.35	6.82	20.7	17.7	17.1	15.7	15.6	125 (0.65)
82		1.46	1.60	2.05	3.13	2.32	3.87	2.57	2.01	
151		0.29	0.44	1.34	3.71	2.94	2.50	2.71	2.44	
135		0.30	0.42	0.90	2.42	1.92	1.76	1.61	1.61	
149/123		0.86	1.76	4.08	9.96	9.76	7.90	6.89	7.72	
118		1.31	1.95	5.33	11.8	10.4	9.35	10.7	9.79	272 (0.23)
146		0.33	0.47	0.99	1.21	1.70	1.65	1.75	1.45	
153/132		1.41	3.08	7.69	20.7	13.5	12.9	12.7	13.1	103 (0.30)
105		0.30	0.84	2.12	4.26	5.60	3.77	3.81	4.24	
141/179		0.17	0.35	0.73	1.77	1.59	1.34	1.43	1.22	
138/160		1.96	3.52	7.93	16.5	13.9	11.6	11.5	11.0	86 (0.62)
187		0.73	1.17	2.57	6.16	4.50	4.23	4.29	3.88	89 (0.53)
128		0.21	0.35	0.82	1.82	1.25	1.10	0.95	0.82	44 (0.76)

TABLE A-12
(continued)

Analyte	Uptake phase (days) 0	3	7	15	30	Depuration phase (days) 3	7	15	30	BHL (R^2)[a]
			ng g^{-1}				ng g^{-1}			
177		0.18	0.43	0.95	2.40	1.82	1.49	1.54	1.56	95 (0.38)
180		0.33	0.24	0.51	0.37	0.19	0.09	0.11	0.18	
Total dichlorobiphenyls										
Total trichlorobiphenyls		5.27	7.58	19.4	37.4	32.5	38.3	28.6	21.0	
Total tetrachlorobiphenyls		14.4	20.9	51.7	135	125	118	106	85.2	
Total pentachlorobiphenyls		7.44	14.8	37.4	89.4	83.8	74.5	73.7	68.6	
Total hexachlorobiphenyls		5.77	10.7	25.1	58.8	47.7	41.9	40.8	40.4	
Total heptachlorobiphenyls		1.35	2.04	4.40	9.70	7.13	6.19	6.27	5.89	
Total octachlorobiphenyls		0.09	0.09	0.14	0.12	0.16	0.27	0.11		
Total nonachlorobiphenyls										
Total decachlorobiphenyl										
Total PCBs		34.3	56.1	138	330	296	279	255	221	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-13

Polynuclear Aromatic Hydrocarbon Concentrations in Oysters During Exposure in the Laboratory to a Mixture of Selected PAHs and Following Depuration in Contaminant-Free Aquariums.

Analyte	Uptake phase (days)					Depuration phase (days)				BHL (R^2)[a]
	0	3	7	15	30	3	7	15	30	
	ng g^{-1}					ng g^{-1}				
Naphthalene	22	26	13	9.4	10	9.8	16	8.6	8.6	
2-Methylnaphthalene	16	31	21	9.0	8.7	5.9	9.5	5.2	6.8	
1-Methylnaphthalene	9.0	27	16	6.2	7.7	5.3	9.7	5.0	5.0	
Biphenyl	8.3	64	56	37	16	10	11	7.3	6.4	
2,6-Dimethylnaphthalene	15	57	50	35	13	6.9	12	7.2	7.4	
Acenaphthylene	7.3	30	27	24	22	12	13	9.1	5.7	
Acenaphthene	4.0	48	48	48	65	9.1	8.8	8.3	5.3	
2,3,5-Trimethylnaphthalene	26	69	73	61	51	21	23	15	11	16 (0.75)
Fluorene	9.3	54	57	48	44	9.1	12	7.9	7.0	
Phenanthrene	14	69	64	46	49	25	31	28	22	
Anthracene	16	33	42	50	70	24	29	19	13	16 (0.68)
1-Methylphenanthrene	62	119	115	92	86	37	60	22	19	16 (0.72)
Fluoranthene	11	84	91	76	144	44	68	15	11	9 (0.78)
Pyrene	16	86	82	88	83	31	47	8.7	8.4	9 (0.75)
Benz(a)anthracene	8.3	85	67	154	305	279	228	94	93	16 (0.81)
Chrysene	7.7	52	55	112	286	268	276	144	124	22 (0.86)
Benzo(b)fluoranthene/ Benzo(k)fluoranthene	3.3	183	153	300	744	705	742	570	330	25 (0.94)

TABLE A-13
(continued)

Analyte	Uptake phase (days) 0	3	7	15	30	Depuration phase (days) 3	7	15	30	BHL (R^2)[a]
	ng g^{-1}					ng g^{-1}				
Benzo(e)pyrene	4.0	104	92	154	430	436	446	262	174	21 (0.93)
Benzo(a)pyrene	6.3	29	16	35	97	61	82	25	17	12 (0.87)
Perylene	3.0	8.9	12	22	47	42	45	27	13	15 (0.96)
Indeno[1,2,3-c,d]pyrene	10	73	47	102	200	171	137	71	28	10 (1.00)
Dibenzo(a,h)anthracene	3.0	19	9.6	26	45	36	24	13	6.5	11 (0.97)
Benzo(g,h,i)perylene	7.0	36	27	65	144	161	133	94	43	16 (0.96)
Total 2-Rings	97				106				44	
Total 3-Rings	113				337				72	
Total 4-Rings	43				818				237	
Total 5-Rings	23				1370				541	
Total 6-Rings	17				344				71	
Total PAHs	293				2970				965	

[a] R^2 = square of correlation coefficient for regression equation.

TABLE A-14

Polynuclear Aromatic Hydrocarbon Concentrations in Oysters During Exposure in the Laboratory to a Mixture of Selected PAHs and Following Depuration in Contaminant-Free Aquariums.

Analyte	Uptake phase (days)					Depuration phase (days)				BHL (R^2)[a]
	0	3	7	15	30	3	7	15	30	
	ng g^{-1}					ng g^{-1}				
Naphthalene	22	21	10	9.3	7.9	12	16	17	13	
2-Methylnaphthalene	16	36	16	10	9.2	9.9	16	14	9.1	
1-Methylnaphthalene	9.0	29	12	7.7	5.5	5.8	9.2	9.5	6.8	
Biphenyl	8.3	94	49	40	19	9.8	7.9	6.7	5.0	
2,6-Dimethylnaphthalene	15	81	39	28	27	12	12	13	5.8	
Acenaphthylene	7.3	50	27	36	37	18	11	12	5.1	
Acenaphthene	4.0	74	54	87	49	8.3	7.4	4.8	3.9	
2,3,5-Trimethylnaphthalene	26	99	68	56	78	34	28	14	6.9	10 (0.91)
Fluorene	9.3	90	43	62	51	11	12	8.6	5.5	
Phenanthrene	14	113	77	56	60	38	37	24	21	
Anthracene	16	49	64	81	118	55	45	14	8.9	8 (0.89)
1-Methylphenanthrene	62	125	123	114	144	78	73	12	8.2	7 (0.88)
Fluoranthene	11	84	118	162	194	79	77	11	7.8	7 (0.85)
Pyrene	16	81	120	138	150	74	63	9.4	5.4	6 (0.89)
Benz(a)anthracene		33	85	169	519	342	238	127	46	9 (0.99)
Chrysene		20	59	120	357	304	198	155	62	12 (0.98)
Benzo(b)fluoranthene/ Benzo(k)fluoranthene		58	144	317	859	662	521	474	148	13 (0.95)

TABLE A-14
(continued)

Analyte	Uptake phase (days)					Depuration phase (days)				BHL (R^2)[a]
	0	3	7	15	30	3	7	15	30	
	ng g^{-1}					ng g^{-1}				
Benzo(e)pyrene		31	88	170	491	452	303	227	120	15 (0.98)
Benzo(a)pyrene		12	27	57	202	60	37	48	12	9 (0.78)
Perylene		5.2	13	21	65	40	18	20	5.8	10 (0.89)
Indeno[1,2,3-c,d]pyrene		14	42	90	329	113	47	81	14	8 (0.78)
Dibenzo(a,h)anthracene		5.6	16	26	72	24	9.4	17	4.5	10 (0.69)
Benzo(g,h,i)perylene		7.4	21	47	136	85	51	50	17	11 (0.92)
Total 2-Rings	97				146				45	
Total 3-Rings	113				459				53	
Total 4-Rings	43				1,220				120	
Total 5-Rings	23				1,690				290	
Total 6-Rings	17				466				31	
Total PAHs	293				3,980				540	

[a] R^2 = square of correlation coefficient for regression equation.

VITA

José Luis Sericano was born in Puerto Belgrano, Buenos Aires, República Argentina, on October 10, 1953. He is the son of Vicente Luis and Margarita Sericano. He attended public schools and graduated from Colegio Nacional Punta Alta, Punta Alta, República Argentina, in December 1971. He attended Universidad Nacional del Sur, Bahía Blanca, República Argentina, and graduated as Químico, Lic. en Bioquímica and Lic. en Química in August 1975, December 1976 and August 1977, respectively. He enrolled in the Graduate College at Texas A&M University in August 1983 and received a M.S. in Oceanography in May, 1986. In February 1981, he married Nélida María Cavallín and their family consists of one son, Mauro Luis, born in January 1982 and one daughter, Gisella María, born in June 1987.

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