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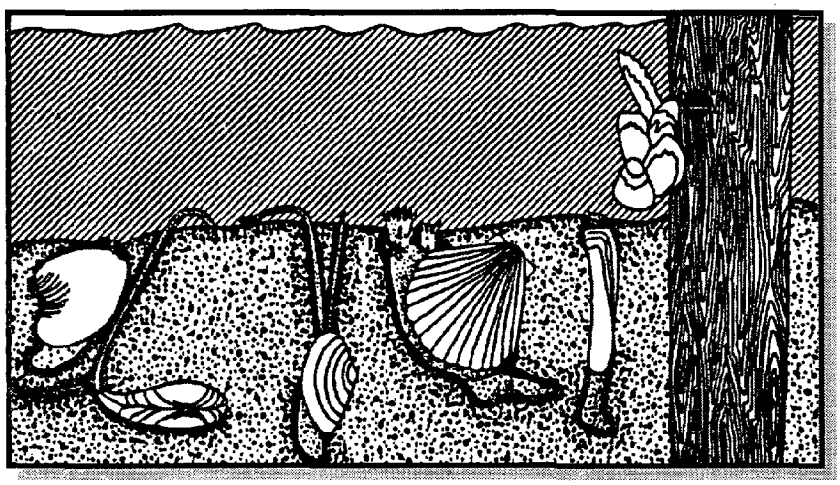
NOAA STATUS AND TRENDS
Mussel Watch Project
Technical Report
Year VIII

The Geochemical and Environmental Research Group
Texas A&M Research Foundation



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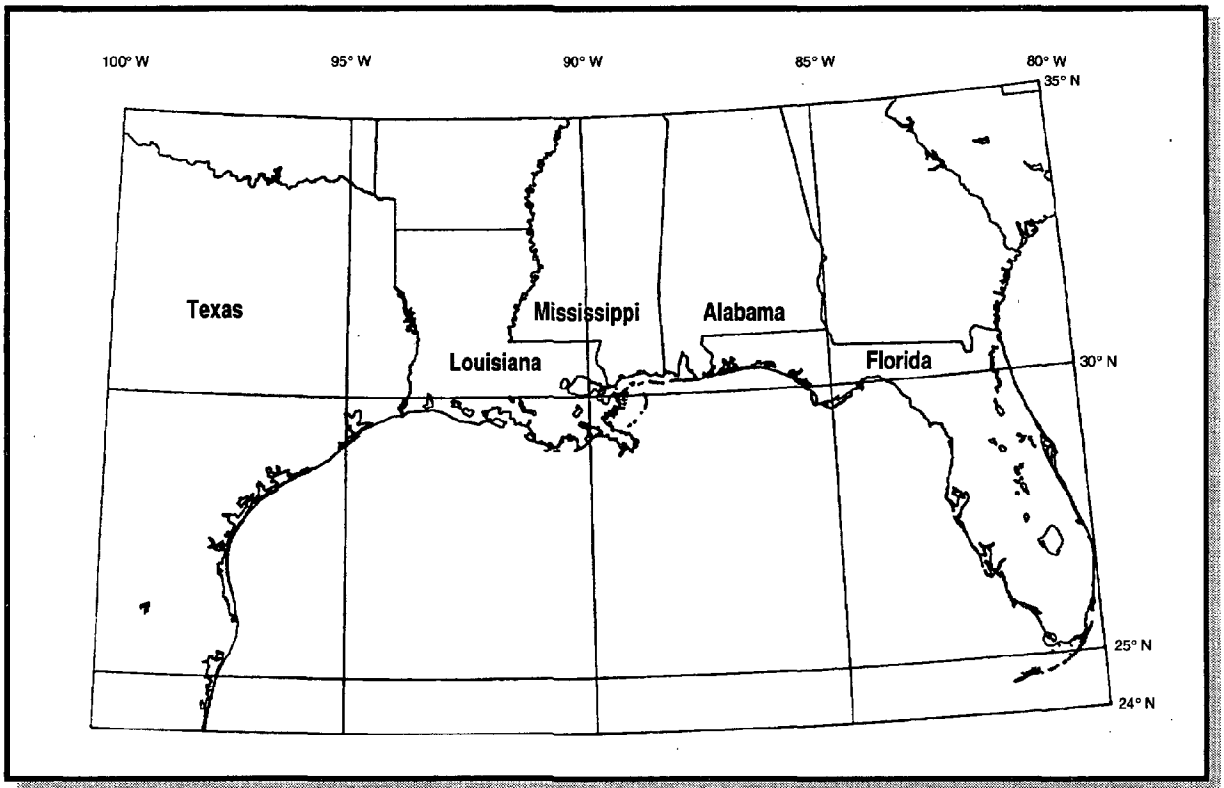

September 1994

NOAA NATIONAL STATUS AND TRENDS

Mussel Watch Project

Year 8 Technical Report

Prepared by

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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 8 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 8 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).

Year 8 - Sixty-eight (68) existing sites were sampled. Only one oyster analysis was conducted at each of the existing sites on a composite from the three stations. Sediments were not collected at any sites.

Details of the sample collection and location of field sampling sites are contained in a separate report titled "Field Sampling and Logistics in Year 8".

The oyster and sediment samples were analyzed for contaminant concentrations [trace metals, polynuclear aromatic hydrocarbons (PAH), pesticides and polychlorinated biphenyls (PCBs)], 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 8 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 on-going 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 eight 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 eight-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 further 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 8 of the NS&T Gulf of Mexico projects. The other two reports are entitled:

- Analytical Data, Year 8
- Field Sampling and Logistics, Year 8

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

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Reprint 1

Sources of Local Variation in Polynuclear Aromatic Hydrocarbon Pesticide Body Burden in Oysters (Crassostrea virginica) from Galveston Bay, Texas

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

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SOURCES OF LOCAL VARIATION IN POLYNUCLEAR AROMATIC HYDROCARBON AND PESTICIDE BODY BURDEN IN OYSTERS (*CRASSOSTREA VIRGINICA*) FROM GALVESTON BAY, TEXAS

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Abstract—1. Eggs and sperm contain significantly more PAH (polynuclear aromatic hydrocarbon) than somatic tissues in oysters (*Crassostrea virginica*) taken from Galveston Bay.

2. The quantity of gonadal material was the most important correlate of PAH body burden.

3. Eggs, but not sperm, were enriched in chlorinated compounds (e.g. DDD, chlordane), while both eggs and sperm were enriched in total PCBs relative to somatic tissue.

4. Oysters may lose up to 50% of their total body burden of certain PAHs and pesticides in a single spawn.

INTRODUCTION

Bivalve molluscs have frequently been used as indicator organisms in studies monitoring levels of contaminants in the environment. These organisms are utilized because of their ability to accumulate and concentrate both metal and organic contaminants enabling them to serve as long-term integrators of their environment (Phillips, 1977). One such program is the NOAA Status and Trends (NS&T) Program ("Mussel Watch") designed to monitor changes in environmental quality along the Atlantic, Pacific and Gulf coasts of the United States by measuring levels of chemical contaminants in fish, bivalves, and sediments and identifying biological responses to those contaminants (e.g. Wilson *et al.*, 1992, 1990; Sericano *et al.*, 1990; Presley *et al.*, 1990).

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

In the Gulf of Mexico, the mollusc used for monitoring by NOAA is the oyster *Crassostrea virginica*. Analysis of the first 4 yr of NS&T data has shown that the body burden of polynuclear aromatic hydrocarbons (PAHs) and pesticides in oysters is correlated with latitude in the Gulf of Mexico. Contaminant body burdens average higher at higher latitudes. Wilson *et al.* (1990) suggested that the latitudinal temperature gradient in the Gulf produced variation in reproductive effort and that this variation in reproductive effort affected PAH body burden sufficiently to override the effect of local variation in contaminant loading. Wilson *et al.* (1992), in a more thorough analysis, showed that PAH body burden responds to climate change and that biological factors are the likely intermediaries between the climate's effect on temperature and freshwater inflow and the final body burden of PAHs.

Two likely intermediaries are spawning and disease. Spawning has frequently been forwarded as an important route of depuration (Marcus and Stokes, 1985; Jovanovich and Marion, 1987; Cossa, 1989) because lipid loss peaks at this time (Chu *et al.*, 1990). Parasites and pathogens are less frequently implicated (Khan, 1987), but parasites and pathogens should have an effect; if for no other reason, they frequently reduce spawning frequency or the number of gametes per spawn (Akberali and Trueman, 1985; Ford and Figueras, 1988; Barber *et al.*, 1988). In oysters, both spawning frequency and disease are significantly affected by temperature and salinity (Hofmann *et al.*, 1992; Soniat and Gauthier, 1989) and thus could serve as important intermediaries by which variation in climate might affect contaminant body burden.

Climate exerts its influence over large geographic scales. Biological parameters capable of responding to climate change and, thus, affecting contaminant body burden on a large geographic scale, should certainly do so as well on a local scale. Accordingly,

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

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

spawning frequency and disease should be important sources of local (within population) variability in contaminant body burden. Monitoring programs typically sample infrequently (NS&T samples once per year) so that the basis for within-sample variability is an important consideration. Accordingly, the primary purpose of this study was to examine sources of local variability in PAH body burden at any sampling period. Some analyses of chlorinated pesticides and PCBs were also conducted.

Unfortunately, the variables likely of most importance in determining local variability in body burden, spawning frequency and the time since the last spawn, are variables that cannot be readily measured even in a temporally-intensive sampling program because continuous (or dribble) spawning is a frequent condition at latitudes south of Chesapeake Bay, including the entire Gulf of Mexico (Hofmann *et al.*, 1992). Consequently, more readily measured variables must be used as surrogates for the more desirable variables. Thus, we examined a series of indices related to reproductive state, including stage of reproduction and the quantity of gonadal material present, and a series of indices related to health, namely digestive gland atrophy, condition and *Perkinsus marinus* infection intensity. *Perkinsus marinus*, an endoparasitic protozoan, is responsible for high mortality (typically >50%) in market-sized oysters in the Gulf each year (Hofstetter, 1977; Osburn *et al.*, 1985; Ray, 1987) and is known to delay reproduction (White *et al.*, 1988; Wilson *et al.*, 1988). Digestive gland atrophy is a putatively pathogenic condition (e.g. Marigómez *et al.*, 1990; Moore *et al.*, 1989) common in Gulf coast oysters (Gauthier *et al.*, 1990).

METHODS

Within-population differences in body burden

Oysters were collected in September, 1990, from Confederate Reef in the West Bay extension of Galveston Bay. Confederate Reef oysters normally have a relatively high PAH body burden in comparison to the Gulf-wide mean (Sericano *et al.*, 1990; Wade *et al.*, 1988). September is near the end of the spawning season; most individuals should have spawned at least twice over the 4 previous months. The oysters were placed on ice and returned to the laboratory. Maximum length and wet weight were determined. The condition of each meat was rated on a semiquantitative scale from 1 (very good) to 9 (very poor), according to Quick and Mackin (1971). A small section of gonadal tissue was taken and fixed in Davidson's fixative (Fig. 28 in NOAA, 1983). A small section of mantle tissue was removed for determination of *P. marinus* infection following Ray (1966). The remaining tissue was placed in a precombusted mason jar with a teflon-lined screw cap and frozen for PAH analyses.

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

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

Table 2. The scale used for digestive gland atrophy

Assigned numerical value	Description
0	Normal
1	Less than one-half atrophied
2	About one-half atrophied
3	Greater than one-half atrophied
4	Completely atrophied

GERG, 1990). Only a brief overview will be given here.

Samples were extracted with methylene chloride after drying with Na_2SO_4. The samples were then purified by silica/alumina column chromatography. In order to remove lipids, a high-performance liquid chromatography separation was performed. Purified extracts were then analyzed by gas chromatography with a mass spectrometry detector, GC/MS/SIM for PAHs and GC-ECD for chlorinated pesticides and PCBs. All concentrations are reported as nanograms of analyte per gram dry weight of sample, or ppb. Concentrations in the procedural blanks were, in all cases, below reporting levels for each individual analyte. The accuracy and precision of these methods have been established by several intercalibration exercises overseen by the U.S. National Institute of Standards and Technology.

Oyster gonadal tissue surrounds much of the body mass and, thus, is difficult to excise cleanly and weigh (Kennedy and Battle, 1964; Morales-Alamo and Mann, 1989). Thus, a quantitative gonadal index based on gonad weight, as is frequently used in invertebrates and fish, is not available. Accordingly, a polyclonal rabbit anti-oyster egg antibody was used to quantify the amount of egg protein present (Choi *et al.*, 1993). A single radial immunodiffusion assay (Mancini *et al.*, 1965; Garvey *et al.*, 1977) was performed to quantitate egg protein using 1.5% agarose in barbitone buffer (0.01 M sodium barbital, 0.0022 M barbital, 0.01% sodium azide as preservative, pH 8.6). Two millilitres of the rabbit serum containing anti-oyster antibody was mixed in 18 ml of the agarose gel and cast on a 10 × 10 cm glass plate. Four millilitre diameter wells were made on the plate using a gel puncher and 20 μl of oyster egg standard (0.05 mg ml^{-1} to 3.2 mg ml^{-1}) or the sample were placed in the wells and incubated in a humid chamber for 48 hr at room temperature. After incubation, the plate was pressed, dried, stained with 0.5% (w/v) Coomassie Brilliant Blue, and destained with 50% EtOH and 10% acetic acid. Diameters of the precipitation rings were measured to the nearest 0.1 mm. A standard curve was constructed by plotting concentration of the egg standard against the diameter squared of the precipitation rings, and the concentration of each sample was read from the curve.

Removal of the body section for histological analysis biases both the total PAH concentration and the gonadal quantity as measured by us. Sericano *et al.* (in press b) showed that the effect of this bias on PAH content is an expected 10–20% reduction in measured body burden. For gonadal quantity, the percent reduction can be expected to be considerably higher. Readers are cautioned not to accept the reported measures of gonadal quantity as true measures of completely intact oysters. However, as most oysters were similar in size, the bias introduced in both measures would be equivalent over all samples and thus not compromise the data analysis.

Body burden of eggs and sperm

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

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

Oyster egg filtrates were washed 4 times by resuspending the filtrates into 30 ml of PBS and centrifuging at 700 *g* for 10 min. During each washing, tissue debris and other impurities sedimented on the egg pellets were removed by pasteur pipette. After the final washing, the egg pellets were resuspended into an equal volume of PBS. Five millilitres of the resuspension was transferred to a 15 ml centrifuge tube, 7 ml PBS added to resuspend the eggs, and the suspension centrifuged at 500 *g* for 15 min. Any remaining tissue debris layered on the egg pellet was removed using a pasteur pipette. Egg pellets from 10–20 oysters were pooled in a 50 ml centrifuge tube and sedimented by centrifugation (700 *g* for 15 min). Oyster egg pellets were then resuspended into an equal volume of PBS. A 60% Percoll solution (4:6 PBS/100% Percoll) (100% Percoll is 9:1 Percoll stock: 10X PBS) was prepared. Five millilitres of egg suspension was mixed with 35 ml 60% Percoll and centrifuged at 900 *g* for 20 min. Oyster eggs formed an aggregate at the top of the centrifuge tube after centrifugation. Purified eggs were harvested from the tube and washed twice by centrifuging at 700 *g* for 10 min.

Oyster sperm filtrates were washed 4 times with PBS by centrifuging at 700 *g* for 15 min. Tissue debris found at the top of the oyster sperm pellet was removed using a pasteur pipette during each washing step. After the final washing, the sperm extracts were resuspended into an equal volume of PBS. 70% Percoll was prepared and 35 ml 70% Percoll was mixed with 5 ml sperm suspension and centrifuged at

900 *g* for 20 min. Oyster sperm were found at the bottom of the centrifuge tube and other impurities found at the top of the Percoll as a float. Purified oyster sperm were pooled from 20–30 oysters and washed twice with PBS by centrifuging at 800 *g* for 15 min.

Because an involved procedure of this sort could lead to significant contamination, each solution was subjected to chemical analysis. No solutions were found to be significantly contaminated by PAHs, pesticides or PCBs.

RESULTS

Within-population differences in PAH body burden

Forty oysters were analyzed (30 females and 10 males). We present the means and ranges of the variables measured in Table 3. The mean length for the group was 8.0 cm, wet weight 9.6 g, condition code 4.3 (fair plus), *Perkinsus marinus* infection intensity 1.45 (light plus), and digestive gland atrophy 2.1 (about half atrophied). The sample contained individuals covering nearly the entire range of condition codes, two-thirds of the range of possible *P. marinus* infection intensities, six of eight possible gonadal states and all stages of digestive gland atrophy. The variability in this data set is typical of single collections of oysters in the Gulf of Mexico region (Wilson *et al.*, 1990).

By sex, the lengths of females and males were fairly close (7.9 cm vs. 8.1 cm); however, females were heavier than males (9.9 g vs. 8.6 g). The weight difference is considerable since females are actually 0.2 cm shorter on average. Condition code for both sexes was also fairly close (4.6 for males vs. 4.2 for females) as was digestive gland atrophy (1.8 for males and 2.2 for females). *Perkinsus marinus* infection intensity differed substantially with males at 0.77 and females at 1.67. Most animals were nearly ready to spawn or spawning. Reproductive stage was similar: 5.3 and 5.6 for males and females, respectively. When measured quantitatively, the 30 females averaged 6.29 mg eggs per female (equivalent to about 4.8×10^5 fully-developed eggs per female). As a section of gonad was removed for histology, these values underestimate female fecundity.

Although we explored the entire suite of PAHs per NOAA's Status and Trends protocol (GERG, 1990), we only report data for the five most important PAHs: fluoranthene, phenanthrene, pyrene, naphthalene and chrysene. Males and females had similar body burdens except for fluoranthene where females had about one-third more. Means for both sexes ranged from 12.0 ng g dry wt^{-1} for phenanthrene to 49.0 ng g dry wt^{-1} for fluoranthene.

A Spearman's rank analysis showed that many of the biological variables were correlated as might be expected. Accordingly, prior to considering their relationship with the PAHs, the relationships among

Table 3. Means and ranges of PAH concentration and the biological parameters measured

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

ND, *not determined.*

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

Variable	R^2	Explanatory variable ($N = 39$)
Perkinsus marinus infection intensity	0.18	Condition code Wet weight Sex***
Digestive gland atrophy	0.14	Length Condition code Gonadal stage**
Sex	0.21	Length Condition code *P. marinus* infection intensity***
Gonadal stage	0.34	Condition code* Wet weight**** Digestive gland atrophy*
Condition code	0.15	Gonadal stage* Wet weight** Digestive gland atrophy

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

Variable	R^2	Explanatory variable ($N = 10$)
Gonadal stage	0.70	Length Wet weight *Perkinsus marinus* infection intensity***
Condition code	0.20	Length Wet weight *Perkinsus marinus* infection intensity
Perkinsus marinus infection intensity	0.74	Length* Wet weight** Digestive gland atrophy****
Digestive gland atrophy	0.80	*Perkinsus marinus* infection intensity**** Length** Wet weight**

the biological variables themselves must be understood. Because of the many significant correlations among them, we chose to identify the best 3-variable model explaining variation for each of the important biological variables, as detailed in Tables 4 to 6. Because gonadal quantity was measured in only 30 of the 40 individuals and only in females, we examined the data with and without this variable included. The variables examined were length, wet weight, *Perkinsus marinus* infection intensity, digestive gland atrophy, sex, condition code, gonadal stage and gonadal quantity.

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

Considering both sexes together, condition code and sex were the most important variables correlating with the PAHs (Table 7). Among the females, gonadal quantity had a significant effect in three of five cases (Table 8): fluoranthene, pyrene and chrysene. Each of the contaminant's concentrations was higher in females having more eggs. Digestive gland atrophy was also a significant correlate of chrysene. Female oysters having a higher degree of atrophy had more chrysene. If gonadal quantity was removed, few significant correlations remained. Among the males, digestive gland atrophy was significantly correlated in three of five cases (Table 9). PAH concentration was lower in male oysters characterized by a greater degree of digestive gland atrophy. Condition code was significant in two of five cases; higher condition code (less healthy) occurred with higher PAH concentration.

Body burden of eggs and sperm

Samples of pure eggs and sperm, collected from oysters taken earlier in the spawning season than those supporting the previous data, had significantly higher PAH levels than somatic tissue for all five

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

Variable	With gonadal quantity ($N = 23$) R^2	Explanatory variable	Without gonadal quantity ($N = 29$) R^2	Explanatory variable
Gonadal stage	0.54	Length Condition code** Wet weight***	0.47	Condition code Wet weight***** Digestive gland atrophy
Condition code	0.23	Length Gonadal stage** Wet weight***	0.11	Gonadal stage Wet weight Digestive gland atrophy
Perkinsus marinus infection intensity	0.22	Length Gonadal stage Digestive gland atrophy	0.06	Condition code Gonadal stage Digestive gland atrophy
Digestive gland atrophy	0.16	*Perkinsus marinus* infection intensity Length Wet weight	0.11	Condition code Wet weight Gonadal stage
Gonadal quantity			0.07	*Perkinsus marinus* infection intensity Wet weight Digestive gland atrophy

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

Variable	R^2	Explanatory variable
Fluoranthene	0.20	Length
		Perkinsus marinus infection intensity
Phenanthrene	0.11	Sex**
		Condition code
		Gonadal stage
Naphthalene	0.20	Sex
		Condition code***
		Gonadal stage
Pyrene	0.19	Sex
		Length
		Perkinsus marinus infection intensity
Chrysene	0.14	Sex**
		Perkinsus marinus infection intensity
		Wet weight
		Sex

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

Variable	R^2	Explanatory variable
Fluoranthene	0.49	Length
		Gonadal stage
		Digestive gland atrophy*
Phenanthrene	0.67	Condition code**
		Gonadal stage
		Digestive gland atrophy
Naphthalene	0.73	Condition code***
		Gonadal stage*
		Digestive gland atrophy
Pyrene	0.68	Length
		Gonadal stage
		Digestive gland atrophy***
Chrysene	0.59	Condition code
		Gonadal stage
		Digestive gland atrophy*

PAHs (Table 10). A factor of 5 difference was typical. Total PCBs were concentrated in eggs and sperm by a factor of about 5 over the somatic tissue. The chlorinated compounds like lindane, chlordane, dieldrin and DDT (plus breakdown products) were concentrated in eggs by about 4 times, but tended to be equivalent to or lower than the somatic tissue in sperm.

DISCUSSION

Spawning as a route of depuration

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

(1) Both eggs and sperm contain significantly more PAH and PCB than somatic tissue. Eggs also contained more chlorinated pesticides. The concentration factor is sufficient to conclude that over half of the PAH body burden, and somewhat less of the pesticide body burden, could be in gonadal tissue prior to spawning. Eggs and sperm had PAH concentrations 5 times higher than somatic tissue, 3–4 times higher for pesticides, and the gonadal tissue can account for 25% of animal dry weight prior to spawning (Choi *et al.*, 1993; Klinck *et al.*, 1992).

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

(3) Sex was an important determinant of body burden. PAH and PCB concentrations differed between sexes in some cases, chlorinated pesticide concentrations were dramatically lower in male gametes, and the factors correlating with body burden differed. Health-related factors were much more important in males. Factors decreasing health probably also decrease spawning frequency. The most important correlation occurred with digestive gland atrophy; however in males, digestive gland atrophy was highly inversely correlated with *Perkinsus marinus* infection intensity, so the two parameters behaved similarly in explaining the variation in PAH body burden among oysters taken from the same site. PAHs were lower

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

	With gonadal quantity		Without gonadal quantity	
Variable	R^2	Explanatory variable	R^2	Explanatory variable
Fluoranthene	0.37	Condition code	0.18	Length
		Wet weight		Condition code
		Gonadal quantity***		*Perkinsus marinus* infection intensity
Phenanthrene	0.18	*Perkinsus marinus* infection intensity	0.16	Condition code
		Digestive gland atrophy		*Perkinsus marinus* infection intensity
		Gonadal quantity		Digestive gland atrophy
Naphthalene	0.21	Length	0.27	Length***
		Digestive gland atrophy		*Perkinsus marinus* infection intensity
		Gonadal quantity		Digestive gland atrophy*
Pyrene	0.31	Gonadal quantity**	0.20	Length
		Digestive gland atrophy		Condition code
		Gonadal stage		*Perkinsus marinus* infection intensity
Chrysene	0.51	Length***	0.25	*Perkinsus marinus* infection intensity*
		Digestive gland atrophy**		Wet weight*
		Gonadal quantity*****		Digestive gland atrophy

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

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

with lower *P. marinus* infection intensity and *P. marinus* is known to slow reproduction in oysters (Wilson *et al.*, 1988; White *et al.*, 1988).

Reproduction, health and body burden

The importance of reproduction in molluscs in controlling or affecting body burden is open to disagreement. Mix *et al.* (1982) and DiSalvo *et al.* (1975) found PAHs no more concentrated in *Mytilus edulis* gonadal material than somatic tissue (purified eggs were not measured), but noticed a significant drop in body burden during the spawning season. Sericano *et al.* (in press b) found that the central body region including the gonad contained proportionately more PAH in oysters. Lee *et al.* (1972), Fortner and Sick (1985) and Solbakken *et al.* (1982), as examples, found the hepatopancreas to be an important depot for PAHs in bivalves; however, gonadal material, and in particular, gametes, were not separately measured. In scallops where gonads can be separated from the somatic tissue by dissection, Friocourt *et al.* (1985) found gonadal material enriched in PAHs over muscle but not digestive gland tissue. Rossi and Anderson (1977) observed spawning to be an important depuration route in a polychaete *Neanthes arenaceodentata.*

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

Correlations were found, indicating the importance of reproductive state and health on body burden. The amount of variation explained among individuals in their PAH body burdens was generally low; however, this probably emphasizes the previous point, that each of the measured variables are themselves relatively poor indicators of how recently and how frequently each animal had spawned. Stegeman and Teal (1973) emphasized the importance of the total exposure history of any individual organism in determining body burden. One aspect of this exposure history is the time since the last significant depuration event due to spawning.

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

Our data clearly support the importance of reproduction, at least in oysters, during the summer and fall. We suggest that the weak evidence for the importance of reproduction in most time series of contaminant body burden generally stems from three factors: collection of animals out of spawning season when little gonadal material is present, failure to analyze purified gametes which are the primary ve-

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

	Group A		Group B		Group C		Group D		Group E	
	Eggs	Tissue	Eggs	Tissue	Eggs	Tissue	Sperm	Tissue	Sperm	Tissue
Lindane	9.4	2.1	5.5	2.2	8.2	1.8	<1.0	2.2	<1.0	2.2
Total BHCs	14.7	5.0	9.5	5.2	14.0	3.9	<1.0	5.2	2.4	5.2
α-Chlordane	6.5	3.8	5.0	3.8	5.1	2.4	<1.0	4.5	3.6	4.5
Dieldrin	6.3	2.2	6.1	1.9	5.8	1.7	<1.0	1.8	1.7	1.8
4,4' DDE	32.1	9.1	26.0	8.2	26.7	7.5	4.1	11.9	6.6	11.9
4,4' DDD	12.3	3.7	11.7	3.2	12.5	3.1	<1.0	3.6	3.5	3.6
Total PCBs	132.6	36.5	147.8	33.5	113.0	29.6	114.2	53.8	102.3	53.8

hicle of depuration during spawning, and the poor understanding of the dynamics of uptake after spawning. We suggest that the timing of the last spawning event prior to sampling—animals recover their body burden within a month or less after a depuration event (Sericano *et al.*, in press)— and the degree of gonadal development (e.g. Hofmann *et al.*, 1992) are important variables affecting PAH body burden in oysters.

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

Variation between compounds

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

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

Reproduction and the latitudinal gradient in body burden

The data suggest one explanation for the latitudinal gradient in PAH and pesticide body burden observed in the Gulf of Mexico (Wilson *et al.*, 1990) and the relationship of PAH body burden and climate change (Wilson *et al.*, 1992). Slight variations in temperature, as affected by climate change, or varying average temperature across latitudes will vary the reproductive season, the annual reproductive effort, and the frequency of spawning in oysters (Hofmann *et al.*, 1992). Small changes in temperature produce large changes in reproductive effort. As a result, body burdens will vary even under similar exposure levels, and this variability may be considerable if a substantial fraction of the body burden is lost in spawning.

Wilson *et al.* (1990) found the latitudinal gradient in PAH body burden to be stronger than the latitudinal gradient in pesticide body burden. We found gonadal material concentrated much more highly in PAHs than pesticides and some pesticides are not concentrated in male gonadal material at all. Our data would suggest that temperature, and therefore latitude, should have a much greater impact on PAHs through reproduction than on pesticides, in agreement with the findings of Wilson *et al.* (1990). Taken together, our data and those of Wilson *et al.* (1990, 1992) suggest that interpretation of the results of monitoring studies such as the Status and Trends program using bivalves requires that close attention be paid to the reproductive state and health of the sampled populations.

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Reprint 2

Sediment Contaminants in Casco Bay, Maine: Inventories, Sources, and Potential for Biological Impact

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Sediment Contaminants in Casco Bay, Maine: Inventories, Sources, and Potential for Biological Impact

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An inventory-based approach to environmental assessment that determines concentrations of sedimentary contaminants, defines their origins, and assesses the potential for biological impact is illustrated in Casco Bay, ME. The most widespread contaminants in Casco Bay are petroleum and petroleum byproducts. The highest concentrations of contaminants are associated with population centers, effluent outfalls, and spills. The majority of PAH in sediments are the product of high-temperature combustion processes. PAH concentrations at sites in close proximity to Portland exceed values believed to produce toxic responses in marine benthic organisms. In contrast, PCB, DDTs, and chlordane concentrations in the sediments are below concentrations thought to produce toxic effects in marine organisms. Metal concentrations in sediments are also below those that elicit biological responses. The geographic distribution of contaminants is initially controlled by the proximity to sources, and the regional differences in concentrations are the result of sediment accumulation patterns. Detrital (terrestrial), autochthonous marine, pyrogenic, and petroleum sources for PAH, alkanes, and trace metals are defined.

Introduction

The systematic inventory of contaminants within coastal environments is often a first step in developing a logical and effective approach to preserving, protecting, and/or reclaiming resources impacted by human activities. While bulk inventories of chemicals alone cannot predict biological impacts or "ecosystem health", this first-order evaluation of the presence and magnitude of contamination can indicate which processes are most influential in controlling ecosystem exposure. Cause and effect must be linked by careful consideration of contaminant input, transport, ultimate fate, and biological impact. High-quality analyses, intensive sampling, and an evaluation of a broad spectrum of contaminants can contribute to defining those processes or activities most closely linked to detrimental or unwanted impacts. Innate in this type of approach is the generation of large, complex multicomponent data sets that must be fully integrated and rigorously evaluated. An approach utilizing comprehensive chemical inventories and a detailed statistical analysis of the data is reported for a study of Casco Bay, ME, sediments. Surficial sediments were evaluated as a long-term accumulator of contaminants, which are probably the main avenue of chronic exposure of the associated ecosystem.

Site Description

Casco Bay is situated along the Atlantic Coast of Maine and is bounded by Cape Small to the northeast and Cape Elizabeth to the southwest (Figure 1). The bay has a wealth of natural resources and marine habitats that support a rich and diverse ecosystem. The bay proper is a 400-km^2 embayment of the Gulf of Maine which includes Portland Harbor, a major docking facility and the principal fishing port of Maine. More than 300 mi of coastline and nearly 400 islands are encompassed by the bay (*1*).

Methods

Sediment samples were analyzed for trace metals, aliphatic and polycyclic aromatic hydrocarbons, pesticides and PCBs (Table 1). Matrix spikes, laboratory sample duplicates, and laboratory blanks were processed with each batch of samples (10–20 samples/batch). Duplicates were produced by subsampling in the laboratory. Standard reference materials (National Institute of Standards and Technology) were analyzed to audit the performance of the analytical methods. The quality assurance standards are those of the NOAA's National Status and Trend Program, of the EPA's Environmental Monitoring and Assessment Program-Near Coastal (EMAP-NC) and of the U.S. Fish and Wildlife Service (FWS) for trace contaminant analyses (*2*). These methods have undergone extensive intercalibration with EPA, NOAA, NIST and FWS. Detailed methods are provided elsewhere (*3*).

Sample Collection. Sediment samples were collected in August 1991 (Figure 1). Station locations were chosen to provide good areal coverage, sediments of different ages (including erosional features), and representative coverage of benthic communities. Bathymetry and sediment texture also guided site selection. The sampling sites are designated as CS, EB, IB, OB, SW, and WB (i.e., Cape Small, East Bay, Inner Bay, Outer Bay, Shallow Water, and West Bay, respectively). A number identifies the location within the bay. Samples were taken with either a Smith–McIntyre grab sampler, a ponar grab sampler, or by hand. All samples were carefully inspected to ensure that undisturbed sediments were collected.

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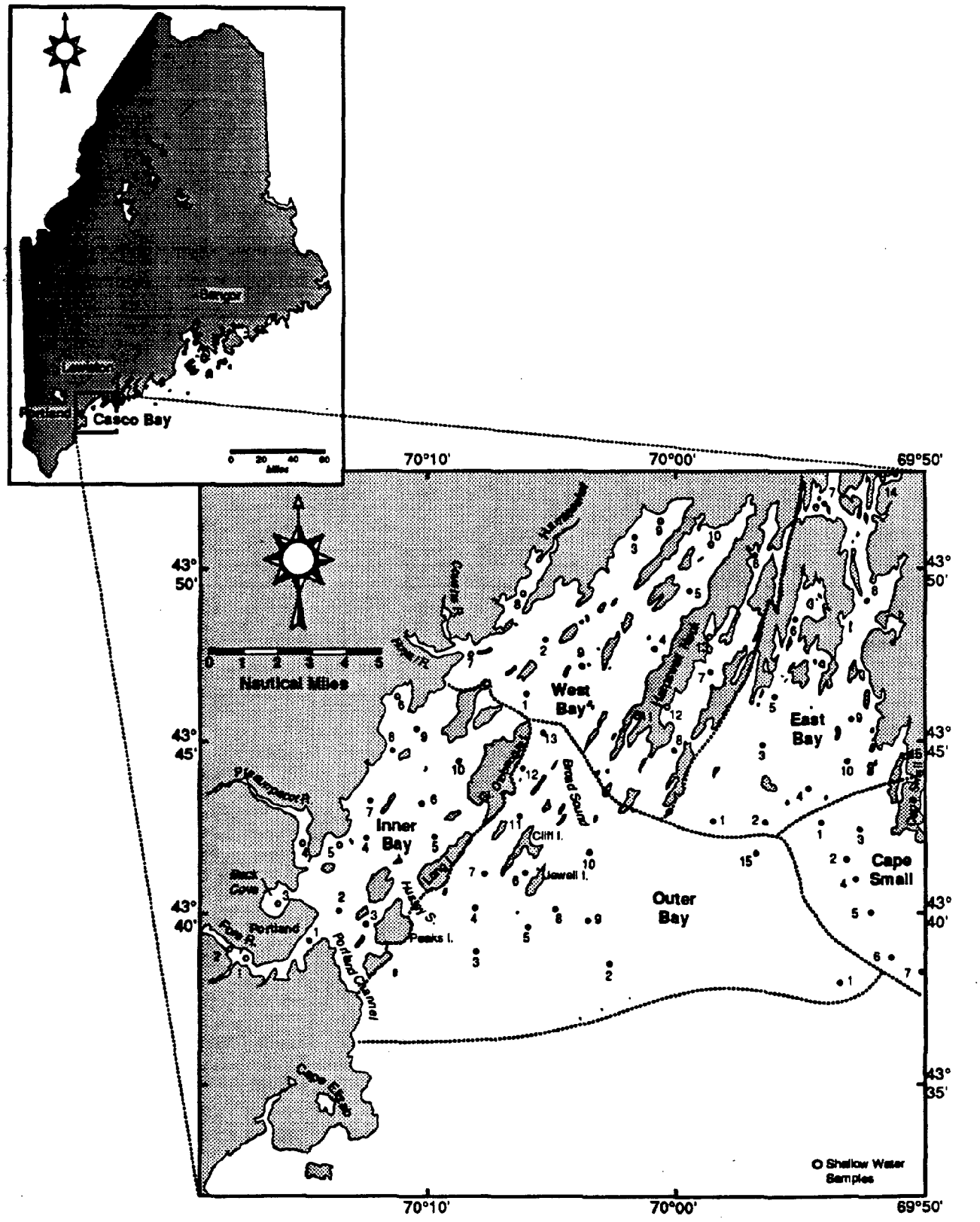

Figure 1. Location map for the Casco Bay study.

Hydrocarbons, Pesticides, and PCBs. The extraction method is that of Wade *et al.* (*2*). A total of 10 g of freeze-dried sediment was Soxhlet-extracted with methylene chloride and concentrated in Kuderna-Danish tubes. The extracts were fractionated by alumina:silica gel (80–100 mesh) chromatography. The extracts were sequentially eluted from the column with 50 mL of pentane (aliphatic fraction) and 200 mL of 1:1 pentane–dichloromethane (aromatic/PCB/pesticide fraction) and concentrated for instrumental analysis.

Table 1. Analytes Measured in Casco Bay Estuary Program[a]

Total Metals

cadmium	chromium	mercury
copper	silver	arsenic
lead	zinc	selenium
nickel	iron	

Hydrocarbons

naphthalene	phenanthrene	benzo[*k*]fluoranthene
2-methylnaphthalene	anthracene	benzo[*a*]pyrene
1-methylnaphthalene	2-methylphenanthrene	benzo[*e*]pyrene
biphenyl	fluoranthene	perylene
2,6-dimethylnaphthalene	pyrene	indeno[1,2,3-*cd*]pyrene
acenaphthylene	benz[*a*]anthracene	dibenz[*a,h*]anthracene
acenaphthene	chrysene	benzo[*g,h,i*]perylene
fluorene	benzo[*b*]fluoranthene	

In Addition

extended PAHs (alkylated homologues useful in differentiating oil from combustion sources)
aliphatic fraction quantitation including C_{12}–C_{34} *n*-alkanes, pristane, phytane, and the unresolved complex mixture

PCBs

congener-specific analysis of 20 individual PCBs including quantitative estimates of the amount of arochlor mixtures

Pesticides

aldrin	endosulfan I	hexachlorobenzene
α-BHC	endosulfan II	2,4′-DDE
β-BHC	endosulfan sulfate	2,4′-DDD
δ-BHC	endrin	2,4′-DDT
γ-BHC	endrin aldehyde	4′,4-DDD
α-chlordane	heptachlor	4′,4-DDE
γ-chlordane	heptachlor epoxide	4′,4-DDT
dieldrin	toxaphene	

Ancillary Parameters

(1) TOC was determined by combustion in a Leco carbon analyzer to CO_2 and subsequent quantitation by IR
(2) grain size (sand, silt, and clay) was determined by the Folk settling method
(3) organic nitrogen was determined by a Kjeldahl digestion
(4) % solids (dry weight) are determined and reported for all samples

[a] Note: Organic analyte concentrations are reported on the basis of dry weight of sediment and are corrected for surrogate recoveries.

Aliphatic hydrocarbons (*n*-C_{13}–*n*-C_{34}), pristane, and phytane were analyzed by gas chromatography (HP-5980) in the splitless mode with flame ionization detection (FID). A 30 m × 0.32 mm i.d. fused-silica column with DB-5 bonded phase (J&W Scientific, Inc.) provided component separations. The FID was calibrated at five concentrations, and deuterated *n*-alkanes were used as surrogates and internal standards. Aromatic hydrocarbons were quantified by gas chromatography with mass spectrometric detection (HP-5890-GC and HP-5970-MSD). The samples were injected in the splitless mode onto a 30 m x 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, and the molecular ions for each of the PAH analytes were used for quantification. The GC/MS was calibrated by the injection of standards at five concentrations. Analyte identifications were based on the retention time of the quantitation ion for each analyte and a series of confirmation ions. Deurated aromatic compounds were used for surrogates and internal standards.

Pesticides and PCBs were separated by gas chromatography in the splitless mode using an electron capture detector (ECD). A 30 m × 0.32 mm i.d. fused-silica column with DB-5 bonded phase (J&W Scientific, Inc.) provided component separations. Four calibration solutions were used to generate a nonlinear calibration curve. A sample containing only PCBs was used to confirm the identification of each PCB congener. The surrogates DBOFB (dibromooctafluorobiphenyl), PCB-103 and PCB-198 for pesticide and PCB analysis were added during the extraction. The internal standard, TCMX (tetrachloro-*m*-xylene), was added prior to GC/ECD analysis. The chromatographic conditions for the pesticide–PCB analysis were 100 °C for 1 min, then 5 °C/min until 140 °C, hold for 1 min, then 1.5 °C/min to 250 °C, hold for 1 min, and then 10 °C/min to a final temperature of 300 °C, which was held for 5 min.

Trace Metals. The major analytical technique used for trace metal determination was atomic absorption spectrophotometry (AAS) in the flame mode for those elements in high enough concentration. Graphite furnace (GC/AAS) or cold vapor techniques were used when necessary. Samples were pressure-digested in 50-mL closed all-Teflon "bombs" (Savillex Co.; Brooks *et al.*, 1988). Sediment aliquots (ca. 200 mg) were digested at 130 °C in a mixture of nitric, perchloric, and hydrofluoric acids. A saturated boric acid solution was then added to

complete the dissolution. Various dilutions were made on the clear digest solutions to bring them within the calibration of the AAS. Standard reference materials and blanks were digested and analyzed with every batch of samples.

Concentrations of Fe, Mn, and Zn were determined by flame AAS using a Perkin-Elmer Model 306 instrument, following the manufacturer's recommendations with only slight modifications. Calibration curves were constructed from commercial standards. Concentrations of Ag, As, Cd, Cr, Cu, Ni, Pb, and Se were determined with a Perkin-Elmer Zeeman 3030 instrument equipped with an HGA-600 furnace and AS-60 autosampler. Matrix modifiers and analytical conditions for the furnace and spectrophotometer were based on the manufacturer's recommendations, with modifications as appropriate to maximize sensitivity and minimize interferences. Mercury was determined by cold vapor AAS following a slightly modified EPA Method 245.5 aqua-regia/permanganate digestion. A headspace sampling procedure was used to remove Hg from the digest in contrast to the more common stripping procedure. A UV monitor (Laboratory Data Control Co.) with a 30-cm path length cell was used for Hg detection and quantification.

Organic Carbon and Grain Size. Organic carbon (OC) was determined by detection of CO_2 by an infrared spectrometer after combustion in an O_2 stream (LECO WR-12 total carbon system). Samples were acidified using dilute HCl in methanol and then dried. Method blanks and duplicate samples were analyzed every 20 samples. Data are reported as micrograms of carbon per gram of dry weight. All glassware and utensils are preheated prior to use.

Sediment grain size was determined by the procedure of Folk (*4*), utilizing sieving to separate gravel and sand fractions from the clay and silt fractions. The latter fractions were subsequently separated by the pipet (settling rate) method. Detailed descriptions of the methods utilized in measuring OC and grain size are reported in Brooks *et al.* (*5*).

Principal Components Analysis (PCA). The organic and inorganic data were analyzed using PCA (*6*). The results of PCA are highly dependent on the pretreatment or scaling of the data matrix. The data for this study consist of a wide variety of analytes that range several orders of magnitude in their absolute values. Because PCA is a least-squares method, variables with large variance will have large loadings. To avoid this bias, the entire data matrix was first scaled by dividing each variable by the standard deviation. This scaling assigns every variable a variance of 1.0 so that each variable has the same influence in the PCA model. The technique of cross-validation was used to establish the significance of each principal component (*7*). PCA was performed on a personal computer using the program SIRIUS (Pattern Recognition Systems A/S, Bergen, Norway).

Results

Hydrocarbons. Aliphatic hydrocarbons were detected at all stations sampled. The majority of resolved alkanes had odd-carbon chain lengths with 23–33 carbons indicative of plant biowaxes (Figure 2; refs 8–10). N-C_{15}, *n*-C_{17}, *n*-C_{19}, *n*-C_{21}, and pristane were often more abundant than the co-occurring even carbon-numbered normal alkanes and phytane, suggesting a phytoplankton input (*8-10*). Total alkanes and unresolved complex mixture (UCM) concentrations varied from 151 to 10 078 ppb dry wt and from 2 to 335 ppm dry wt, respectively. PAHs were also detected at all locations sampled. The predominant PAHs are highly condensed ring structures with few alkylations indicating a pyrogenic or combustion source (Figure 3; refs 11–14). Four-ring and larger PAHs account for more than 60% of sedimentary PAHs in Casco Bay. Total PAH concentrations varied from 16 to 20 798 ppb dry wt.

The western part of Casco Bay (Inner Bay) is most highly contaminated with PAH. Sediments from the Fore River area and locations close to Portland contain the highest concentrations of PAH. In general, contaminants decrease in concentration with distance from populated areas. However, regionally elevated PAH concentrations are also present at a few sites in East Bay and Cape Small. One station in the Cape Small (CS-4) region was unusual compared to other sites in the region. Most Cape Small stations contained <1.0% organic carbon and more than 65% sand, whereas sediment from station CS-4 contained 2.7% organic carbon and only 29.9% sand. Total alkanes, UCM, and total PAH concentrations were elevated at this location as well. Sediments at station EB-9 also had high concentrations of total PAH. An organic carbon content of 4.6% at EB-9 is the highest for all of the sediments sampled.

PCBs and Pesticides. Total PCB concentrations for the study area range from 0.4 to 485 ppb dry wt with a median concentration of 15 ppb. Total PCBs are highest in the Inner Bay in close proximity to Portland. Concentrations are lowest in Cape Small and West Bay with a few anomalous values in East Bay. The site from Cape Small with a total PCB concentration of 40 ppb dry wt has a higher TOC content (2.8%) than other samples from Cape Small.

Total DDT concentrations for the study area range from below the method detection limit (0.25 ppb) to 21 ppb dry weight. The DDTs were dominated by the *p,p'*-isomers. This is expected since technical-grade DDT is primarily the *p,p'*- isomer (75–85%). In the environment, DDT is metabolized to DDD and DDE. In some samples, DDD is the major metabolite while in other samples DDE predominates. Samples from the Inner Bay and associated shallow water sites exhibit DDD > DDE while at most other locations DDE > DDD. There is a relatively high percentage of undegraded DDT in Casco Bay sediments. The geographic distribution of total DDT concentrations is similar to that found for PCBs. The Inner Bay has the highest concentration in Casco Bay. East Bay and Outer Bay have intermediate concentrations, West Bay has lower concentrations, and the Cape Small region has the lowest concentrations.

The highest values of total chlordane are at Inner Bay sites. East Bay and Outer Bay sites are intermediate, while West Bay and Cape Small sites exhibit the lowest concentrations. Total chlordane concentrations range from below the method detection limit (0.25 ppb) to 4.9 ppb dry wt. Other organochlorine pesticides including aldrin, BHC, dieldrin, endosulfan (I, II, and sulfate), endrin, endrin aldehyde, heptachlor, heptachlor epoxide, toxaphene, and hexachlorobenzene were near or below the method detection limit (<0.25 ppb).

Trace Metals. Sediment trace metal data show considerable geographic variation with generally higher values

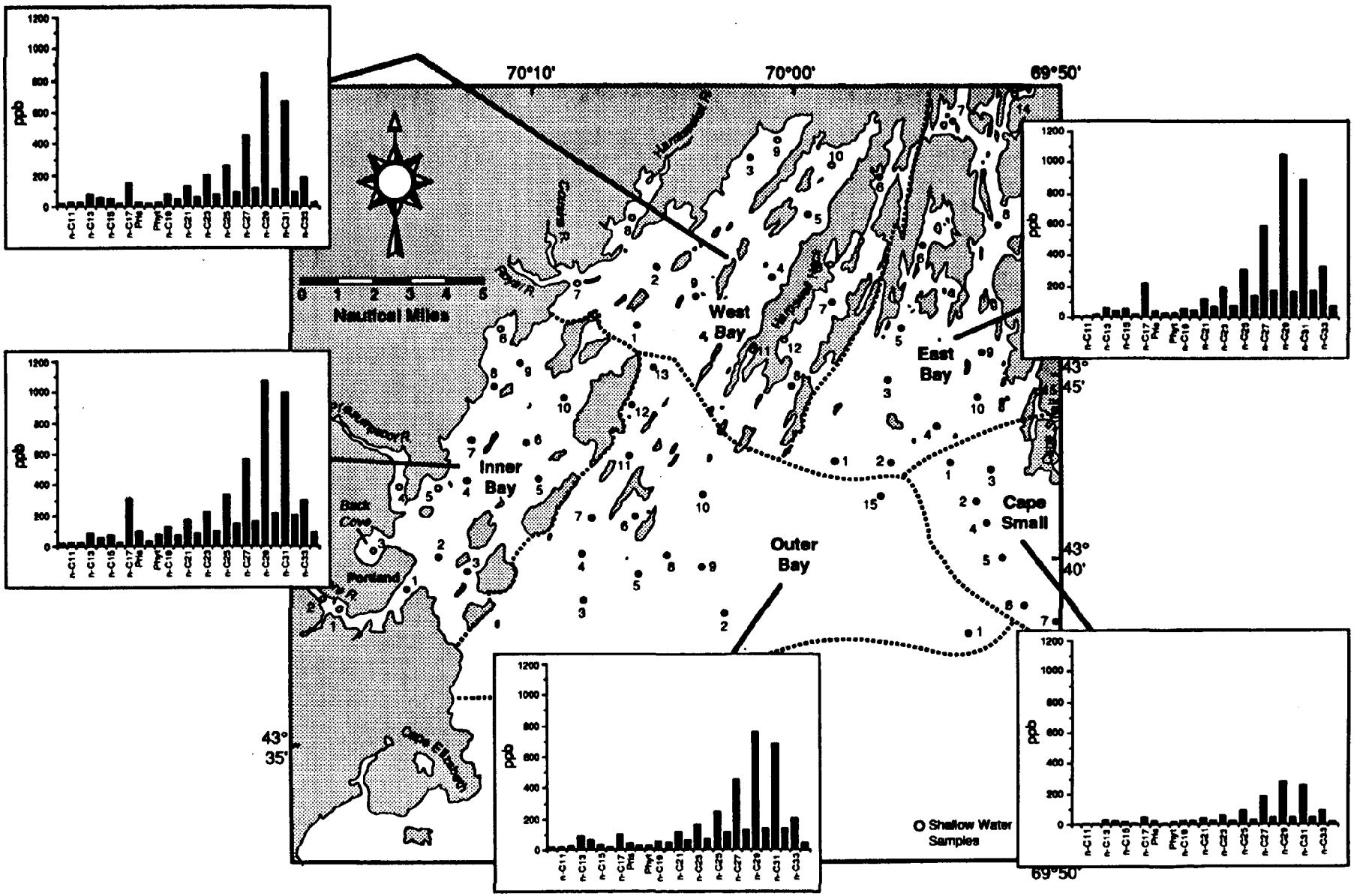

Figure 2. Average normal alkane and isoprenoid concentrations (ppb dry weight, surrogate corrected) and distributions in sediments from Casco Bay.

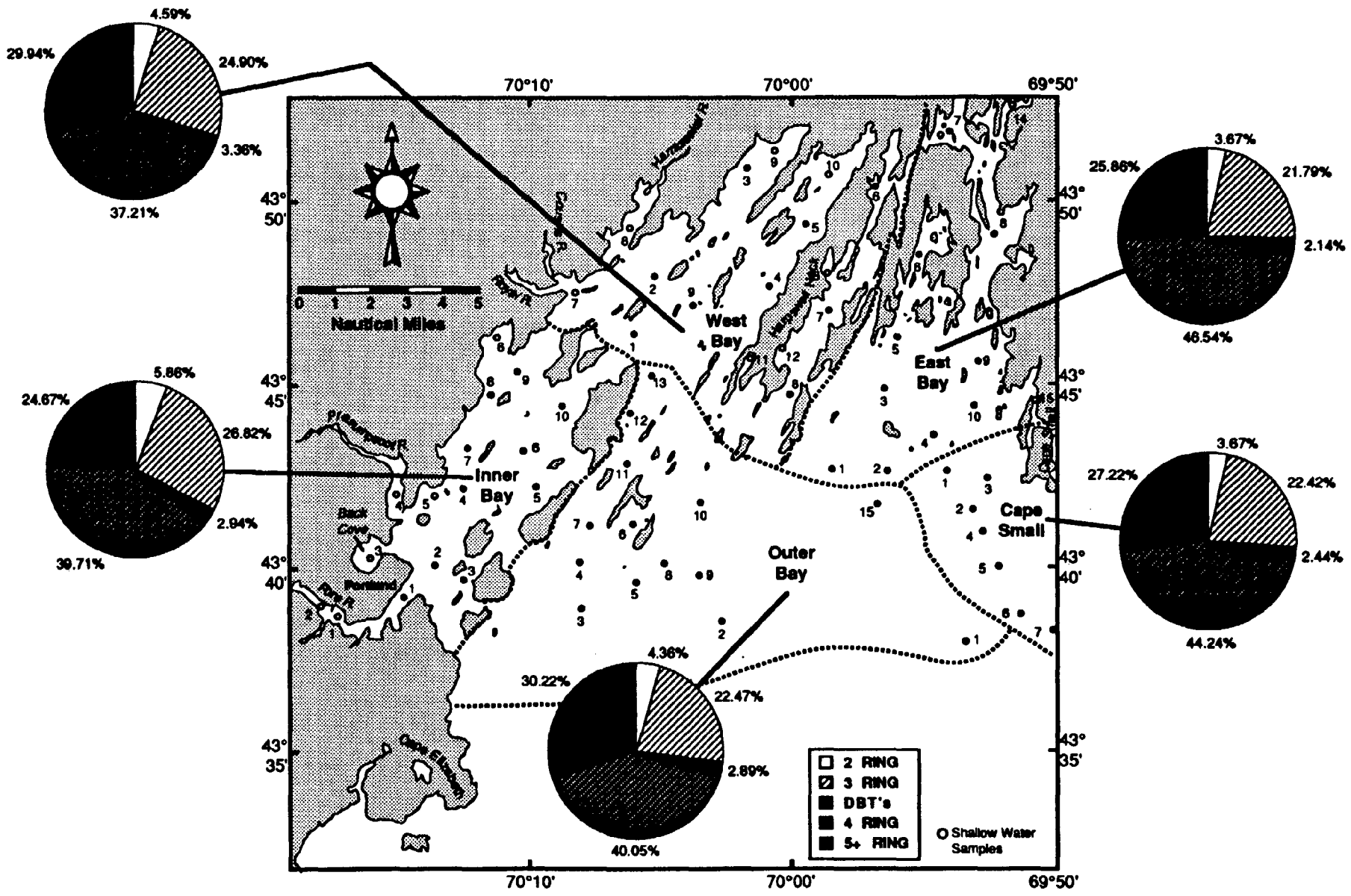

Figure 3. Average PAH compositions in sediments by region within Casco Bay.

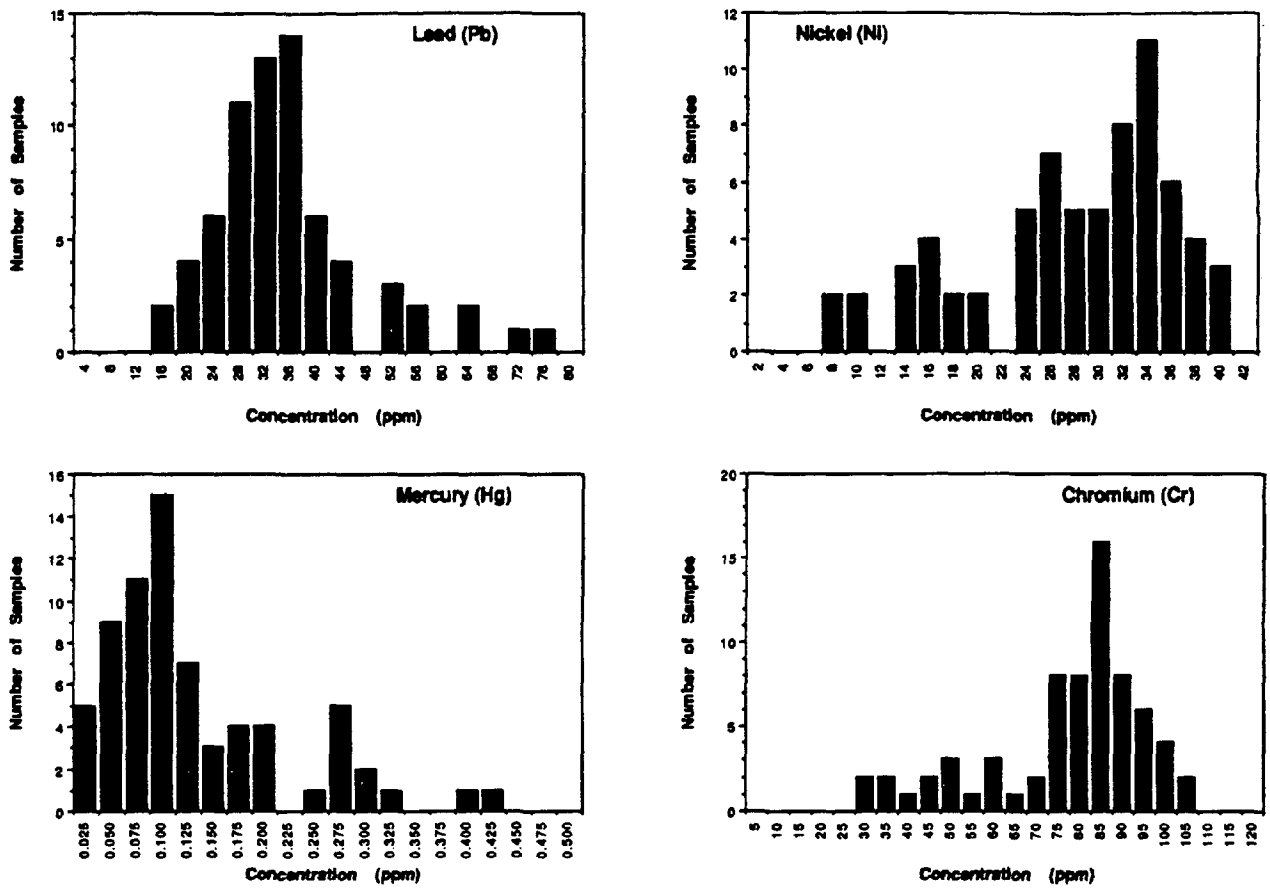

Figure 4. Frequency plots of lead, nickel, mercury and chromium concentrations (ppm dry weight) in sediments from Casco Bay.

in the Inner Bay. Sediment variability in trace metal content is largely due to variations in mineralogy and grain size. The data for Ag, Cd, Pb, and Hg show positive skewness on frequency plots (Figure 4), whereas the other metals are more normally distributed or, as in the case of Cr and Ni, exhibit negative skewness. Positive skewness suggests additions of metals by human activities to a normally distributed background. Negative skewness is most likely caused by the uneven geographic distribution of quartz or other low-metal minerals.

Human activities also contribute trace metals to the environment. Cross-plots of Fe or Al versus trace metals often identify anomalous concentrations that lie off the best-fit line for background data (Figure 5). When Casco Bay Cr and Ni data are plotted vs Fe, the data fall along a best-fit line with an approximately 0 intercept, suggesting a natural distribution. On the other hand, cross-plots of Zn, Pb, and Fe indicate that some samples are enriched in metals (Figure 5). The enriched samples are from the Inner Bay near Portland.

Based on the definition of O'Connor (*15*) "high" values are as follows: Cd, 1.3 ppm; Cr, 230 ppm; Cu, 87 ppm; Pb, 87 ppm; Hg, 0.51 ppm; Ag, 1.2 ppm; and Zn, 280 ppm for sediments which are 100% silt and clay. For a sediment consisting partly of quartz sand, these values would be reduced proportionally (e.g., multiplied by 0.75 for a sediment with 25% sand). Few of the metal concentrations detected in Casco Bay would be classified as high on this basis. Whereas some of the Cd, Pb, Ag, Zn, and Hg values found in Casco Bay sediments suggest an influence from human activities, they are not high in comparison to samples collected away from obvious point sources of pollutant inputs (*15*).

Discussion

Regional Distribution. One or more anthropogenic contaminants (trace metals, PCBs, DDTs, chlordane, or PAH) were detected at all locations sampled in Casco Bay. The geographic distribution of contaminants is initially controlled by the regional occurence of sources. The most elevated contaminants are derived from the utilization of fossil fuels. The predominant sources of PAH are combustion processes associated with urbanized and industrialized locations. The Inner Bay region directly offshore of Portland contains the highest levels of trace metals, PCBs, DDTs, chlordanes, and hydrocarbons. In general, for contaminants other than PAH, the levels of contamination would not be considered high on a national basis (*15*). PAH concentrations are high in the Inner Bay and are comparable to other contaminated estuaries (*15, 16*).

To compare the distribution of all of the contaminants measured, each site was ranked from 1 to 65 based on the abundance of each suite of contaminants. If a contaminant was below the MDL, it was given a ranking of 1. If multiple stations had the same concentrations, they were given the same relative ranking. Organic and inorganic contaminants were assessed separately, the cumulative rankings for each site were summed, and the sites were sorted from low to high values (Tables 2 and 3). This approach provides an indication of where contaminants are geographically located. Based on these variations, the highest 25% of organic contaminants are located at 10 Inner Bay, two

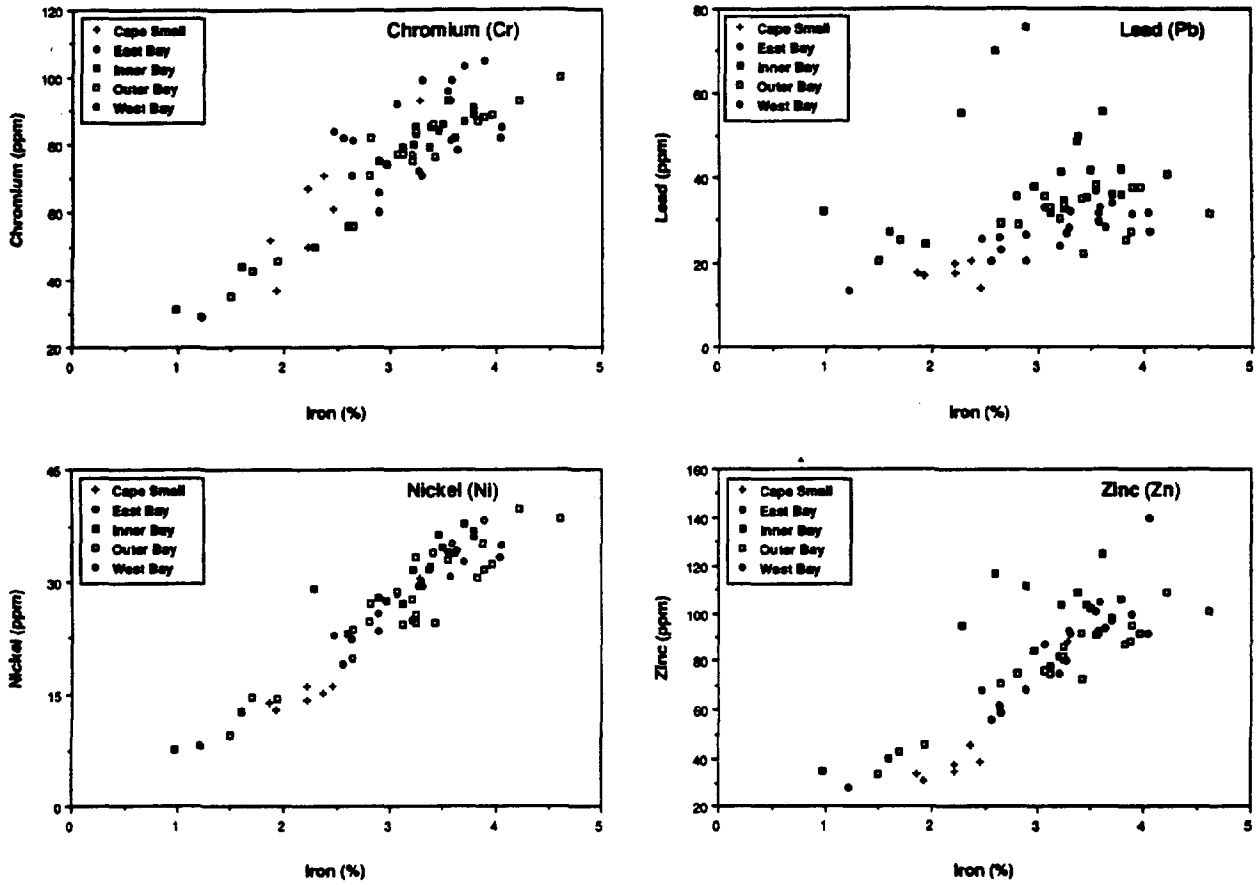

Figure 5. Relationship between chromium, lead, nickel, and zinc concentrations (ppm dry weight) and iron content (% dry weight) in sediments from Casco Bay.

Outer Bay, three East Bay, and one Cape Small sites. Eight of the 10 most highly contaminated stations are located in the Inner Bay region, including the six highest stations. The lowest levels of organic contaminants are in the Cape Small and West Bay regions. High levels of a variety of organic contaminants tend to occur at the same location.

For inorganic contamination, only those metals believed to be influenced by anthropogenic inputs were used to rank the sample locations, i.e., Ag, Cd, Pb, Zn, and Hg. Based on the summation of inorganic contaminant rankings, 25% of the locations with the highest levels were as follows: 12 Inner Bay, three East Bay, and one Outer Bay locations. Nine of the 10 highest locations are in the Inner Bay region, including the eight highest stations. Lowest metal concentrations occur in the Cape Small region. Eleven stations are ranked in the highest 25% on both the inorganic and organic contaminant rankings (Figure 6). They are almost exclusively Inner Bay locations, i.e., 9 of 11.

Principal Components Analysis. A total of four significant principal components (PC) were extracted from the Casco Bay data. PC 1 accounts for 48.9% of the total variance. The loadings for this PC show the sand content of the sediments inversely correlated with all other measured variables. PC 1 is inversely correlated with sand content and positively correlated with the TOC content of the sediments (Figure 7). This principal component reflects differences in the concentration of the targeted analytes due to variations in sediment texture. This finding is more significant than might appear at first consideration, as it implies that regional differences in concentrations result in part from sediment accumulation patterns. Thus, areas of fine-grained sediment accumulation such as the Inner Bay have high scores for PC 1 and exhibit high concentrations, while sediments in areas that are characterized by a dynamic physical environment and little sediment accumulation such as the Outer Bay have low scores for PC 1 and exhibit lower concentrations. It is also notable that both the organic and inorganic contaminants exhibit the same general trend. Shallow water samples SW-1 and SW-2 were identified as outliers because their compositions were anomalous relative to the other sediments (extreme enrichment in PAH and PCB, respectively). These samples were excluded from the PCA analysis.

PC 2 (12.3% of the total variance) and PC 3 (6.1% of the total variance) are related to the *composition* of organic and inorganic contaminants in the sediments. Since principal components are orthogonal, the processes governing PC 2 and PC 3 are independent of PC 1. Hence, the information contained in these principal components is more representative of contaminant sources in the sediments and is not related to absolute concentrations.

PC 2 is correlated positively with the Fe and saturated hydrocarbon content of the sediments (Figure 8). This most likely reflects a detrital component enriched in plant wax *n*-alkanes and inorganic clastics derived from continental erosion (*8-10*). A loadings cross-plot for PC 2 versus PC 3 (Figure 9) shows that, although all *n*-alkanes are positively loaded in PC 2, C_{23}, C_{25}, C_{27} and C_{29} *n*-alkanes have the highest loadings, consistent with this interpretation. Figure 9 also shows that nearly all the aromatic

Table 2. Casco Bay Estuary Program Site Rankings Based on Organic Contaminant Data, 1991 (ppb dry wt surrogate corrected)

station no.	total PAHs (ppb)	total PAH ranking	total chlordane (ppb)	total chlordane ranking	total DDTs (ppb)	total DDT ranking	total PCBs (ppb)	total PCB ranking	total organic ranking
CS-1	93	2	0.01	1	0.01	1	0.6	2	6
CS-7	16	1	0.02	3	0.02	2	0.4	1	7
CS-3	515	6	0.02	2	0.10	4	2.0	5	17
WB-3	421	4	0.07	4	0.18	5	2.6	6	19
SW-8	445	5	0.16	12	0.47	8	1.6	3	28
SW-10	595	8	0.11	6	0.30	6	4.5	9	29
CS-2	362	3	0.24	19	0.04	3	1.7	4	29
CS-6	672	9	0.15	10	0.50	9	3.8	7	35
SW-12	1094	16	0.23	16	0.72	10	5.5	11	53
WB-6	774	11	0.23	15	0.94	14	6.0	13	53
SW-9	734	10	0.23	17	0.73	11	8.1	17	55
WB-2	146	22	0.16	11	1.01	15	7.2	14	62
SW-5	911	13	0.15	9	1.63	26	7.3	16	64
WB-8	1112	18	0.11	5	1.52	25	8.4	18	66
SW-7	807	12	0.25	20	1.70	29	5.2	10	71
SW-13	961	14	0.19	14	1.23	21	9.8	23	72
CS-5	546	7	1.32	53	0.33	7	3.9	8	75
WB-7	1329	20	0.12	7	1.36	23	10.2	25	75
OB-1	1433	21	0.41	27	1.09	18	7.2	15	81
OB-11	1312	19	0.24	18	1.11	19	11.6	28	84
OB-7	1650	30	0.45	30	1.03	16	5.5	12	88
SW-14	1059	15	0.25	21	1.94	34	9.1	21	91
EB-1	2230	37	0.60	35	0.86	13	9.0	20	105
EB-2	2875	45	0.57	33	0.82	12	8.9	19	109
WB-4	1495	24	0.56	31	1.83	31	11.5	27	113
SW-6	1526	26	0.30	23	2.29	41	10.0	24	114
WB-5	1102	17	0.57	32	1.91	33	14.1	34	116
EB-4	2791	44	0.16	13	1.37	24	14.3	35	116
OB-4	1964	36	0.64	37	1.26	22	9.6	22	117
OB-6	1631	28	0.13	8	2.33	43	18.8	42	121
OB-13	1568	29	0.85	42	1.69	28	11.5	26	125
OB-8	1865	33	0.39	25	1.72	30	17.4	39	127
EB-10	4545	55	0.43	28	1.12	20	13.5	31	134
WB-9	1901	34	0.33	24	2.28	40	16.3	38	136
WB-1	1490	23	0.91	43	2.42	45	11.8	29	140
OB-12	1696	31	0.74	39	2.00	35	14.4	36	141
SW-11	1501	25	0.98	46	3.10	49	13.9	32	152
OB-5	2964	48	0.60	34	1.65	27	18.9	43	152
IB-9	1945	35	0.78	41	3.56	50	13.4	30	156
EB-3	2939	46	1.06	47	2.26	39	14.0	33	165
IB-5	2545	40	0.96	45	2.40	44	15.8	37	166
OB-9	2706	41	0.77	40	2.08	36	22.2	49	166
EB-8	3459	52	0.26	22	2.81	48	19.6	46	168
EB-5	2944	47	0.40	26	2.55	47	23.7	50	170
SW-15	7180	59	1.60	55	1.07	17	17.9	40	171
SW-4	1530	27	1.12	48	3.93	54	19.1	44	173
OB-2	1817	32	1.89	59	2.31	42	18.1	41	174
OB-10	2269	39	1.25	51	2.09	37	20.0	48	175
IB-6	3068	49	0.62	36	2.53	46	27.9	53	184
OB-15	4004	54	1.13	49	2.17	38	19.4	45	186
IB-8	2723	42	0.93	44	4.44	57	19.9	47	190
CS-4	7454	61	0.71	38	1.89	32	40.0	59	190
OB-3	3727	53	0.43	29	4.12	55	30.7	54	191
IB-10	2737	43	1.13	50	3.69	51	27.9	52	196
EB-6	2233	38	1.72	57	3.86	52	35.7	57	204
EB-7	4872	56	1.30	52	3.86	53	23.9	51	212
IB-4	3273	51	1.39	54	7.63	59	31.8	55	219
IB-7	3109	50	1.84	58	4.70	58	33.7	56	222
EB-9	7340	60	1.91	60	4.16	56	37.3	58	234
IB-2	6392	58	1.63	56	9.91	61	47.6	61	236
IB-3	5059	57	2.49	61	9.02	60	42.2	60	238
SW-1	20748	65	3.47	63	10.10	62	72.3	62	252
IB-1	9174	63	2.89	62	14.50	63	79.2	64	252
SW-3	7517	62	4.91	65	20.42	65	77.1	63	255
SW-2	12588	64	3.98	64	16.81	64	485.0	65	257

hydrocarbons measured are loaded negatively in PC 2. One exception is the alkylated chrysenes, which show a slight positive loading in PC 2. Thus, PC 2 can also be regarded as a saturate/aromatic hydrocarbon ratio. These results indicate that saturated and aromatic hydrocarbons in Casco Bay have different origins, which is generally consistent with the known geochemistries of these classes of compounds.

PC 3 differentiates individual saturated and aromatic hydrocarbons based on molecular weight (Figure 9). Most

Table 3. Casco Bay Estuary Program Site Rankings Based on Selected Metal Data, 1991 (ppm dry wt)

station no.	Ag (μg/g)	Ag ranking	Cd (μg/g)	Cd ranking	Hg (μg/g)	Hg ranking	Pb (μg/g)	Pb ranking	Zn (μg/g)	Zn ranking	total ranking
CS-7	0.05	1	0.069	5	<0.006	1	17.1	3	31	2	12
CS-3	0.06	1	0.053	3	0.008	1	17.6	4	35	4	13
CS-2	0.07	1	0.060	4	0.019	2	17.8	5	34	3	15
CS-1	0.05	1	0.071	6	<0.006	1	14.1	2	39	6	16
CS-5	0.09	3	0.036	1	0.031	3	20.0	6	38	5	18
CS-6	0.07	1	0.051	2	0.046	6	20.8	9	46	9	27
SW-8	0.09	3	0.150	14	0.019	2	20.5	7	34	3	29
SW-15	0.08	2	0.192	21	0.048	7	13.6	1	28	1	32
SW-7	0.07	1	0.155	15	0.032	4	24.7	13	46	9	42
EB-4	0.10	4	0.076	7	0.058	10	23.3	11	59	11	43
EB-10	0.08	2	0.121	10	0.069	15	20.6	8	56	10	45
OB-11	0.10	4	0.168	17	0.049	8	25.5	14	43	8	51
EB-1	0.11	5	0.127	12	0.059	11	26.2	16	62	12	56
WB-3	0.11	5	0.258	28	0.031	3	20.5	7	69	14	57
EB-2	0.11	5	0.175	19	0.077	20	25.8	15	68	13	72
SW-5	0.12	6	0.245	27	0.062	13	27.5	20	40	7	73
OB-1	0.14	8	0.118	9	0.065	14	27.7	21	88	27	79
WB-6	0.11	5	0.088	8	0.057	9	31.7	30	92	29	81
WB-8	0.13	7	0.293	30	0.077	20	26.8	17	68	13	87
SW-10	0.16	10	0.486	48	0.037	5	22.2	10	73	16	89
WB-7	0.11	5	0.312	32	0.071	17	27.1	18	80	20	92
SW-9	0.17	11	0.400	38	0.037	5	25.5	14	87	25	93
OB-15	0.16	10	0.155	15	0.102	28	29.3	24	75	17	94
SW-12	0.25	16	0.355	35	0.048	7	29.4	25	71	15	98
SW-4	0.19	12	0.213	24	0.097	27	32.0	32	35	4	99
SW-14	0.16	10	0.414	40	0.082	22	24.3	12	75	17	101
SW-13	0.15	9	0.125	11	0.073	18	31.5	28	101	36	102
OB-10	0.14	8	0.156	16	0.081	21	33.8	38	82	22	105
OB-2	0.12	6	0.133	13	0.058	10	37.7	49	92	29	107
OB-13	0.15	9	0.268	29	0.082	22	30.6	27	82	22	109
OB-8	0.14	8	0.176	20	0.087	24	35.7	43	76	18	113
SW-6	0.13	7	0.435	45	0.061	12	31.7	30	78	19	113
OB-5	0.15	9	0.200	22	0.085	23	34.7	40	81	21	115
OB-4	0.17	11	0.226	25	0.104	29	33.1	36	75	17	118
WB-2	0.17	11	0.358	36	0.076	19	29.7	26	92	29	121
WB-1	0.15	9	0.430	42	0.087	24	28.4	22	93	30	127
OB-7	0.16	10	0.245	27	0.113	32	35.8	44	75	17	130
WB-4	0.17	11	0.444	46	0.082	22	28.6	23	94	31	133
WB-9	0.36	21	0.302	31	0.087	24	31.9	31	93	30	137
OB-9	0.17	11	0.174	18	0.113	32	38.3	51	91	28	140
CS-4	0.20	13	0.208	23	0.190	43	32.4	34	88	27	140
WB-5	0.15	9	0.529	52	0.069	16	27.4	19	140	45	141
SW-11	0.16	10	0.239	26	0.096	26	37.6	48	95	32	142
IB-5	0.20	13	0.325	33	0.094	25	38.1	50	84	23	144
EB-3	0.19	12	0.431	43	0.112	31	33.2	37	87	26	149
EB-9	0.19	12	0.401	39	0.148	36	32.1	33	92	29	149
OB-12	0.19	12	0.434	44	0.118	33	35.1	41	92	29	159
OB-6	0.26	17	0.592	58	0.106	30	32.8	35	86	24	164
EB-7	0.20	13	0.608	59	0.153	37	31.6	29	100	35	173
OB-3	0.20	13	0.327	34	0.141	35	40.7	52	109	41	175
IB-10	0.23	14	0.501	50	0.170	39	36.0	45	98	34	182
EB-8	0.23	14	0.720	60	0.181	42	34.1	39	97	33	188
IB-6	0.25	16	0.392	37	0.195	44	41.2	53	104	38	188
IB-8	0.24	15	0.573	56	0.168	38	35.3	42	104	38	189
EB-6	0.29	19	1.320	63	0.137	34	33.2	37	105	39	192
IB-9	0.23	14	0.557	53	0.173	40	36.2	46	106	40	193
EB-5	0.23	14	0.794	61	0.176	41	37.0	47	101	36	199
IB-7	0.32	20	0.424	41	0.234	45	42.1	55	106	40	201
SW-1	0.46	23	0.488	49	0.264	46	55.5	58	95	32	208
IB-4	0.27	18	0.571	55	0.274	49	41.5	54	102	37	213
IB-2	0.46	23	0.524	51	0.271	48	49.9	57	109	41	220
IB-3	0.39	22	0.574	57	0.264	46	48.5	56	109	41	222
SW-2	0.57	24	0.478	47	0.392	50	70.3	60	117	43	224
IB-1	0.57	24	0.564	54	0.269	47	55.6	59	125	44	228
SW-3	0.78	25	0.908	62	0.424	51	75.6	61	112	42	241

n-alkanes in the range C_{10}–C_{22} are positively loaded in PC 3, as are the more highly alkylated (C_2 and higher) two- and three-ring aromatics: naphthalenes, fluorenes, phenanthrenes, and dibenzothiophenes. Pristane, phytane, and UCM hydrocarbons are also loaded positively in PC 3. In contrast, *n*-alkanes in the range C_{23}–C_{34} along with C_{15} and C_{17} are loaded negatively in PC 3. Aromatic hydrocarbons loaded negatively in PC 3 include most parent two- and three-ring compounds, their methyl-substituted homologs, and most four- and five-ring aromatic compounds.

Together, the loadings for PC 2 and PC 3 discriminate sources of organic and inorganic materials in the Casco Bay sediments. Hydrocarbons loaded positively in PC 2

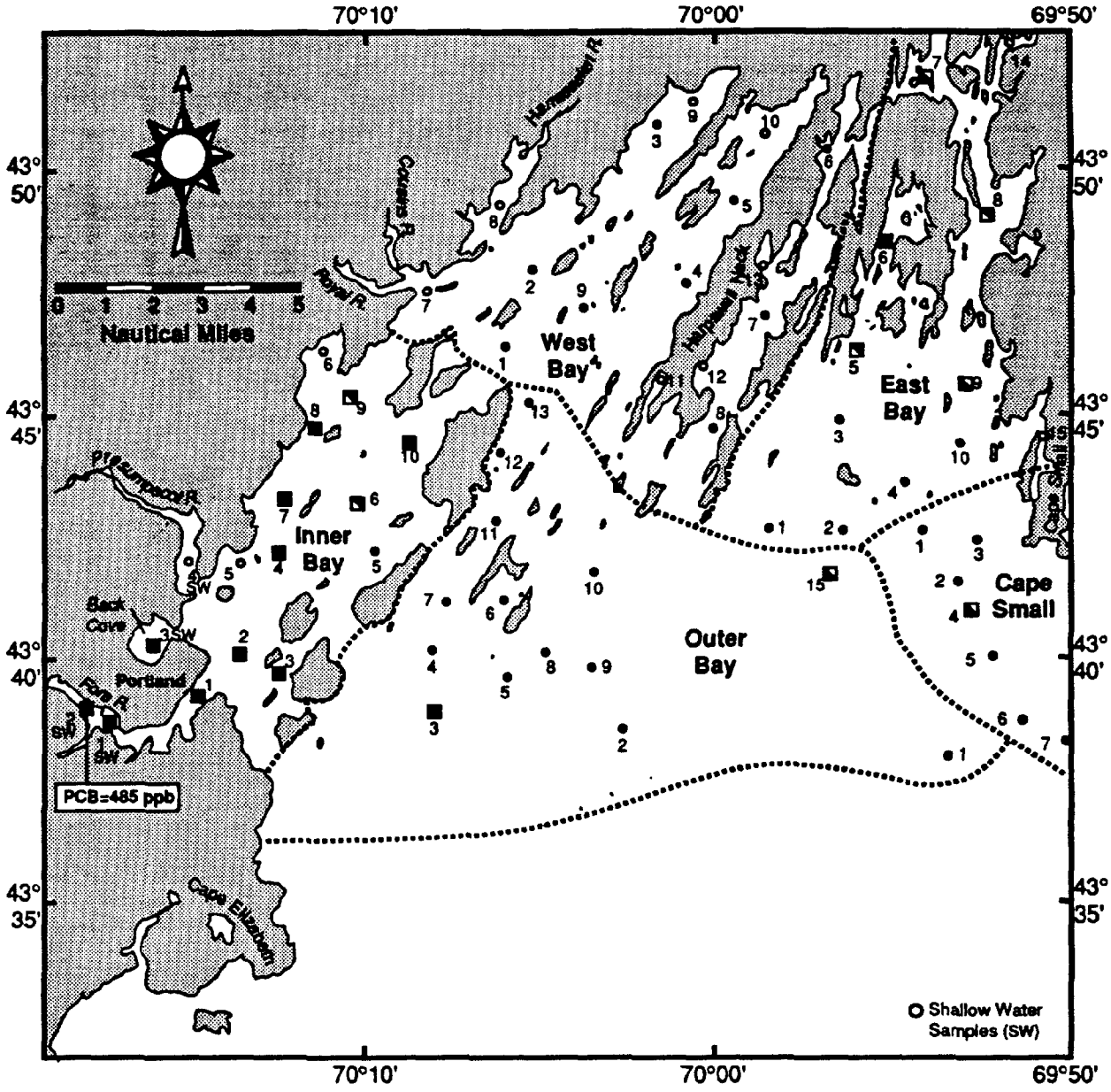

Figure 6. Location of the 25% high organic (▣) and inorganic (▣) concentrations in sediments from Casco Bay.

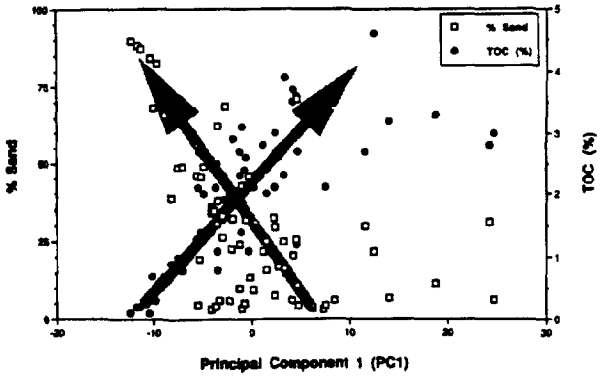

Figure 7. Relationship between PC 1, TOC (%), and sand content (%) for Casco Bay sediments.

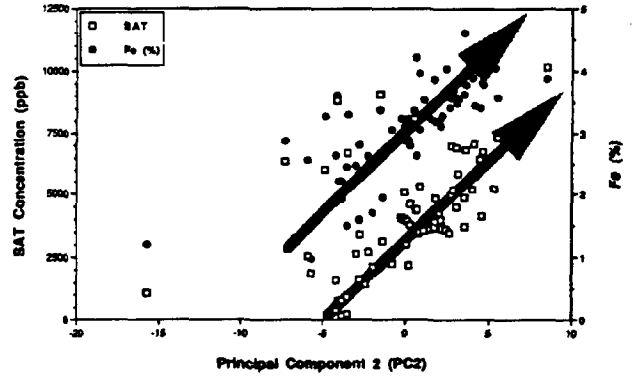

Figure 8. Relationship between PC 2, Fe content (%), and saturated aliphatic hydrocarbons (ppb) for Casco Bay sediments.

and negatively in PC 3 (lower right quadrant, Figure 9) include compounds of algal (C_{15} and C_{17}) and higher plant (C_{23}–C_{31}) origin (*8–10*). Other similarly loaded variables include Fe, Ni, Se, As, Cr, and percentage silt and clay (Figure 9). These distributions represent terrigenous detrital and autochthonous marine inputs. TOC is sim-

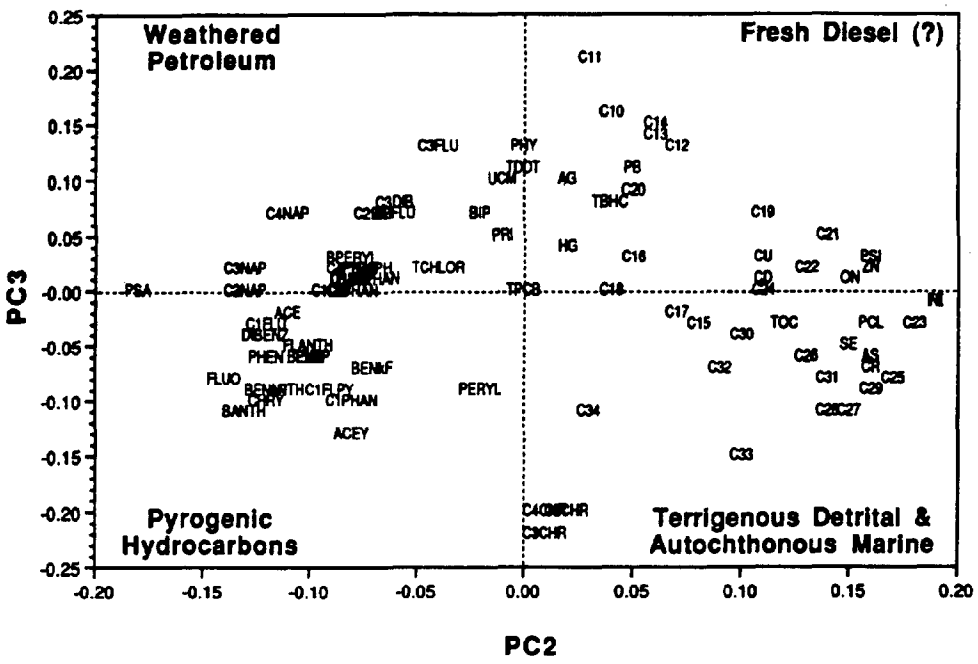

Figure 9. Relationship between PC2 and PC3 for PCA of Casco Bay contaminant data.

ilarly loaded, suggesting that biogenic materials are an important contributor to the organic richness of the sediments (*17*). Hydrocarbons loaded negatively in both PC 2 and PC 3 (lower left quadrant, Figure 9) consist primarily of four- and five-ring aromatics that are generated from both natural and anthropogenic combustion processes. A combustion origin for these hydrocarbons is also supported by the covariance of the parent two- and three-ring aromatics which are structurally stable at high temperatures (*11–14*). The departure of the alkylated chrysenes from this trend suggests either a biogenic source for these compounds or possibly some interference in their analysis from biogenic material. Hydrocarbons loaded negatively in PC 2 and positively in PC 3 (upper left quadrant, Figure 9) include two- and three-ring aromatics containing a C_2 or greater alkylation. These compounds are the most abundant aromatic hydrocarbons in petroleum and petroleum byproducts. Pristane and UCM are similarly loaded, suggesting a weathered petroleum origin (*18, 19*). The source represented by the hydrocarbons that are loaded positively in both PC 2 and PC 3 (upper right quadrant, Figure 9) is equivocal. These consist primarily of *n*-alkanes in the range C_{10}–C_{22}, which might represent a relatively unweathered petroleum product, i.e., diesel fuel. Alternatively, the covariance of these hydrocarbons with the metals Pb, Ag, and Hg and total DDTs and BHC concentrations (Figure 9) suggest possible inputs from runoff associated with either agricultural or industrial activities. Principal component 4 (5.4% of the total variance) is characterized by high positive loadings for most of the chlorinated hydrocarbons analyzed and is less straightforward to interpret. It should be noted that the organochlorine compounds are generally low and near the method detection limit, thus indicating a relatively "noisy" data set.

Based on these interpretations, the distribution of samples in a scores cross-plot of PC 2 versus PC 3 (Figure 10) can be used to assess the regional influence of a variety of sources. Sediments exhibiting a predominantly biogenic influence from detrital and autochthonous sources (positive scores for PC 2, negative scores for PC 3) are found in the upper East Bay (EB-3, -5, -6, -7, and -8), and also at Outer Bay sites OB-1 and OB-12 and Inner Bay site IB-9. In contrast, the lower East Bay (EB-1, -2, -4, -9, and -10), as well as Outer Bay site OB-15 and shallow water site SW-15, contains a greater component originating from pyrogenic sources (negative scores for PC 2 and PC 3). Site CS-4 in Cape Small exhibits a composition similar to the lower East Bay sites. These distributions are significant in that the sites that are similar in composition are geographically clustered. This suggests subtle differences in the principal sources of hydrocarbons in the upper and lower East Bay.

Sites characterized by inputs of weathered petroleum (negative scores for PC 2, positive scores for PC 3) include the Inner Bay and shallow water sites nearest the city of Portland (IB-1 and -2 and SW-3, -4, and -5). This is probably the result of chronic inputs from runoff and point sources associated with urban activities. Surprisingly, however, the sandy sediments from Cape Small (CS-1, -2, -3, -5, -7, and, to a lesser extent CS-6) have contaminant compositions that are nearly identical to site IB-1. This is illustrated in the scores cross-plot in Figure 10, where the majority of Cape Small sites plot intermediate between the lower East Bay and shallow water sites SW-3 and SW-4 from the Inner Bay. This likely reflects aromatic hydrocarbon inputs from both pyrogenic and petroleum sources at these locations and suggests that, despite significantly lower concentrations, the assemblage of contaminants in Cape Small sediments is similar to those at some contaminated Inner Bay sites. Sites showing a relative enrichment in C_{10}–C_{22} *n*-alkanes (positive scores for PC 2 and PC 3) include nearly all the West Bay sites and shallow water sites SW-9, -10, -11, and -13 within the West Bay. Several nearby sites also exhibit a similar composition. These include Outer Bay site OB-13 and Inner Bay sites IB-6 and IB-10. Thus, although the origin of this compositional feature is uncertain, it appears to manifest itself over a limited portion of Casco Bay, suggesting a localized source. Several Outer Bay sites (OB-

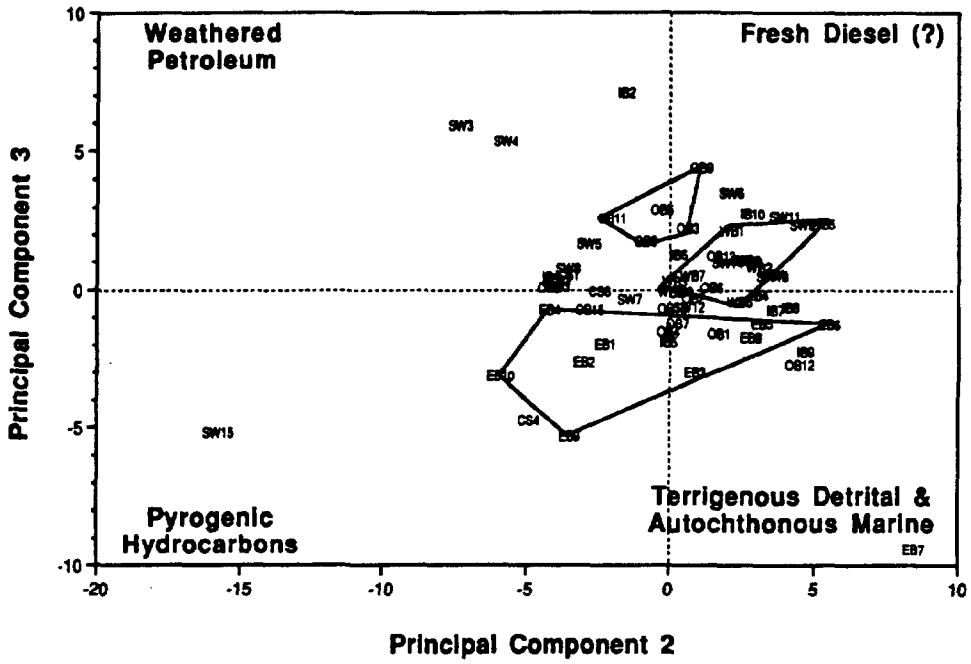

Figure 10. Suggested model for determining the source of hydrocarbons and trace metals in Casco Bay sediments.

Table 4. Comparison of ER-L, ER-M, Apparent Effects Thresholds, and Washington State Sediment Quality Criteria Concentrations for Selected Chemicals in Sediments and Values Measured in Casco Bay (after Long and Morgan, 1990; Washington State Dept. of Ecology Sediment Management Standards, Chapter 173-204 WAC)

						Casco Bay regions[f]									
						Inner Bay		West Bay		East Bay		Cape Small		Outer Bay	
chemical analyte	ER-L[a]	ER-M[b]	AET[c]	deg of confidence[d]	WSSQC[e]	min	max	min	max	min	max	min	max	min	max
						Trace Elements (ppm dry wt)									
arsenic	33	85	50	L/M	5.7	1.62	16.00	4.76	19.60	3.20	19.60	5.03	13.70	5.03	20.50
cadmium	5	9	5	H/H	5.1	0.213	0.908	0.088	0.529	0.076	1.320	0.036	0.208	0.036	0.592
chromium	80	145	NA[h]	M/M	26.0	31.00	91.00	35.00	100.00	29.00	105.00	37.00	93.00	43.00	93.00
copper	70	390	300	H/H	390	7.92	48.40	6.98	26.20	5.59	27.90	2.52	21.60	6.94	26.20
lead	35	110	300	M/H	450	27.50	75.60	20.50	37.60	13.60	37.00	14.10	32.40	25.50	40.70
mercury	0.15	1.3	1	M/H	0.41	0.061	0.424	0.019	0.096	0.048	0.181	<0.010	0.190	0.049	0.141
nickel	30	50	NSD[g]	M/M	NA	7.81	37.80	9.67	38.60	8.36	38.40	12.90	30.60	14.50	39.80
silver	1	2.2	1.7	M/M	6.1	0.12	0.78	0.07	0.36	0.08	0.29	<0.07	0.20	0.10	0.26
zinc	120	270	160	H/H	410	35.00	125.00	34.00	140.00	28.00	105.00	31.00	88.00	43.00	109.00

[a] ER-L, effects range-low. [b] ER-M, effects range-median. [c] AET, apparent effects threshold. [d] L, low; M, medium; H, high. [e] WSSQC, Washington State Sediment Quality Criteria, calculated ppb dry wt based on 2% TOC. [f] ppm dry weight. [g] NSD, not sufficient data. [h] NA, not available.

3, -5, -8, -9, and -11) exhibit a composition intermediate between the Inner Bay sites characterized by weathered petroleum and the West Bay sites enriched in lower molecular weight *n*-alkanes.

Potential for Biological Effects. Biological effects or sediment quality were not directly measured in this study. However, the concentrations of most organic contaminants detected are below the concentration levels that are believed to evoke toxic responses in marine benthic organisms (Tables 4–6). Long and Morgan (*9*) conducted an extensive review of articles that provide both concentrations of contaminants in sediments and observed biological effects. Six different approaches used in these studies were briefly described and reviewed. It was concluded that each approach had strengths and weaknesses, i.e., there is no perfect method for determining specific threshold concentrations for contaminants in sediment. They therefore derive consensus values by considering data from all of the studies reviewed. Sediment concentrations shown by the studies to cause biological effects, and judged to be valid, were ranked from low to high. A 10th and 50th percentile were then determined. Those were designated "effects range low" and "effects range median" (ER-L and ER-M). The Washington State Sediment Quality Criteria, the summary of data from Long and Morgan (*16*), and the Casco Bay results are compared in Tables 4–6.

The total PAH concentrations present in Inner Bay sediments are above the PAH concentrations thought to produce toxic responses in marine benthic organisms, i.e., total PAH ≥35 000 ppb (Table 4). Bioavailability and not necessarily absolute concentration are compared and also a factor in determining whether a contaminant evokes a biological response. For example, the mode of occurrence of PAH has been shown to vary widely depending on the original source (*19*). Coal or soot-associated combustion-derived PAHs are often tightly bound or occur in the interiors of particles. This mode of occurrence renders these PAHs largely inert as far as biological effects. In contrast, liquid hydrocarbons such as oil or creosote contain PAHs that are readily available to organisms and would be expected to induce toxicological effects. A majority of

Table 5. Comparison of ER-L, ER-M, Apparent Effects Thresholds, and Washington State Sediment Quality Criteria Concentrations for Selected Chemicals in Sediments and Values Measured in Casco Bay (after Long and Morgan, 1990; Washington State Dept. of Ecology Sediment Management Standards, Chapter 173-204 WAC)

chemical analyte	ER-L[a]	ER-M[b]	AET[c]	deg of confidence[d]	WSSQC[e]	Casco Bay Regions[f] Inner Bay min	Inner Bay max	West Bay min	West Bay max	East Bay min	East Bay max	Cape Small min	Cape Small max	Outer Bay min	Outer Bay max
Polychlorinated Biphenyls (ppb)															
total PCBs	50	400	370	M/M	240	7.31	484.97	1.58	16.32	8.89	37.30	0.44	40.02	5.50	30.67
DDT and Metabolites (ppb)															
DDT	1	7	6	L/L		0.49	4.28	<0.20	0.96	0.40	2.01	<0.20	0.86	0.47	1.52
DDD	2	20	NSD[g]	M/L		0.67	15.09	0.08	1.49	0.31	1.98	<0.07	0.62	0.34	2.04
DDE	2	15	NSD	L/L		0.18	3.84	<0.06	1.14	0.07	0.48	<0.06	0.40	0.06	0.63
total DDT	3	350	NA[h]	M/M		1.63	20.42	<0.20	3.10	0.82	4.16	<0.20	1.89	1.03	4.12
Other Pesticides (ppb)															
lindane	NA	NA	NSD	NA		<0.07	0.48	<0.07	0.22	<0.07	0.35	<0.07	0.11	<0.07	0.34
chlordane	0.5	6	2	L/L		0.15	4.91	0.07	0.98	0.16	1.91	<0.07	1.32	0.13	1.89
heptachlor	NA	NA	NSD	NA		0.08	0.13	<0.04	0.05	<0.04	0.13	<0.04	<0.04	<0.04	0.04
dieldrin	0.02	8	NA	L/L		<0.16	0.94	<0.16	<0.16	<0.16	0.43	<0.16	2.46	<0.16	1.40
aldrin	NA	NA	NSD	NA		<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28	<0.28
endrin	0.02	45	NSD	L/L		<0.06	0.84	<0.06	0.21	<0.06	0.17	<0.06	<0.06	<0.06	0.55
mirex	NA	NA	NSD	NA		<0.04	0.29	<0.04	0.08	<0.04	0.49	<0.04	0.66	<0.04	0.16

[a] ER-L, effects range-low. [b] ER-M, effects range-median. [c] AET, apparent effects threshold. [d] L, low; M, medium; H, high. [e] WSSQC, Washington State Sediment Quality Criteria, calculated ppb dry wt based on 2% TOC. [f] ppm dry weight. [g] NSD, not sufficient data. [h] NA, not available.

Table 6. Comparison of ER-L, ER-M, Apparent Effects Thresholds, and Washington State Sediment Quality Criteria Concentrations for Selected Chemicals in Sediments and Values Measured in Casco Bay (after Long and Morgan, 1990; Washington State Dept. of Ecology Sediment Management Standards, Chapter 173-204 WAC)

chemical analyte	ER-L[a]	ER-M[b]	AET[c]	deg of confidence[d]	WSSQC[e]	Casco Bay regions[f] Inner Bay min	Inner Bay max	West Bay min	West Bay max	East Bay min	East Bay max	Cape Small min	Cape Small max	Outer Bay min	Outer Bay max
Polynuclear Aromatic Hydrocarbons (ppb dry wt surrogated corrected)															
acenaphthene	150	650	150	L/L	320	2	81	<1	3	2	19	<1	13	2	6
anthracene	85	960	300	L/M	4 400	6	255	3	15	8	107	<1	99	14	50
benz[a]anthracene	230	1600	550	L/M	2 200	30	655	12	56	34	481	1	360	48	173
benzo[a]pyrene	400	2500	700	M/M	1 980	43	741	17	100	50	498	1	433	62	209
benzo[e]pyrene	NA[h]	NA	NSD[g]	NA		37	514	14	74	37	276	1	271	48	140
biphenyl	NA	NA	NSD	NA		3	29	<2	7	4	12	<2	10	4	12
chrysene	400	2800	900	M/M	2 200	44	766	19	74	47	530	1	398	53	192
dibenz[a,h]anthracene	60	260	100	M/M	240	3	105	3	41	7	58	<0	64	11	73
2,6-dimethylnaphthylene	NA	NA	NSD	NA		4	130	1	9	3	28	<1	17	5	14
fluoranthene	600	3600	1000	H/H	3 200	90	1444	34	144	82	639	2	522	118	304
fluorene	35	640	350	L/L	460	4	201	1	7	4	96	<1	27	6	15
1-methylnaphthalene	NA	NA	NSD	NA		3	81	1	7	3	31	<1	20	5	11
2-methylnaphthalene	65	670	300	L/M	760	5	95	2	11	5	37	<1	34	8	17
1-methylphenanthrene	NA	NA	NSD	NA		10	311	5	14	0	68	<1	49	8	33
naphthalene	340	2100	500	M/H	7 400	8	135	2	14	7	46	<2	41	12	26
perylene	NA	NA	NSD	NA		17	216	9	56	31	110	<4	94	21	77
phenanthrene	225	1380	260	M/M	2 000	42	1036	17	71	41	550	1	269	57	160
pyrene	350	2200	1000	M/M	20 000	82	1552	31	137	78	560	2	562	1127	302
2,3,5-trimethylnaphthalene	NA	NA	NSD	NA		3	187	1	4	2	34	<1	9	3	6
total PAH	4000	35 000	22 000	L/L		911	20 748	421	1901	1059	7340	16	7454	1312	4004

[a] ER-L, effects range-low. [b] ER-M, effects range-median. [c] AET, apparent effects threshold. [d] L, low; M, medium; H, high. [e] WSSQC, Washington State Sediment Quality Criteria, calculated ppb dry wt based on 2% TOC. [f] ppm dry weight. [g] NSD, not sufficient data. [h] NA, not available.

the PAHs in this study are combustion related and thus may be in a sequestered form that significantly reduces their toxicity.

Long and Morgan (*16*) estimated that median concentrations of total PCB above 400 ppb dry wt elicits a toxic response in most benthic organisms. For this study, only one site (SW-2) is above this threshold. The DDT concentrations are low compared to concentrations known to cause a toxic response in most benthic organisms (*16*). Chlordane concentrations are "low" based on the definition of O'Connor (*15*) and should pose little or no threat of toxic biological effects (*16*).

A number of different approaches to determining the trace metal concentrations in sediments which lead to a biological response have been used, resulting in a large and confusing literature. Thomas (*20*) briefly describes eight different approaches to setting toxicity criteria for sediments, but no actual data are presented. Pavlov (*21*) compared results from one of these approaches, the equilibrium partitioning approach, to results from other commonly used methods. He shows that the concentration of a given metal needed to elicit a biological response, as determined by equilibrium partitioning and other methods, does not vary widely (except for Hg). The threshold concentrations for toxicity are much higher than those found in Casco Bay sediment.

None of the metal concentrations in the Casco Bay sediments are as high as Long and Morgan's (*16*) ER-M,

and only a few are as high as the ER-Le. For example, Casco Bay chromium concentrations are as high as 105 ppm, whereas the ER-L is 80 ppm. Many uncontaminated sediments from other parts of the world, however, contain chromium concentrations higher than 105 ppm, and it is unlikely that chromium in Casco Bay sediment would cause a biological effect. The same can be said for nickel and zinc, where Casco Bay concentrations are as high as 40 and 140 ppm compared to ER-Le of 30 and 120 ppm, respectively. A few mercury concentrations in Casco Bay are also higher than the ER-L but are much lower than those of highly contaminated sediments from Hudson-Raritan, Long Island Sound, Boston Harbor and elsewhere (*15*). It is unlikely that mercury in Casco Bay sediment is causing an effect on marine organisms. As with PAH, bioavailability is an issue in determining trace metal toxicity.

Conclusions

Detailed, high-quality assured analysis of a broad spectrum of contaminants can be utilized to understand the dynamics of pollutants in coastal environments. The potential processes implicated in releasing these contaminants to the marine environment can be identified and their relative importance can be estimated. Statistical analysis of contaminant concentrations can be used to identify geographically consistent contaminant profiles and suggest the source of these pollutants. This approach was applied to Casco Bay, ME.

Anthropogenic contaminants are widespread throughout Casco Bay, but in most cases occur at exceedingly low concentrations. The focus of contamination is in the Inner Bay region directly associated with the densest population centers and industrialization. Multiple processes add contaminants to Casco Bay, and these chemicals have accumulated in bay sediments. Localized accumulations of various chemicals do occur, but even these areas are mostly below levels suspected of evoking toxic biological responses. In order to more specifically assign the sources of the observed contaminants, intense localized sampling and analysis of effluents and runoff patterns would be needed. To determine sediment quality, bioassays of sediments at suspect sites should be conducted to directly assess the potential for biological impacts.

Acknowledgments

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Reprint 3

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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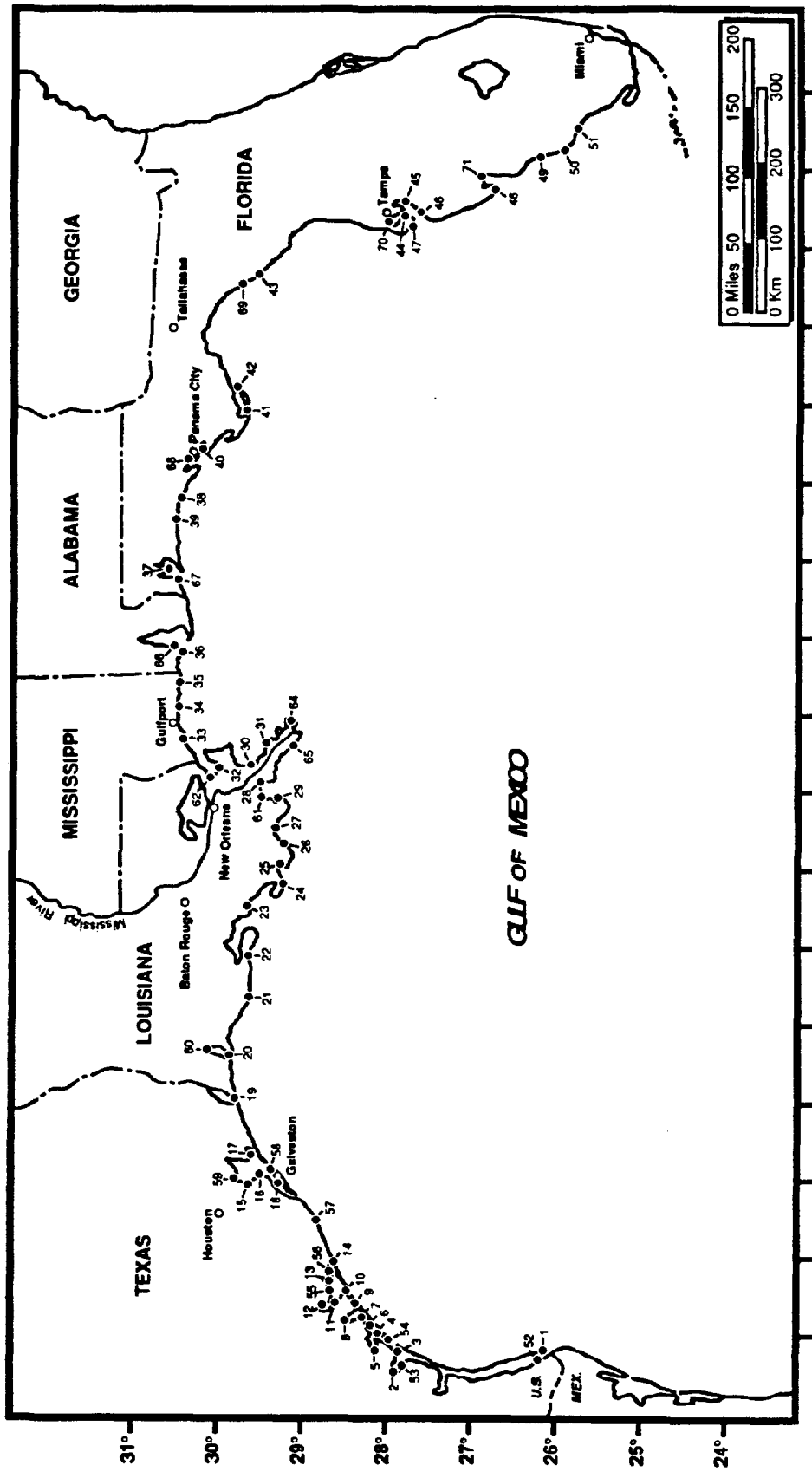

Fig. 1. Location of NS&T Mussel Watch Sites in the Gulf of Mexico (Sericano *et al.*, 1990).

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	84	43	44 ± 50
22	VBSP	189	31	20	118	79	79 ± 108
24	ABOB	20	28	192	115	32	22 ± 42
25	CLCL	20	54	20	20	20	
26	TBLB	20	49	306	37	20	40 ± 162
27	TBLF	101	50	83	20	25	
61	BBTB	—	—	20	—	—	
28	BBSD	963	5 480	44	25	57	963 ± 1 020
29	BBMB	1 080	1 380	1 460	1 150	822	

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)
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,

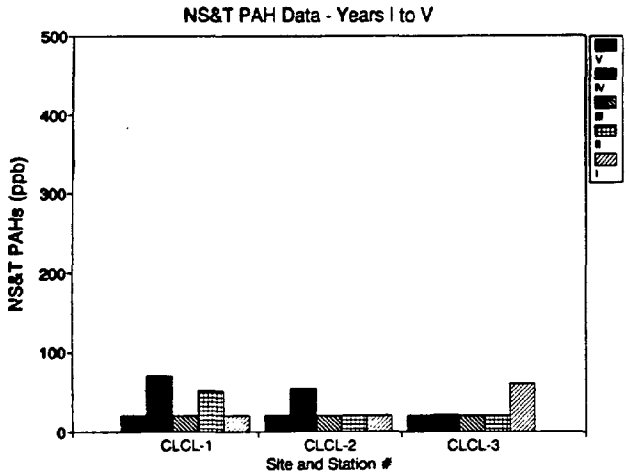

Fig. 2. Total NS&T PAH concentration distribution during the first five years for all three stations; Caillou Lake in Louisiana (Site 25—CLCL).

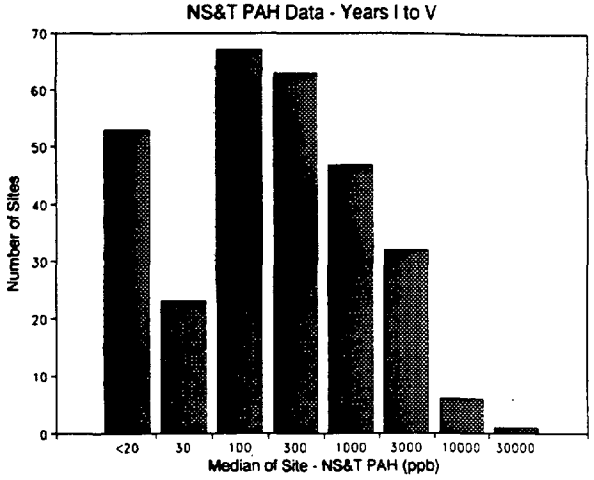

Fig. 4. Frequency distribution of the median total NS&T PAH (tPAH) concentration in the Gulf of Mexico during the first five years of the program.

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.

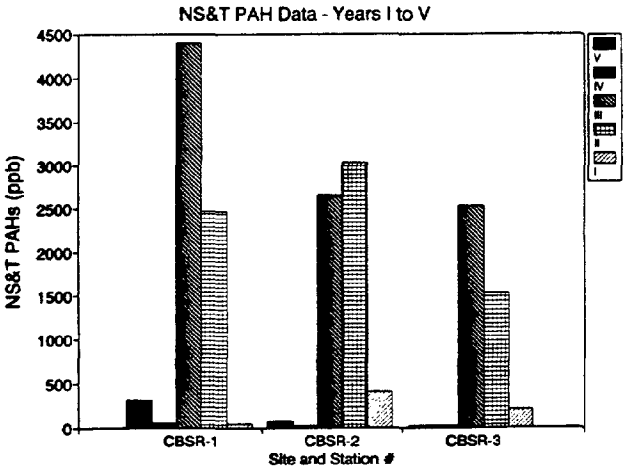

Fig. 3. Total NS & T PAH concentration distribution during the first five years for all three stations; Choctawatchee Bay off Santa Rosa (Site 38—CBSR).

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

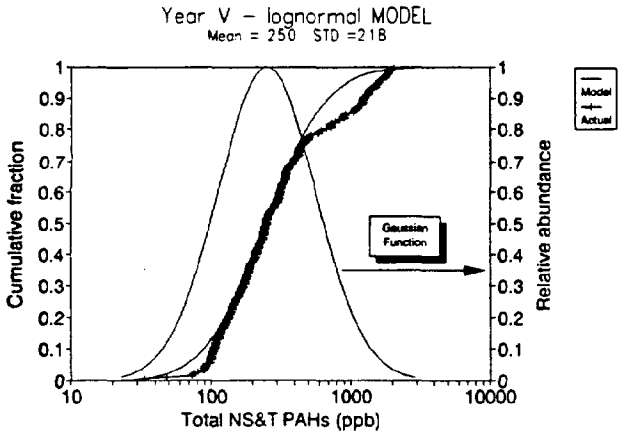

Fig. 5. Plot of the cumulative frequency distribution for Year V total NS&T PAH (tPAH) concentration, compared to the Gaussian curve and its cumulative frequency distribution generated from a lognormal model with a mean of 250 ppb and standard deviation of 218.

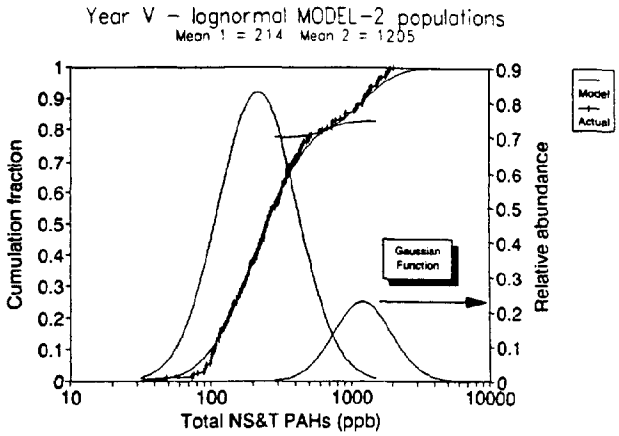

Fig. 6. Plot of the cumulative frequency distribution for Year V NS&T PAH (tPAH) concentration, compared to the Gaussian curves and their cumulative frequency distributions generated from a two population lognormal model with a mean of 214 ppb for Population 1 and a mean of 1205 ppb for Population 2.

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

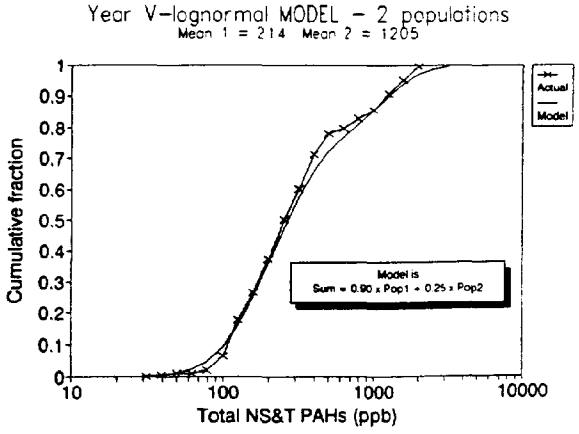

Fig. 7. Comparison of the cumulative frequency distributions for the actual Year V total NS&T PAH (tPAH) concentration data and the cumulative frequency distribution generated from the two population model.

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	869	1030	480	557
90%	1290	1440	2090	1300	1180
95%	1670	2840	3020	2300	1750
98%	1920	5630	4550	3740	2450

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 log-normal 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.

ACKNOWLEDGEMENTS

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Reprint 4

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MODELING OYSTER POPULATIONS II. ADULT SIZE AND REPRODUCTIVE EFFORT

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ABSTRACT A time-dependent model of energy flow in post-settlement oyster populations is used to examine the factors that influence adult size and reproductive effort in a particular habitat, Galveston Bay, Texas, and in habitats that extend from Laguna Madre, Texas to Chesapeake Bay. The simulated populations show that adult size and reproductive effort are determined by the allocation of net production to somatic or reproductive tissue development and the rate of food acquisition, both of which are temperature dependent. For similar food conditions, increased temperature reduces the allocation of net production to somatic tissue and increases the rate of food acquisition. This temperature effect, however, is mediated by changes in food supply. Within the Gulf of Mexico, oyster size declines from north to south because increased temperature decreases the allocation of net production to somatic growth. An increase in food supply generally results in increased size as more energy is used in somatic growth; however, at low latitudes, as food supply increases, adult size decreases because the allocation of more net production to reproduction outweighs the effect of increased rates of food acquisition. Variations in temperature and food supply affect reproductive effort more than adult size because the rate of energy flow through the oyster is higher in warmer months when most net production is allocated to reproduction and small changes in temperature substantially change the spawning season. The wide range of reproductive effort expected from small changes in temperature and food supply suggest that comparisons of adult size and reproductive effort between oyster populations can only be made within the context of a complete environmental analysis of food supply and associated physical parameters and an energy flow model.

INTRODUCTION

Populations of any species tend to have a characteristic mean adult size, which is defined as the size reached by the average surviving adult individual in the dominant cohort. When the characteristic adult size is considerably below that characteristic of the population, the population is described as stunted (Hallam 1965). Stunting is generally considered to result from suboptimal conditions such as extreme environments or low food resources.

In the Gulf of Mexico, populations of the American oyster (*Crassostrea virginica*) exhibit a latitudinal gradient in characteristic adult size (Fig. 1, Table 1). Mean adult size decreases with decreasing latitude on the eastern and western coasts of the Gulf. At the extremes of this distribution, most oysters fail to reach the standard size limit of 7.6 cm that is required for commerical exploitation (e.g. Hofstetter 1977, Berrigan 1990). The nearly complete restriction of the Gulf of Mexico oyster fishery to the northern Gulf is the practical result of this trend. Additionally, year-to-year variations in mean adult oyster size show similar variations throughout the Gulf of Mexico (Wilson et al. 1992). That is, the characteristic adult oyster size increases or decreases uniformly among the many populations in the Gulf. Variation in age cannot be completely excluded as a contributor to these trends; however, the annual mortality in oyster populations from predators and disease exceeds 75% throughout the Gulf of Mexico (e.g. Butler 1953a, Moore and Trent 1971, Powell et al. 1992a) and fished and unfished populations were included in the analysis. Accordingly, the oyster populations sampled in the Gulf of Mexico were composed primarily of individuals that were one to two years in age (Wilson et al. 1992). Hence, size rather than age accounts for the trends seen in these populations.

The similar trends on both sides of the Gulf of Mexico in oyster size with latitude and the year-to-year variability in mean adult size suggest that one or more climatic variables limit oyster size. The correlation with latitude suggests temperature as a likely variable. From a physiological perspective, temperature may affect adult size by regulating the division of net production into somatic and reproductive tissue growth and by regulating the relative rates of filtration and respiration. As temperature increases, more net production is allocated to reproduction. Filtration and respiration rates also increase, but the rate of increase in filtration rate is greater (Powell et al. 1992b). Therefore, a complex interaction of temperature with oyster physiology may place an upper limit on adult size.

Related to adult size is the concept of reproductive senility (Peterson 1983) in which fecundity per unit biomass declines at large size or old age. The existence of reproductive senility in oysters remains to be determined. However, respiration rate rises faster than filtration rate with increasing body size (Klinck et al. 1992, Powell et al. 1992b). The different scaling of respiration and filtration with body size suggests that the scope for growth in oysters must eventually be curtailed at large size which will result in declining fecundity per unit biomass (Powell et al. 1992b). Consequently, populations of lower characteristic size may spawn more per unit biomass.

The objectives of this study are to investigate processes that contribute to variation in the characteristic adult size of oyster populations within a particular habitat and over a latitudinal gradient in temperature and to address the possible influence of reproductive senility in oyster populations. These objectives are addressed using an energy flow model (Fig. 2) developed for post-settlement oyster populations. A series of simulations are presented for Galveston Bay, Texas that consider the effect of variations in temperature, food supply and salinity on adult oyster

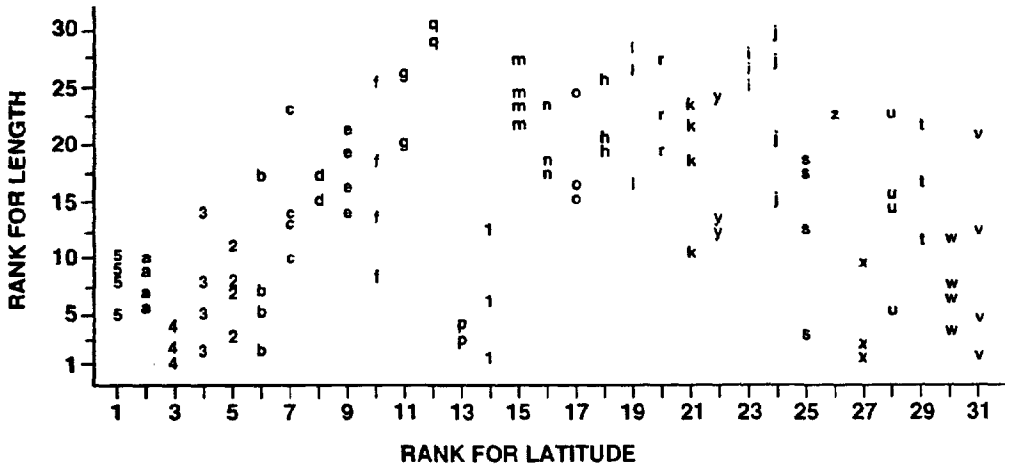

Figure 1. Mean adult oyster size (length) versus latitude plotted as the rank-order of latitude versus the rank-order of size [see Wilson et al. (1992) for details]. The four values for each size and latitude, referenced by letter (a–z) or number (1–5), are those given in Table 1 for 1986 to 1989. Bays with the characteristically smaller sizes are the more southerly bays on either side of the Gulf of Mexico (on the left), the bays in the Florida Panhandle (right), and Tiger Pass and the Mississippi Delta.

TABLE 1.

Oyster population mean length (cm) and fraction of the population in advanced reproductive state (spawning or ready to spawn) for thirty-one bay systems around the Gulf of Mexico that were sampled from 1986 to 1989 as part of the NOAA Status and Trends program. Details of the sampling sites are given in Wilson et al. (1992). Bays are listed beginning with the southern most bay in Texas and proceding clockwise around the Gulf of Mexico. The high fraction ready to spawn in the northern Gulf of Mexico in 1986 (bays l to s) resulted from sampling late in the year. Year and Julian Day were used in the statistical analysis of these data to control for this effect.

	Length				Fraction in Advanced Reproductive State			
Bay Systems	1986	1987	1988	1989	1986	1987	1988	1989
a Laguna Madre	8.16	6.95	6.04	6.03	0.14	0.86	0.27	0.15
b Corpus Christi Bay	7.41	5.67	5.52	7.04	0.13	0.00	0.14	0.23
c Aransas Bay	8.47	8.20	8.19	6.38	0.05	0.02	0.04	0.05
d San Antonio Bay	8.68	8.36	–	–	0.09	0.70	–	–
e Matagorda Bay	9.38	8.30	6.92	7.07	0.20	0.05	0.05	0.21
f East Matagorda Bay	10.13	8.37	6.72	6.29	0.10	0.00	0.14	0.23
g Brazos River	–	–	8.57	7.14	–	–	–	0.33
h Galveston Bay	9.03	8.56	8.55	8.33	0.14	0.09	0.04	0.10
i Sabine Lake	10.44	9.65	9.66	8.40	0.00	0.15	0.00	0.00
j Lake Calcasieu	11.48	8.27	7.99	9.32	0.00	0.00	–	0.00
k Joseph Harbor	8.36	8.79	8.19	7.06	0.67	0.00	–	0.14
l Vermillion Bay	8.72	9.66	9.91	9.06	0.93	0.00	0.25	0.00
m Caillou Lake	9.73	10.36	8.18	8.20	0.83	0.14	0.00	0.13
n Lake Barre/Felicity	8.96	9.22	7.17	7.49	0.97	0.04	0.00	0.21
o Barataria Bay	10.08	9.57	7.04	6.86	0.89	0.00	0.15	0.35
p Tiger Pass	–	–	5.80	5.72	–	–	–	0.27
q Pass a Loutre	–	–	11.23	10.57	–	–	0.00	0.00
r Breton Sound	9.66	8.50	7.71	8.47	0.93	0.07	0.04	0.04
s Lake Borgne	8.94	7.27	7.52	5.68	1.00	0.00	0.07	0.00
t Mississippi Sound	8.40	7.15	7.10	7.20	0.00	0.00	0.00	0.13
u Mobile Bay	8.62	9.03	6.03	6.66	0.13	0.00	0.00	0.13
v Pensacola Bay	9.09	4.55	6.02	6.46	0.08	0.00	0.05	0.09
w Choctawatchee Bay	7.74	4.95	6.67	5.97	0.09	0.00	0.00	0.03
x St. Andrew Bay	6.01	4.81	6.53	6.35	0.64	0.00	0.10	0.06
y Apalachicola Bay	8.43	7.35	8.29	6.64	0.13	0.07	–	0.04
z Apalachee Bay	–	–	–	7.29	–	–	–	0.00
1 Cedar Key	7.44	5.16	6.71	5.39	0.07	0.00	0.08	0.00
2 Tampa Bay	6.58	5.90	6.37	6.44	0.25	0.41	0.23	0.57
3 Charlotte Harbor	6.52	5.30	6.47	6.64	0.00	0.00	0.48	0.27
4 Rookery Bay	6.70	5.26	4.67	5.47	0.00	0.13	0.11	0.13
5 Everglades	8.06	6.56	6.56	5.84	0.08	0.20	0.10	0.00

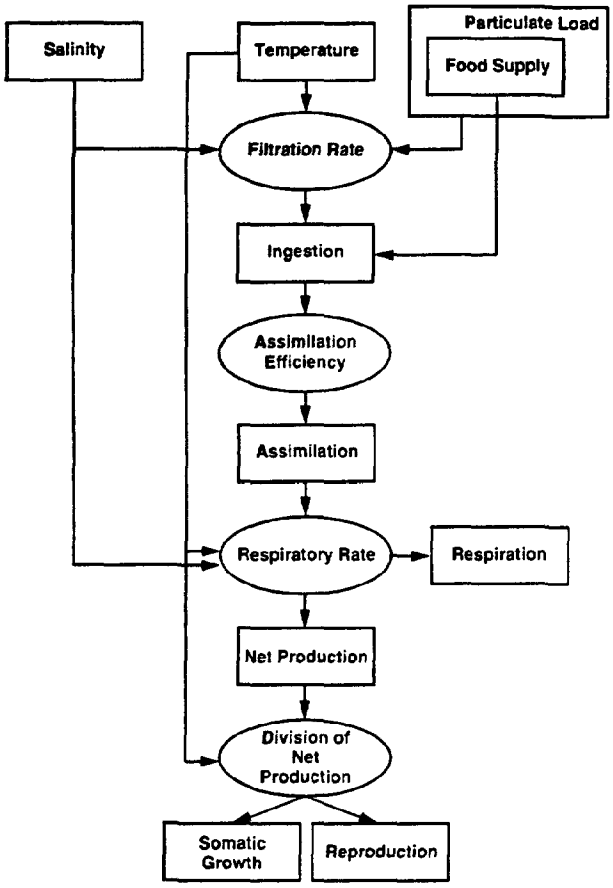

Figure 2. Schematic of the oyster population model.

size. Aside from reductions in oyster growth rate from diseases (Ray and Chandler 1955, Matthiessen et al. 1990) and perhaps genetic differences (Grady et al. 1989, Reeb and Avise 1990) these are likely to be the most important factors controlling size in oyster populations. The effect of latitudinal temperature effects is investigated with simulations that use environmental conditions appropriate for the Laguna Madre, Apalachicola Bay and Chesapeake Bay, as well as Galveston Bay.

THE MODEL

Basic Characteristics

The oyster population model (Fig. 2) is designed to simulate the dynamics of the post-settlement phase of the oyster's life from newly-settled juvenile through adult. Therefore, the oyster's size spectrum was partitioned into 10 size classes (Table 2), that are not equally apportioned across biomass. The lower size limit represents the size at settlement (Dupuy et al. 1977); the upper size limit represents an oyster larger than those normally found in the Gulf of Mexico. In Galveston Bay, for example, the largest oysters routinely collected are 7 to 8 g dry wt (Fig. 3), which corresponds to model size class 9. Thus, the largest size class, 10, is large enough to prevent boundary effects in the model solutions at the upper end of the size-frequency distribution. The boundaries between size classes 4 and 5, 5 and 6, and 6 and 7 represent size limits that have been used or considered for market-size oysters:

TABLE 2.

Biomass and length dimensions of the oyster size classes used in the model. Biomass is converted to size using the relationship given in White et al. (1988), denoted by WPR, and Paynter and DiMichele (1990), denoted by PD. The market-size/submarket-size boundary is about one size class smaller using the conversion from Paynter and DiMichele (1990). The upper size class length conversions obtained from the Paynter and DiMichele (1990) relationship are extrapolations and are, therefore, less accurate, as are the final two conversions obtained from the White et al. (1988) relationship. The range of length to biomass relationships in Galveston Bay, Texas is shown in Figure 3.

Model Size Class	Biomass (g ash free dry wt)	Length (WPR) (mm)	Length (PD) (mm)
1	1.3×10^{-7}–0.028	0.3–25	0.15–21.4
2	0.028–0.10	25–35	21.4–35.7
3	0.10–0.39	35–50	35.7–61.7
4	0.39–0.98	50–63	61.7–89.4
5	0.98–1.94	63–76	89.4–117.6
6	1.95–3.53	76–88	117.6–149.5
7	3.53–5.52	88–100	149.5–178.9
8	5.52–7.95	100–110	178.9–207.1
9	7.95–12.93	110–125	
10	12.93–25.91	125–150	

2.5 in, 3.0 in and 3.5 in, respectively. Adult oysters, those individuals capable of spawning, are defined as individuals weighing more than 0.65 g ash-free dry weight, about 50 mm in length (Hayes and Menzel 1981), although gonadal development has been observed at somewhat smaller sizes (Coe 1936, Burkenroad 1931). Hence, size classes 1 to 3 are juveniles.

The following conversions and scaling factors were used in the oyster model. For simplicity, these are not explicitly shown in the governing equations that are described in the following section. First, all calculations were done in terms of energy (cal m^{-2}). Oyster caloric content was obtained by applying a caloric conversion of 6100 cal g dry wt^{-1} (Cummins and Wuycheck 1971), and the food available to the oysters was converted to caloric equivalents by using 5168 cal g dry wt^{-1}. The model calculations use biomass exclusively (and calories) and so are independent of oyster growth form and length-to-biomass relationships. To relate the biomass size classes, defined in Table 2, to lengths for comparison to the available measurements and the standard measures of fishery management, the length-to-biomass conversion given in White et al. (1988) was used. This conversion is only an approximation, however, given the variation in growth forms found in oysters within bays and throughout their latitudinal range. The model results are presented in terms of biomass, which can be converted to any local specific lengths by using an alternative length-to-biomass relation and the size class boundaries given in Table 2. One example, from Paynter and DiMichele (1990) is shown in Table 2 for comparison.

Second, gains, losses or transfers of energy (or biomass) between oyster size classes were expressed as specific rates (day^{-1}) which were then applied to the caloric content in a size class. For example, ingestion (cal day^{-1}) divided by a caloric value in cal gives a specific rate (cal day^{-1}/cal = day^{-1}), which is then used to calculate incremental changes in a size class. Because the size classes in the model are not of equal size, transfers between size classes were scaled by the ratio of the average weight of the

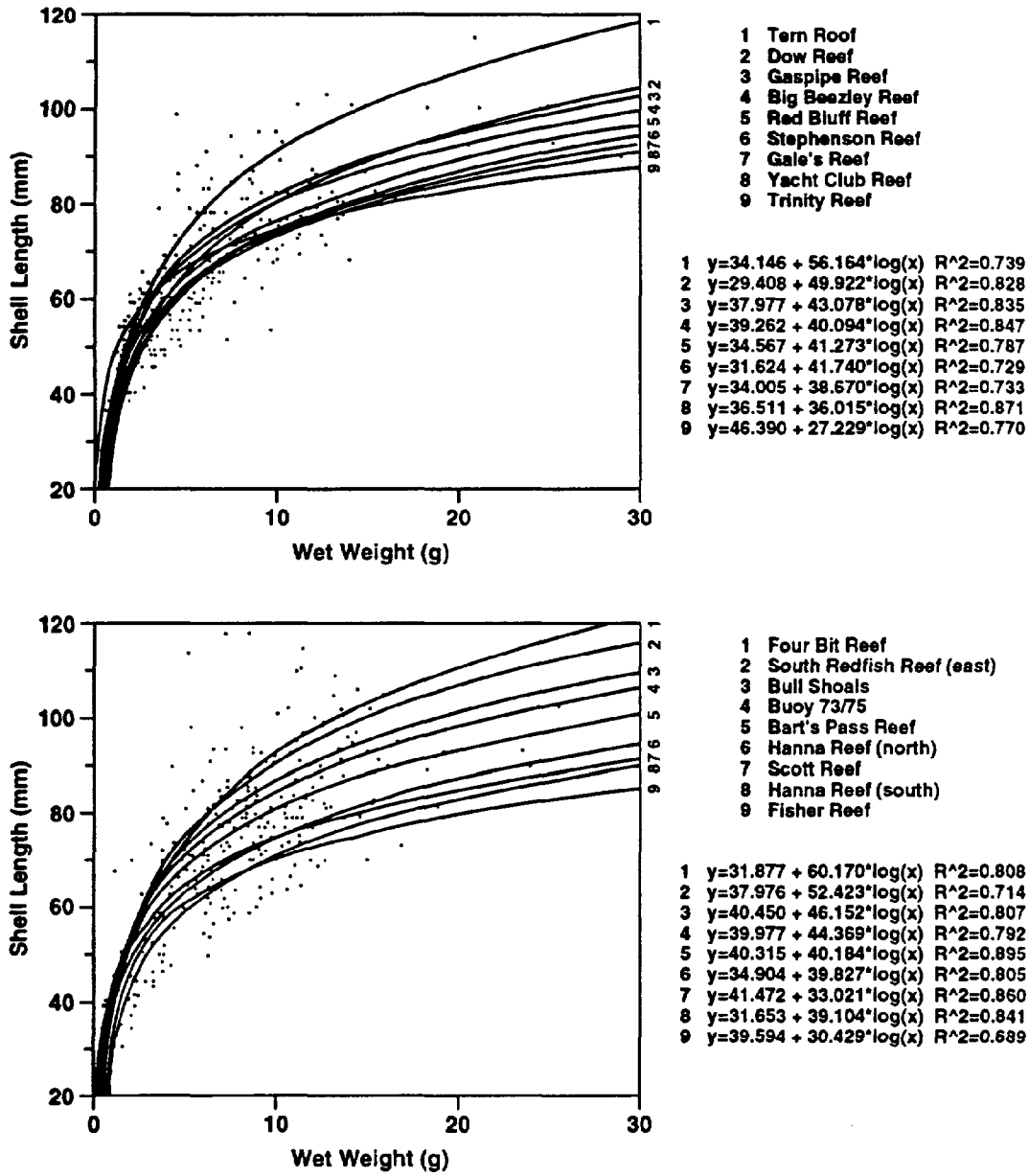

Figure 3. Shell length versus wet weight for oysters collected at eighteen locations in Galveston Bay, Texas. The curves indicate the empirical relationships obtained using the data from the different locations. The numbers on the curves correspond to those for the empirical relationships from each site.

current size class (in g dry wt or cal) to that of the size class from which energy was being gained or to which energy was being lost:

$$\frac{W_j}{W_{j-1}} \text{ or } \frac{W_j}{W_{j+1}}$$

where W is the median value for biomass (in g dry wt) in size class j. This ensured that the total number of individuals in the simulated population was conserved in the absence of recruitment and mortality. Finally, each specific rate for each transfer between size classes was scaled to the relative size of the respective classes:

for transfers up: $W_j/(W_{j+1} - W_j)$
for transfers down: $W_j/(W_j - W_{j-1})$.

Governing Equation

The change in oyster standing stock with time in each size class (O_j) is the result of changes in net production and the addition of individuals from the previous size class or loss to the next largest size class by growth. Excretion was not included since it is a minor component of the oyster's energy budget (Boucher and Boucher-Rodoni 1988). Following White et al. (1988), net production in any size class, NP_j, is the sum of somatic (P_{sj}) and reproductive tissue (P_{rj}) production which is assumed to be the difference between assimilation (A_j) and respiration (R_j):

$$NP_j = P_{sj} + P_{rj} = A_j - R_j. \quad (1)$$

Therefore, a governing equation for each oyster size class can be written as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) \qquad (2)$$

for $j = 1, 10$, with $P_{rj} = 0$ for $j = 1, 3$.

Resorption of either gonadal or somatic tissue results in loss of biomass. When $NP_j < 0$, oysters lose biomass and transfer into the next lower size class. This is an important difference between this size class model and a size class model based on linear dimensions: shell size does not change, however biomass does during periods of negative scope for growth. This is the basis for the use of condition index as a measure of health in oysters (e.g. Newell 1985, Wright and Hetzel 1985). To allow for a negative scope for growth, equation (1) is modified as

$$\frac{dO_j}{dt} = P_{gj} + P_{rj} + (\text{gain from } j-1) - (\text{loss to } j+1) + (\text{gain from } j+1) - (\text{loss to } j-1). \qquad (3)$$

The last two terms on the right side of equation (3) represent the individuals losing biomass and thus, translating down to the next lower size class. Implementation of the model given by equation (3) requires that the processes that result in production and/or loss of somatic and reproductive tissue be described in mathematical terms. The functional relationships used in the model and the rationale for particular choices are given in the following sections.

Filtration Rate, Ingestion and Assimilation

For this model, the filtration rate relationship given by Doering and Oviatt (1986) was adapted to oysters using Hilbert's (1977) biomass-length relationship to obtain filtration rate for each size class as a function of temperature (T) and biomass:

$$FR_j = \frac{K_j^{0.96} T^{0.95}}{2.95} \qquad (4)$$

and

$$K_j = W_j^{0.317} 10^{0.669} \qquad (5)$$

where filtration rate, FR_j, is given as ml filtered $\text{ind}^{-1}\ \text{min}^{-1}$ and W_j is the ash-free dry weight in g for each size class. Powell et al. (1992b) show that equations (4) and (5) yield results comparable to a more general equation derived for all bivalves, including oysters, over the size range appropriate for this model. In addition, equation (4) has the advantage of containing the temperature-dependency described in more detail by Loosanoff (1958), an attribute not present in most other filtration rate equations (Doering and Oviatt 1986). Measurements (Loosanoff 1958) suggest that the rate of increase of filtration rate moderates at temperatures above 25°C, in accordance with a general trend for bivalves described by Winter (1978), and declines above 32°C. However, equation (4) yields realistic values throughout the normal temperature range, so it is used in the model without modification for lower filtration rates at even higher temperatures.

Equation (4) was modified to allow for salinity effects on filtration rate as described by Loosanoff (1953). Filtration rate decreases as salinity drops below 7.5 ppt and ceases at 3.5 ppt. In mathematical terms:

$$\begin{aligned} S \geq 7.5 \text{ ppt} \quad & FR_{aj} = FR_j \\ 3.5 < S < 7.5 \text{ ppt} \quad & FR_{aj} = FR_j(S - 3.5)/4.0 \\ S \leq 3.5 \text{ ppt} \quad & FR_{aj} = 0 \end{aligned}$$

where S is the ambient salinity and FR_j is the rate obtained from equation (4). [Note that the second salinity relationship was misprinted in Powell et al. (1992b) and Hofmann et al. (1992).]

The reduction in feeding efficiency at high particulate loads, characterized by pseudofeces production, was included as a depression in filtration rate rather than as a separate function as used by Soniat (1982). From data presented in Loosanoff and Tommers (1948), total particulate content can be related to a reduction in filtration rate as

$$\tau = (4.17 \times 10^{-4}) 10^{0.418x} \qquad (7)$$

where τ is the total particulate content (inorganic + organic) in g l^{-1} and x is the percent reduction in filtration rate. Solving equation (7) for the percent reduction in filtration rate gives an expression for filtration rate modified by total particulate content, $FR_{\tau j}$, of the form:

$$FR_{\tau j} = FR_{aj}\left[1 - .01\left(\frac{\log_{10}\tau + 3.38}{0.0418}\right)\right]. \qquad (8)$$

Equation (8), if applied to total particulate content (inorganic + organic), approximates the results of Haven and Morales-Alamo (1966) and limits ingestion rate to approximately the maximum value found by Epifanio and Ewart (1977). Therefore, an additional term to lower ingestion efficiency at high food concentrations was not used. We assume all particles are removed by filtration, a slight overestimate (Palmer and Williams 1980), that oysters feed more or less continuously (Higgins 1980a), and that filtration rate does not vary with food availability (Higgins 1980b, Valenti and Epifanio 1981).

Filtration rate times the ambient food concentration gives oyster ingestion. To the extent that oysters can select nitrogen-rich particles from the filtered material for ingestion, equation (8) yields an underestimate of ingestion (Newell and Jordan 1983). Assimilation is obtained from ingestion using an assimilation efficiency of 0.75, an average value obtained from Tenore and Dunstan (1973), Langefoss and Maurer (1975), and Valenti and Epifanio (1981).

Respiration

Oyster respiration, R_j, as a function of temperature and oyster weight in each size class was obtained from Dame (1972) as

$$R_j = (69.7 + 12.6T)W_j^{b-1} \qquad (9)$$

where b has the value 0.26. Equation (9) conforms to the more general relationship for all bivalves obtained by Powell and Stanton (1985).

Salinity effects on oyster respiration over a range of temperatures were parameterized using data given in Shumway and Koehn (1982) as follows:

$$T < 20°\text{C} \qquad R_r = 0.007T + 2.099$$

and

$$T \geq 20°\text{C} \qquad R_r = 0.0915T + 1.324;$$

where R_r is the ratio of respiration at 10 ppt to respiration at 20 ppt:

$R_r = R_{10\ ppt}/R_{20\ ppt}$. Equations (9) and (10) were combined to obtain respiration over a range of salinities as:

$$\begin{array}{ll} S \geq 15 \text{ ppt} & R_j = R_j, \\ 10 \text{ ppt} < S < 15 \text{ ppt} & R_j = R_j(1 + [(R_r - 1)/5((15 - S))]) \\ S \leq 10 \text{ ppt} & R_j = R_j R_r. \end{array}$$

Shumway and Koehn (1982) identified effects of salinity on respiration at 20 ppt; however, we used a 15 ppt cutoff to conform to Chanley's (1958) observations on growth.

Reproduction

For adult oysters ($j = 4, 10$), net production was apportioned into growth and reproduction by using a temperature-dependent reproduction efficiency of the form

$$R_{eff} = 0.054T - 0.729 \quad (12)$$

for January to June and

$$R_{eff} = 0.047T - 0.809 \quad (13)$$

for July to December. Equations (12) and (13) were derived empirically from the field observations of Soniat and Ray (1985). Disagreement exists in the literature concerning the extent to which oyster reproduction is temperature acclimatized (Loosanoff and Davis 1953, Stauber 1950, Loosanoff 1969). However, from the studies of Butler (1955), Kaufman (1979) and Quick and Mackin (1971), acclimatization appears unimportant over the latitudinal range of Chesapeake Bay to the southern Gulf of Mexico. Equations (12) and (13) may not hold north of Delaware Bay.

The portion of new production that goes to reproduction is given by

$$P_{rj} = R_{eff} NP_j, \text{ for } j = 4, 10, \quad (14)$$

Somatic growth is the remaining fraction. In cases where $NP_j < 0$, we assume preferential resorption of gonadal tissue to cover the debt, although some data suggest the contrary (Pipe 1985). Gonadal resorption is commonly observed in stressed oysters (e.g. Gennette and Morey 1971) and in the fall and winter when food is reduced (Kennedy and Battle 1964). For juveniles and adults with no gonadal tissue, resorption of somatic tissue occurs. We assume reduced reproduction at low salinity (Engle 1947, Butler 1949) results from decreased filtration rate and increased respiratory rate and so include no specific relationship for this effect.

Although a considerable literature exists on factors controlling the initiation of spawning (e.g. Stauber 1950, Loosanoff 1965, Dupuy et al. 1977), including empirical temperature-dependent relationships (Loosanoff and Davis 1953, Kaufman 1979), little is understood about factors controlling the frequency of spawning over the entire spawning season (e.g. Davis and Chanley 1956). In our model, spawning occurs when the cumulative reproductive biomass of a size class exceeds 20% of the standing stock; an estimate based on data presented in Gallager and Mann (1986) and Choi et al. (1993).

Model Implementation and Environmental Forcing

The model described by equation (3) was solved numerically using an implicit (Crank-Nicolson) tridiagonal solution technique with a one day time step. The external forcing for the model is from time series that specify ambient temperature, salinity, food concentration and turbidity conditions. Each simulation was run for 6 years which is sufficient time for transient adjustments to disappear and for the oyster population to reach an equilibrium in response to a given set of environmental conditions.

Numerous simulations (not shown) were performed initially using real and idealized time series for the environmental variables. These simulations, some of which are reported by Powell et al. (1992b) and Hofmann et al. (1992), were used to calibrate and verify the transfers between size classes and the overall population characteristics and to provide guidance as to model sensitivity to various parameters. These simulations demonstrated that temperature and food concentration had more of an effect on the structure and character of the simulated oyster populations than variations (i.e. ±10%) in individual model parameters. It should be noted that all of the parameters in the model are specified from either field or laboratory measurements; no free parameters need to be empirically determined. Therefore, the focus of this modeling study is on the effect of variations in environmental conditions on characteristic adult oyster size and fecundity.

The simulations described in the following sections used observed monthly-averaged time series of temperature of two years length from Galveston Bay (Soniat and Ray 1985), the Laguna Madre (Powell et al. 1992b) and Chesapeake Bay (Galtsoff et al. 1947). The temperature values were linearly interpolated to obtain values at one day intervals to be consistent with the time step used in the model. For a six year simulation, the two-year temperature time series was repeated three times.

For most of the simulations described in the following section, salinity values were held constant at 24 ppt to remove the effect of low salinity on oyster respiration and filtration rates and to emphasize temperature effects. For some Galveston Bay simulations, a low salinity (7 ppt) event was imposed and one Chesapeake Bay simulation used the salinity time series given in Galtsoff et al. (1947). Food and turbidity values were specified as described for each simulation. A summary of the environmental conditions used for the simulations is given in Table 3.

RESULTS

Basic Simulation

The time evolution of an oyster population that resulted from the settlement of a cohort of ten individuals in mid-May (day 140) that were subsequently exposed to the monthly-averaged temperatures from Galveston Bay, a constant salinity (24 ppt) and a constant food supply of 0.5 mg l^{-1} was simulated. No recruitment or mortality was allowed so that the same individuals were tracked from settlement onwards, about 5.5 years. This simulation provided a basic case to which other simulations could be compared. Following settlement, the oyster population increases in biomass during the first 1.5 years of the simulation (Fig. 4a) after which it reaches a steady population distribution that is in equilibrium with the imposed environmental conditions. The majority of the population at the end of the simulation is in size classes 5 and 6 (63 to 88 mm). In the first two years of the simulation, gonadal tissue is present in size classes 4 to 6. However, as the population stabilizes, gonadal tissue is confined to size classes five and larger. Gonadal tissue development occurs in the adult size classes throughout the summer and into the fall, with the maximum development as a fraction of body weight occurring in late July of each year.

A fall larval set, exposed to the same environmental conditions, results in a similar population distribution (Fig. 4b). The oyster population stabilizes with the same size-frequency distribu-

TABLE 3.

Summary of the environmental conditions used for the oyster population simulations. Inclusion of a time varying monthly-averaged temperature, salinity, food concentration or turbidity time series is indicated by V. For simulations that used constant salinity or food conditions the values are given in ppt or mg l^{-1}, respectively. Some simulations used an idealized (I) food time series that included increased concentrations in the spring and fall to simulate blooms. Exclusion of an environmental variable is denoted by N.

Area	Temperature	Salinity	Food	Turbidity	Figure
Galveston Bay	V	24	0.5	N	4a, b
Galveston Bay	V	24	1.0	N	5a
Galveston Bay	V	24	1.5	N	5b
Galveston Bay	V	V	V	N	6a
Galveston Bay	V	V	V	V	6b
Galveston Bay	V	7	0.5	N	7a
Galveston Bay	V	7	1.0	N	7b
Galveston Bay	V	7	1.5	N	7c
Chesapeake Bay	V	V	V	N	9a
Laguna Madre	V	24	V	N	9b
Laguna Madre	V	24	0.5	N	10a
Apalachicola Bay	V	24	0.5	N	10b
Chesapeake Bay	V	24	0.5	N	10c
Laguna Madre	V	24	1.0	N	11a
Apalachicola Bay	V	24	1.0	N	11b
Chesapeake Bay	V	24	1.0	N	11c
Laguna Madre	V	24	I	N	13a
Galveston Bay	V	24	I	N	13b
Chesapeake Bay	V	24	I	N	13c

tion and gonadal tissue development is nearly identical. Consequently, a spring settlement is used to initialize the simulations described in the following sections.

Overall, the growth rates, gonadal tissue production and adult size of the simulated oyster populations shown in Figure 4 are in agreement with measurements from Galveston Bay. Some oysters reach size class 5 (63 mm) in about 45 days and size class 6 (76 mm) in about 72 days after settlement. These growth rates are similar to those found for oysters in Galveston Bay and around the Gulf coast in general (Powell et al. 1992a, Ingle and Dawson 1952, Hayes and Menzel 1981). Gonadal tissue production and spawning in oyster populations in the northern Gulf of Mexico is normally restricted to the summer months (Wilson et al. 1990). Consequently, reproductively-advanced oysters make up the majority of the population only from April to October. This same pattern is seen in the simulated population. In Galveston Bay the upper limit on oyster size is 80 to 100 mm and the mean oyster length is about 85 mm (Table 1; Wilson et al. 1992). Adult oyster size at the end of the simulation approaches this value.

Local Controls on Adult Size

Food Supply

Food supply is an important factor governing the growth and development of post-settlement oyster populations. Within any one bay, local conditions can result in large variations in the food concentrations experienced by these populations. To investigate this effect on oyster adult size, constant food supplies that bracketed the range of typical food variations measured in Galveston Bay (Soniat et al. 1984) were tested. The pattern of development for an oyster population exposed to a food supply double that used in the basic simulation (Fig. 5a) is not substantially different. A stable size-frequency distribution develops in about 1.5 years. However, the details of the population do differ. The final size-frequency distribution shows that most of the individuals are in size classes 8 and 9, 100–125 mm. Gonadal tissue development occurs throughout the year, but reaches maximum development in the larger animals in the fall. A further increase in food supply by 50% results in a simulated population that rapidly increases in size (Fig. 5b) and has the majority of the individuals in size class 8 and larger. Development of gonadal tissue occurs in the larger individuals throughout the year. Overall, these simulations demonstrate that oyster size increases with increasing food concentration.

Food supply does not remain constant throughout the year in Galveston Bay at the levels used in the previous simulations. Rather, in many years, food supply shows maximum values in the spring and fall that are associated with the spring and fall plankton blooms and reduced food values in the winter. Hence, a monthly-averaged food time series from Galveston Bay (Soniat et al. 1984) was used with the model. This simulation also used observed salinity values for Galveston Bay. The time varying food supply results in the simulated oyster population shown in Figure 6a. The final adult size for this population is intermediate between that obtained for the constant low and medium food simulations. The majority of the adults are found in size classes 7 and 8 (88–110 mm). Maximum gonadal tissue production is also associated with these size classes and occurs in the late summer and fall. A constant salinity of 24 ppt results in a simulated population (not shown) that is almost identical to that shown in Figure 6a.

Turbidity

In estuarine systems, like Galveston Bay, total seston includes inorganic particles that can interfer with filtration and reduce ingestion rates at high enough concentrations. Hence, the overall food supply is effectively reduced. When monthly-averaged turbidity values (Soniat et al. 1984) from Galveston Bay are included as part of the food supply, the effect is to reduce the overall size of the oyster population and gonadal tissue development (Fig. 6b). The final adult size is reduced to 63 to 88 mm (size classes 5 and 6) and is similar to that obtained at the low constant food supply of 0.5 mg l^{-1}. Gonadal tissue development is confined to a smaller portion of the year.

Salinity

Estuarine systems are frequently characterized by extended periods of low salinity. As many laboratory and field studies have shown, the filtration and respiration rates of oysters are adversely affected at salinities below 7.5 ppt and 15 ppt, respectively. Consequently, episodes of low salinity could result in reduced size and reduced gonadal tissue development. To test the effect of this environmental variable, the development of oyster populations during extended periods of low salinity (7 ppt) over a range of food concentrations was simulated (Fig. 7).

The effect of low salinity is to reduce the overall size of the adult population and to hinder the development of gonadal tissue at a given food concentration. The effect of low salinity is most pronounced at low food concentration (Fig. 7a) where the scope for growth is most reduced. The final adult size is reduced relative to the equivalent high salinity case (cf. Fig. 4a) and gonadal tissue

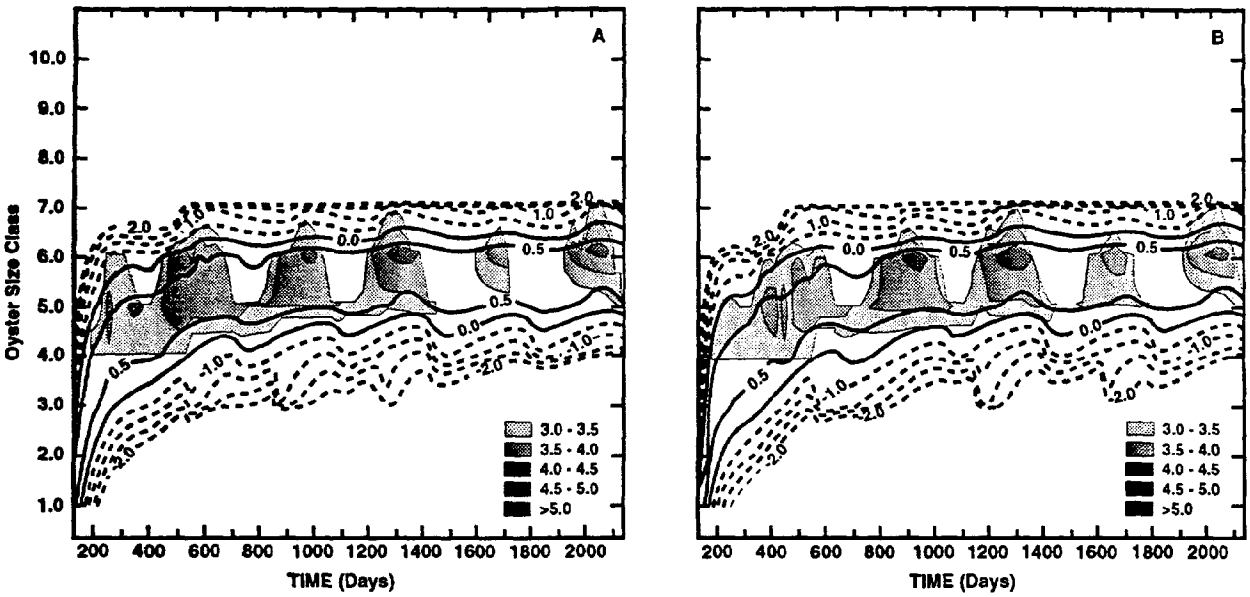

Figure 4. Comparison of the time evolution of oyster populations and gonadal tissue development produced by recruitment of a cohort of ten individuals into size class 1 on A) Julian Day 140 (mid-May) and B) Julian Day 240 (early August). Isolines represent the number of individuals which are given in terms of the logarithm of the number of oysters ($\log_{10} N$). Size class boundaries are defined in terms of biomass (ash free dry weight) as shown in Table 2. Hence, the zero contour corresponds to one individual. Population values less than this are indicated by the dashed lines; solid lines are population values greater than one individual. Shading for the amount of gonadal tissue development represents the logarithm of calories ($\log_{10}$ cal) with the darkest shades corresponding to the highest values. Contour interval is 0.5 for the number of individuals m^{-2} and 1.0 for gonadal tissue production. Numbers of individuals or calories are plotted opposite the size class designations, not halfway between; hence, on day 140 all individuals are in size class 1 opposite the grid mark labeled 1. The caloric values can be expressed as Joules by using a conversion of 4.18 Joules cal^{-1}.

production is less. Similar trends are observed for low salinity conditions at the higher food concentrations (Fig. 7b, c). However, higher food concentrations offset the deleterious effects of low salinity somewhat by providing more energy for growth. Comparison of the simulated populations at low (Fig. 7) and high salinity conditions (Figs. 4 and 5) shows that the effect of reduced salinity is minor relative to that of reduced food. Therefore, the detrimental effects of low salinity on oyster populations can be reduced by high, but not unusually high food supplies.

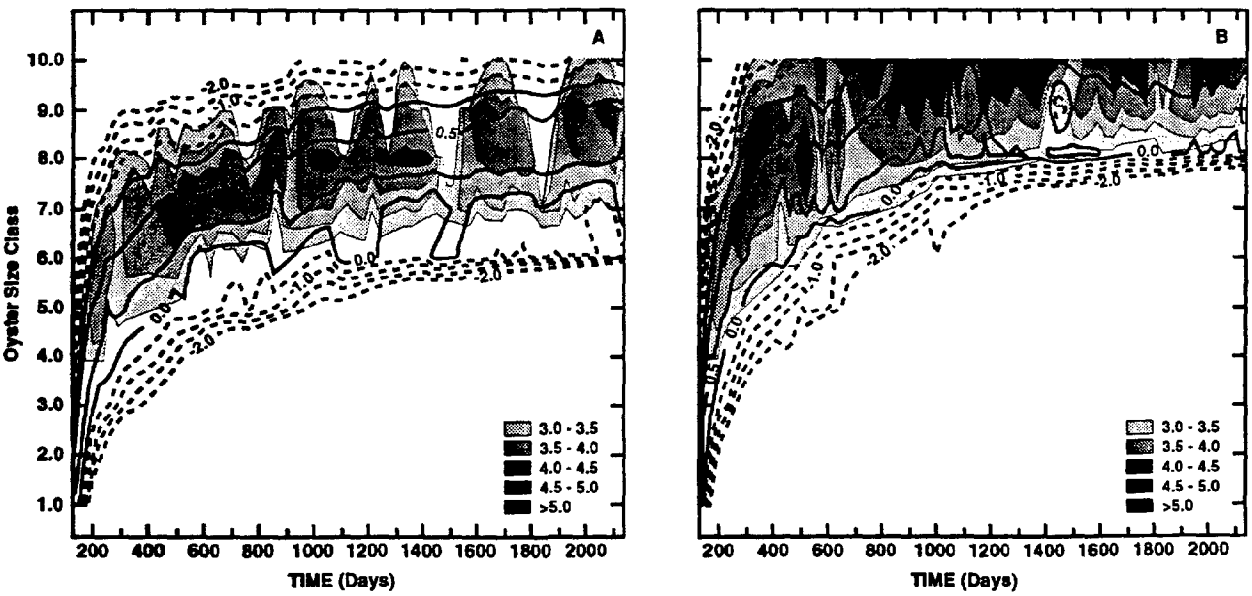

Figure 5. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay environmental conditions and constant food concentrations of A) 1.0 mg l^{-1} and B) 1.5 mg l^{-1}. Otherwise same as Figure 4.

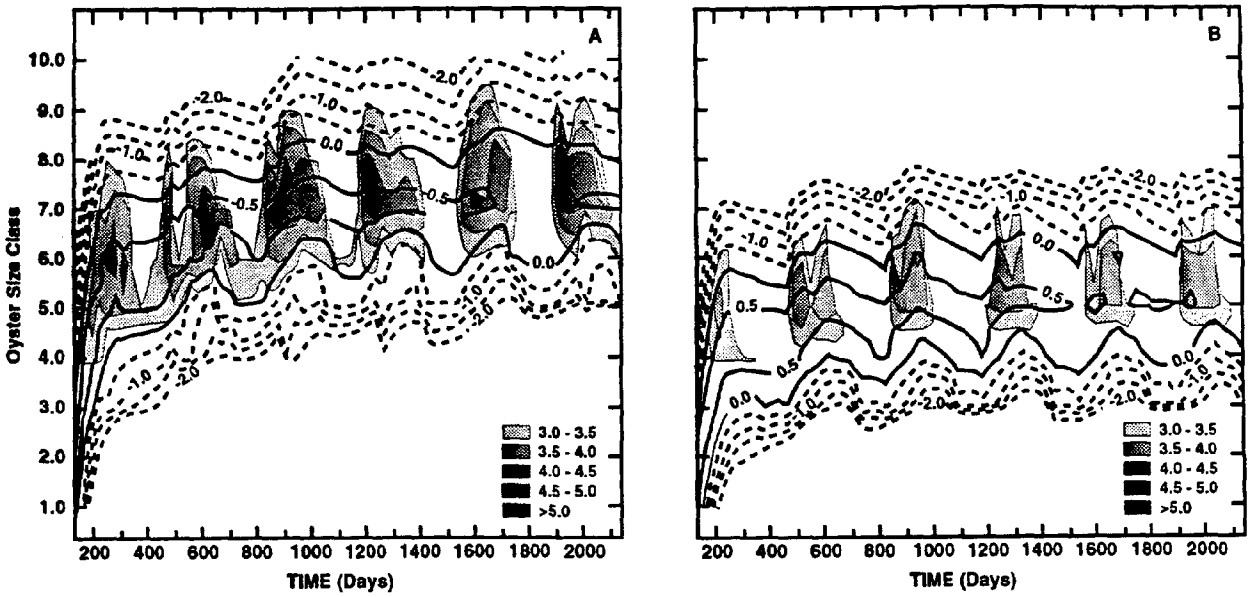

Figure 6. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay environmental conditions and food conditions A) without, and B) with turbidity. Otherwise same as Figure 4.

Latitudinal Controls on Adult Size

Temperature

The monthly temperature distributions that are characteristic of Laguna Madre, Texas (26°N), Galveston Bay, Texas (29°N), Apalachicola Bay, Florida (30°N) and Chesapeake Bay, Virginia (38°N) show that all three bays reach about the same temperature (28°C) in the summer (Dekshenieks et al. 1993). The primary difference over this latitudinal range is in the winter temperatures and duration of cold conditions. To test the effect of temperature on oyster size and gonadal tissue development over such a latitudinal range, a series of simulations that used idealized temperature time series were done. All simulations used six months of warm (28°C) temperature. The remaining six months were set at 25°C, 20°C, 15°C and 10°C to represent winter conditions in the four bays, respectively.

For all the temperature conditions, the mode of the oyster population, after 5.5 years of simulation, was found in size class 7, 88–100 mm (Fig. 8). However, the population distribution about this mode varied considerably from bay to bay. The small temperature difference between winter and summer conditions in Laguna Madre, resulted in the oyster population being dominated by essentially a single size class. Adult size increased between Laguna Madre and Galveston Bay, with about 40% of the population found in size class 8. This model result agrees with observations of increased adult oyster size in Galveston Bay relative to Laguna Madre. However, the simulated size distributions suggest that adult size decreases between Galveston Bay and Chesapeake Bay, which is opposite of the trend seen in the measurements. This difference in simulated and observed adult size arises from the similar time periods used for the warm and cool temperatures.

As a check on the above results, realistic temperature distributions for Chesapeake Bay and Laguna Madre were used with the model (Fig. 9). The simulated population size-frequency distribution for Chesapeake Bay shows that oysters of size classes 6 and 7 (70–100 mm) are produced by the summer of the second year. The juvenile growth rates and adult size obtained from the model agree with those reported for Chesapeake Bay oyster populations by Butler (1953b) and Beaven (1952). Yearly fluctuations in biomass are higher in Chesapeake Bay because scope for growth is negative for longer periods during the winter.

Adult size in Chesapeake Bay (size class 8) is larger relative to that in the Laguna Madre (size class 7). This difference arises despite the shorter growing season in Chesapeake Bay (Butler 1953b). The Chesapeake Bay simulation (Fig. 9a) allows more time at intermediate temperatures where somatic, but not reproductive, tissue is developed. The practical result is a larger adult population. Thus, the temperature range as well as the length of time exposed to a temperature are important determinants of adult size.

Food Supply

A low (0.5 mg l^{-1}) constant supply of food alters the size distribution of adult oysters from Laguna Madre to Chesapeake Bay (Fig. 10). The simulated adult size is essentially the same throughout the Gulf of Mexico. Adult oysters in Laguna Madre (Fig. 10a), Galveston Bay (Fig. 4a) and Apalachicola Bay (Fig. 10b) are found in size class 6. Gonadal tissue production is about the same in the three bays, with that in Laguna Madre being somewhat higher and extending over more of the year. Chesapeake Bay oysters (Fig. 10c) are slightly smaller (size class 5) which results from decreased filtration rate and hence reduced net production in response to the colder winter temperatures in this bay. Winter temperatures in Laguna Madre allow a higher rate of filtration which results in this bay having the largest oysters at the low food levels.

Doubling the available food supply to 1.0 mg l^{-1}, results in the largest oysters being produced at the mid-latitude sites, Galveston Bay (Fig. 5a) and Apalachicola Bay (Fig. 11b). The smaller adult size occurs in Laguna Madre (Fig. 11a) because more of the available food supply is used to produce reproductive rather than somatic tissue. Adult size in Chesapeake Bay (Fig. 11c) is also smaller than that in the mid-latitude bays. However, this arises

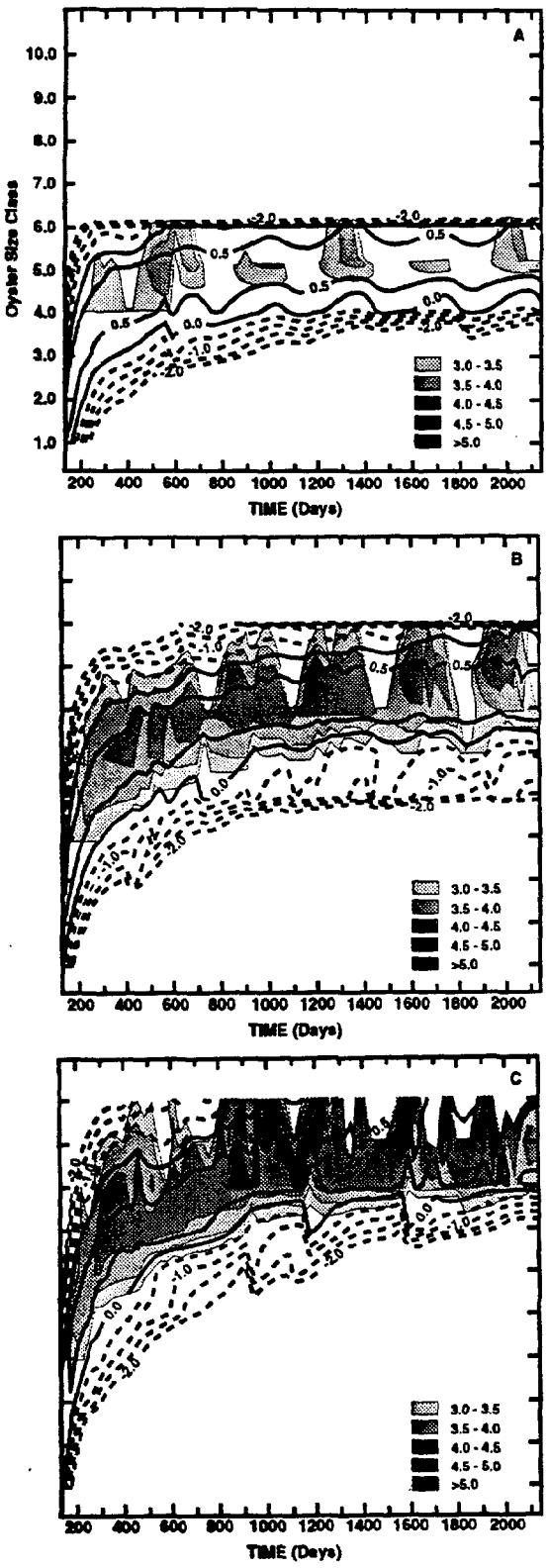

Figure 7. Simulated oyster population distribution and gonadal tissue development that results from Galveston Bay temperatures, low salinity (7 ppt) conditions and food concentrations of A) 0.5 mg l^{-1}, B) 1.0 mg l^{-1}, and C) 1.5 mg l^{-1}. Otherwise same as Figure 4.

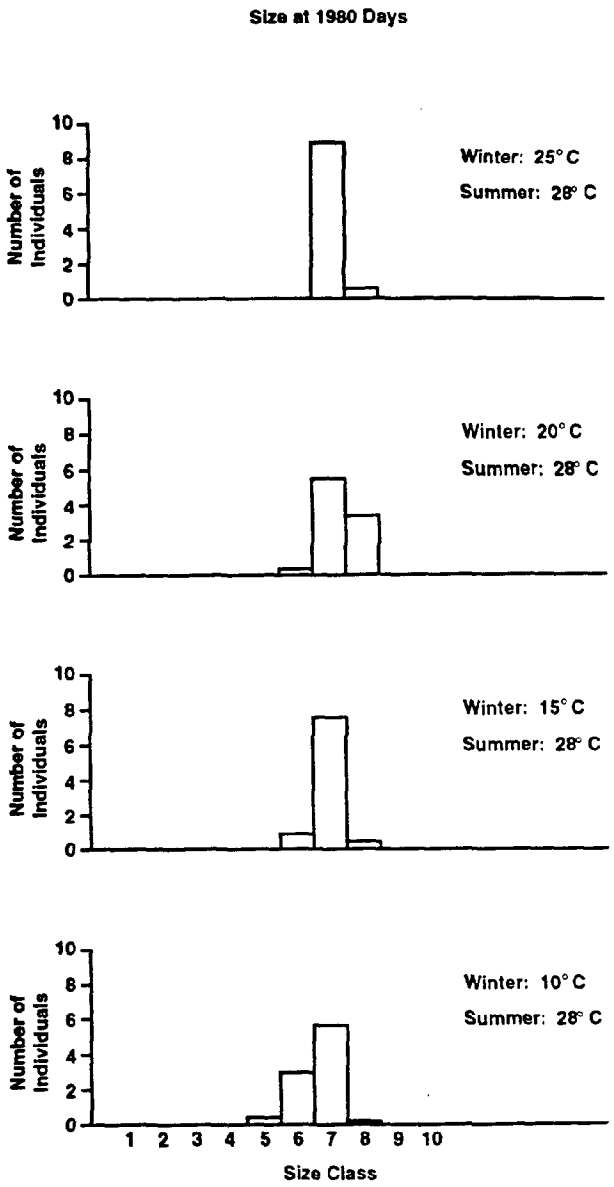

Figure 8. Simulated size frequency distribution from year six for four idealized temperature time series. Other environmental conditions were constant salinity (24 ppt), Galveston Bay food conditions and no turbidity.

due to the colder temperatures which limit winter net production rather than the production of reproductive tissue.

Environmental Controls on Reproductive Potential

The simulations presented in Figures 4–11 show that gonadal tissue development changes for a given set of environmental conditions. This in turn determines the reproductive potential (spawning) of an oyster population. The ability to check the accuracy of the reproductive portion of the population model is limited due to the paucity of observations that provide measurements of oyster reproductive state, oyster size, and environmental conditions concurrently. However, there are some general trends that should appear in the simulated populations.

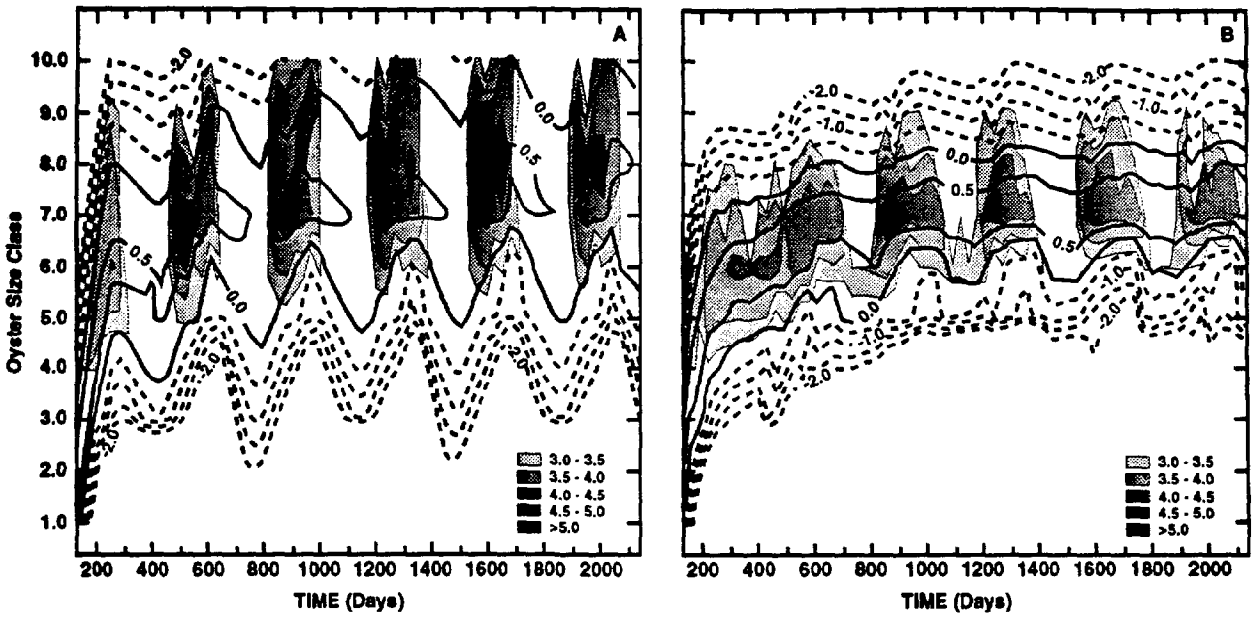

Figure 9. Simulated oyster population distribution and gonadal tissue development that results from temperature, salinity and food conditions characteristic of A) Chesapeake Bay and B) Laguna Madre. Observations on food distributions are lacking for Laguna Madre. Hence, the Galveston Bay food time series was used in this simulation. Otherwise same as Figure 4.

The spawning frequency and pattern associated with the simulated populations from Laguna Madre, Galveston Bay and Chesapeake Bay is shown in Figure 12. In general spawning is associated with the larger size classes and the spawning season tends to be longer at lower latitudes. Also, the most southerly bays tend to have continuous spawning; whereas, that in Chesapeake Bay tends to be confined to discrete pulses. This same trend is observed in the observations from the NOAA Status and Trends program (Table 2). More oysters were found in late reproductive phase, ready to spawn or spawning at lower latitudes.

Spawning season is usually defined by the period of time during which mature eggs are present or by the period of actual spawning. The simulated spawning season, as defined by significant spawning events, is about 100 days in Laguna Madre (Fig. 12a), somewhat shorter in Galveston Bay (Fig. 12b) and even shorter in Chesapeake Bay (Fig. 12c). A tendency towards a spring and fall spawning peak occurs in Galveston Bay (last two years of simulation) and an even stronger tendency towards this occurs in Chesapeake Bay. Significant gonadal material is present for about 200 days (7 months) in Galveston Bay, 160 days (5 months) in Chesapeake Bay, and nearly all year in Laguna Madre. These features of the stimulated spawning season are within the range of values reported for oyster populations and fit the trend toward shorter spawning seasons at higher latitudes (e.g. Hopkins 1935, Stauber 1950, Ingle 1951, Heffernan et al. 1989, and previous references). The development of reproductive material in the simulated oyster populations, from initiation to first spawning, takes about 40 days in Galveston Bay and 60 days in Chesapeake Bay. This is somewhat slower than the 20 to 40 days suggested by Kaufman (1979) and Loosanoff and Davis (1953). However, these time intervals were based on results from constant temperature incubations, which will result in shorter times. Hayes and Menzel (1981) recorded mature gametes in oysters that were 40 to 50 days old, which is similar to what is observed in the simulated populations from Galveston Bay. Egg production, over a two month period, recorded for Delaware Bay oysters held in the laboratory was 3×10^7 to 4×10^7 eggs per female (Davis and Chanley 1955). This study did not report food levels. Egg number, estimated from the simulation results for Chesapeake Bay and Galveston Bay, using the approach described in Klinck et al. (1992), is 1.7×10^8 and 3×10^8 eggs per female, respectively, for a spawning period of about 100 days.

The extent to which these differences and similarities in spawning frequency and pattern result from variations in environmental conditions is discussed in Hofmann et al. (1992). For this study, the interest is in the extent to which these differences and similarities result from variations in adult size. Oyster populations in Laguna Madre (Fig. 13a), Galveston Bay (Fig. 13b) and Chesapeake Bay (Fig. 13c) show a restriction in the period of reproductive effort, as measured by spawn production, over the course of the six-year simulation. This is a consequence of the increased size of the population rather than of increased age. Smaller oysters are more likely to have a positive energy balance and cal allocate a larger fraction of their total assimilated energy to reproduction. As a result, they can spawn more frequently. This trend is independent of the pattern or frequency of spawning and is observed for all ranges of environmental conditions.

A summary of reproductive effort, derived from the simulations, as it relates to average adult size, food supply and latitude is given in Table 4. These results show the strong relationship that exists between reproductive effort, temperature and food supply. Overall reproductive effort is more variable than adult size. For example, in Galveston Bay a reduction in food supply, produced by increased turbidity, gives a 67% reduction in average adult size, but an 85% decrease in reproductive effort (Fig. 6a vs. Fig. 6b). Similarly, the change in temperature that occurs between Galveston Bay and Laguna Madre reduces adult size by 6%, but increases reproductive effort by 23%. Higher temperatures produce higher filtration rates which give increased net production.

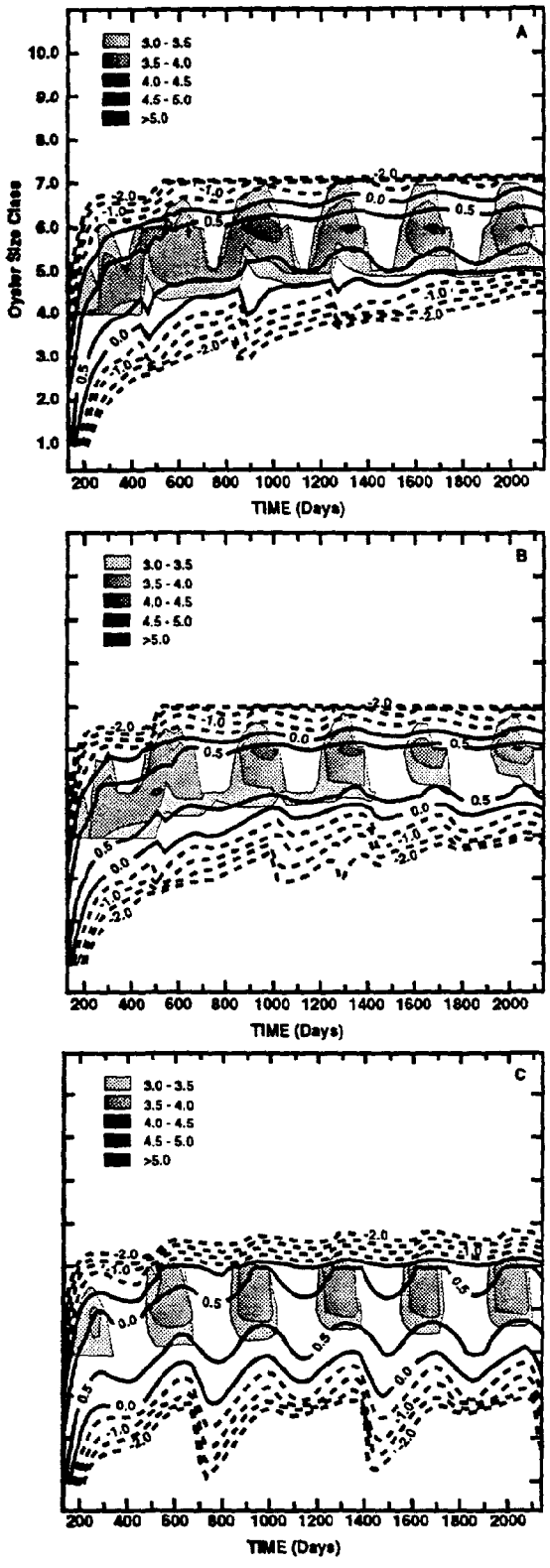

Figure 10. Simulated oyster population distribution and gonadal tissue development that results from constant low food (0.5 mg l^{-1}) supply and environmental conditions characteristic of A) Laguna Madre, B) Apalachicola Bay and C) Chesapeake Bay. Otherwise same as Figure 4.

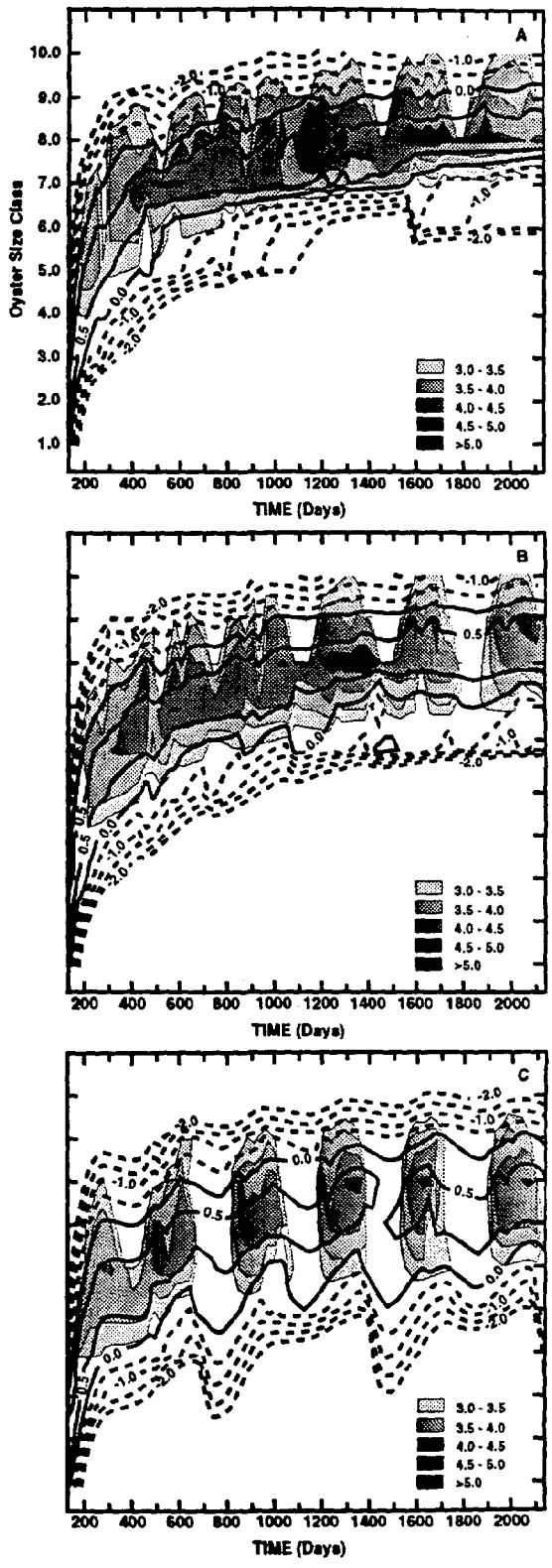

Figure 11. Simulated oyster population distribution and gonadal tissue development that results from medium food (1.0 mg l^{-1}) supply and environmental conditions characteristic of A) Laguna Madre, B) Apalachicola Bay and C) Chesapeake Bay. Otherwise same as Figure 4.

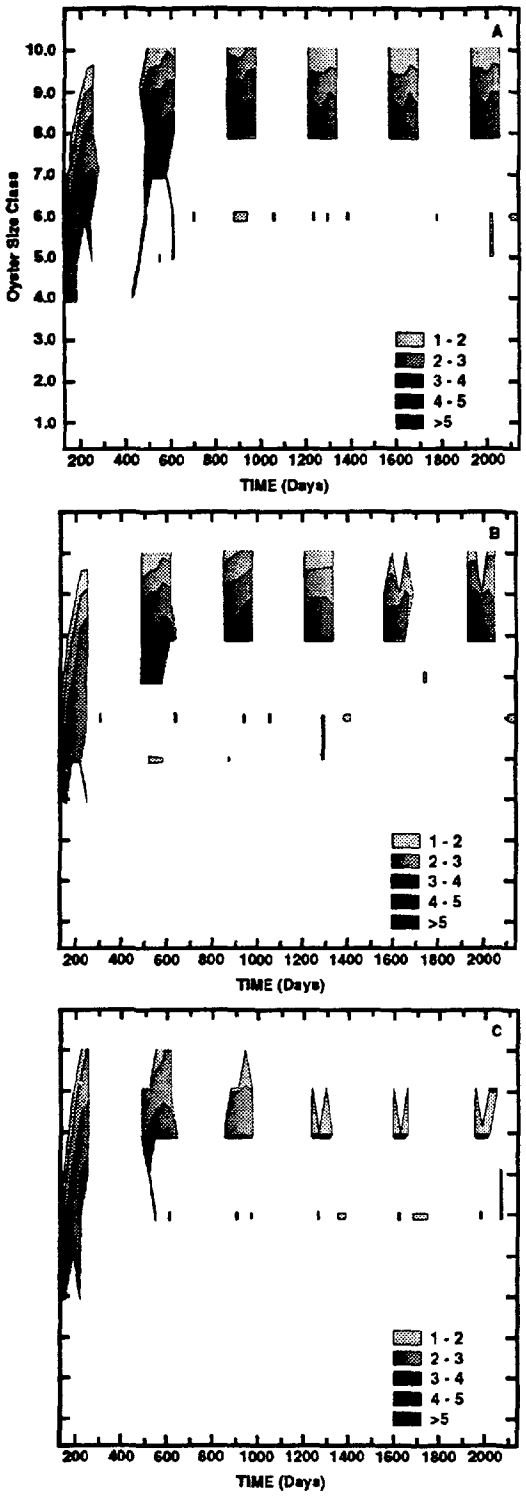

Figure 12. Comparison of spawning intensity versus oyster population size in A) Laguna Madre, B) Galveston Bay and C) Chesapeake Bay. Spawning intensity is shown as $\log_{10}$ calories spawned with a contour interval of 1. Spawning intensity for Laguna Madre and Chesapeake Bay was obtained from the simulated oyster populations shown in Figures 9b and 9a, respectively. The Galveston Bay spawning intensity was obtained from the constant salinity simulation that was essentially identical to the simulation results shown in Figure 6a.

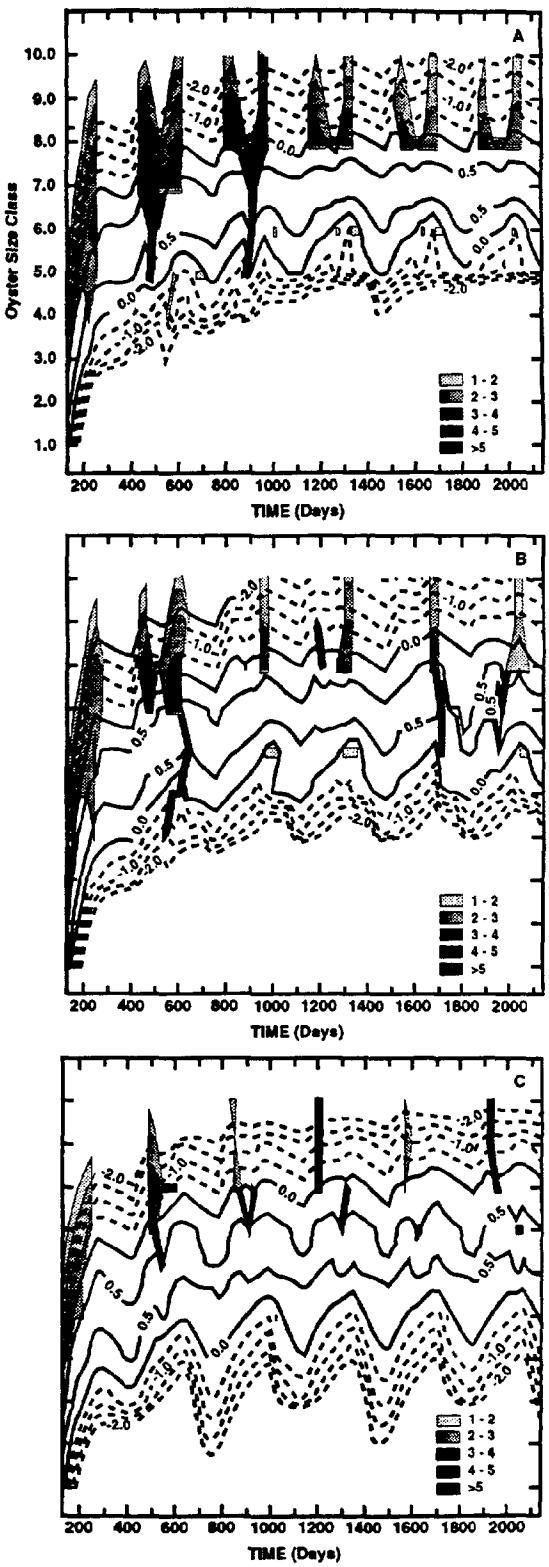

Figure 13. Simulated oyster population distribution and spawn production for A) Laguna Madre, B) Galveston Bay and C) Chesapeake Bay obtained using an idealized food time series. Spawning intensity is shown as $\log_{10}$ calories spawned with a contour interval of 1. Otherwise same as Figure 4.

TABLE 4.

Reproductive effort, average adult size and the ratio of the two calculated from year six of the simulated populations shown in the indicated figures. One simulation used is not shown (NS). This simulation used monthly-averaged temperature and food conditions from Galveston Bay, Texas, a constant salinity of 24 ppt and no turbidity. The results of this simulation were similar to those shown in Figure 6a. Size and reproductive effort are based on simulations that used the environmental time series defined in Table 3. Lower food supply, higher turbidity, or the inclusion of disease (e.g. *Perkinsus marinus*) could be expected to reduce these values.

Location	Reproductive Effort (kcal)	Average Size (g dry wt)	Ratio (kcal:g dry wt^{-1})	Figure Number
Laguna Madre vs.	266.71	4.87	54.77	11a
Galveston Bay	260.92	5.12	50.96	NS
Laguna Madre vs.	218.79	4.62	47.36	13a
Galveston Bay	179.03	4.89	36.61	13b
Galveston Bay vs.	129.77	4.73	27.44	13a
Chesapeake Bay	47.47	4.24	11.19	13c
Galveston Bay vs.	156.49	5.18	30.21	6a
Galveston Bay	24.21	1.81	13.36	6b

However, most of the net production is allocated to reproductive rather that somatic tissue development.

DISCUSSION AND SUMMARY

General Characteristics

Adult size and reproductive effort in oyster populations are determined by the temperature- and season-dependent allocation of net production to somatic and reproductive tissue development which in turn depends upon the temperature regulation of filtration rate. Salinity and turbidity affect oyster physiology through a reduction in the rate of food acquisition and cannot be distinguished from a simple reduction in food supply. Although respiration rate varies non-linearly with body mass and is affected by salinity, the overall effect of environmental conditions on respiration rate is small and can be ignored, in most situations.

A summary of simulated adult oyster size that results from variations in local and latitudinal controls on growth is given in Figure 14. These simulations considered only environmental control on oyster biomass. Oyster growth form is extremely plastic, although Kent (1988) argues for some predictable influences of local habitat. Nevertheless, the shell length achieved in the various simulated populations may vary over a wide range (Table 2). Unfortunately, much of the available oyster measurements are in terms of shell length or condition index rather than biomass. In this discussion, except where noted, oyster size is considered strictly in terms of biomass, and where needed, conversions to length are done as shown in Table 2.

The simulations indicate that adult oysters in Chesapeake Bay tend to be about the same size in terms of biomass as those in Galveston Bay (Fig. 14a), when presented with equivalent food supplies, salinities and levels of turbidity, despite the difference in temperature regimes. Water temperatures in Chesapeake Bay tend to be colder for longer periods than in Galveston Bay. Thus, the temperature-dependent control on the allocation of net production results in more going to somatic rather than reproductive tissue development.

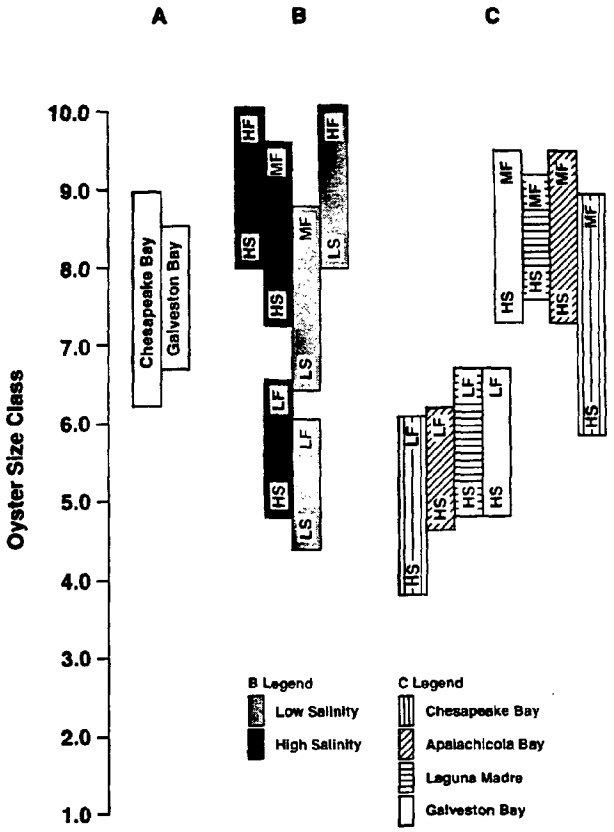

Figure 14. Comparison of adult size from year six of the simulations from A) Galveston and Chesapeake Bays (Figs. 6a and 9a), B) Galveston Bay for high and low salinity at a range of food concentrations (Figs. 4a, 5 and 7) and C) four bays and a range of food concentrations. High and low salinity values are 24 ppt and 7 ppt and are designated by HS and LS, respectively. Designations for high (1.5 mg l^{-1}), medium (1.0 mg l^{-1}), and low (0.5 mg l^{-1}) food concentrations are HF, MF and LF, respectively.

Variations in local environmental conditions also affect adult oyster biomass. Low salinity conditions in an environment such as Galveston Bay can result in reduced adult size (Fig. 14b). However, the effect of low salinity can be compensated for by increases in food supply. Low salinity conditions combined with high food conditions can result in adult biomass that is similar to that obtained during high salinity conditions. The largest reduction in adult oyster size occurs when low salinity is combined with a restricted food supply.

The importance of food in determining adult biomass over a latitudinal range is illustrated in Figure 14c. For all bays, low food conditions produced adult oysters that were about the same size, size classes 5 to 6. The only exception is Chesapeake Bay where somewhat smaller, size class 4, adult oysters are produced by low food conditions. Medium food conditions result in larger adult oysters for all bays with minimal overlap with the size produced by low food conditions. Galveston and Apalachicola Bays have similar sized adult oyster populations. Individuals in Laguna Madre tend to be a bit smaller. The warmer temperatures in Laguna Madre result in more of net production going to form reproductive tissue, thereby producing more spawn and smaller individuals. Chesapeake Bay populations show a wider range of adult size, but

many individuals reach adult size typical of the lower latitude sites despite the cooler temperatures and more restricted growing season (e.g. Butler 1953b).

Adult Size (Biomass)

The shape of the growth curve for bivalves—whether size continuously increases at some declining rate or asymptotes to some maximum size (e.g. Levinton and Bambach 1970)—is probably more a function of environment than genetics. It is significant that the simulated oyster populations reached sizes characteristic of populations throughout the latitudinal range from Laguna Madre to Chesapeake Bay solely on the basis of physiology and environment. No upper limit for oyster growth or adult size was included in any of the formulations used to describe oyster physiology. Limitations on size in the simulated populations come from the balance between winter and summer somatic production less the energy expended in reproduction:

$$P_{gj_{summer}} - P_{gj_{winter}} = A_j - P_{rj}. \quad (15)$$

In adult oysters, net production is normally negative in the winter and for the most part is balanced by somatic growth in the spring and fall. Cessation or slowing of growth in the summer (e.g. Beaven 1950) in disease-free oyster populations is normally due to reproduction and spawning which accounts for most of the net production in older animals. Hence, the relationship given above should result in a stable, but seasonally-oscillating, variation in adult oyster size. In the simulated population distributions, the balance between winter loss in net production and spring-summer-fall gain begins in the second or third year depending on the ambient temperature and food supply. Exceptions to this occur only when food supply is very high.

Growth rate in the hard clam, *Mercenaria mercenaria*, has a concave parabolic relationship with temperature (Ansell 1968). Growth rates are lowest at low and high seasonal temperatures and maximum at intermediate temperatures. Multiplying equations 4 and 12, and assuming a food supply adequate to minimize the effect of respiration on the energy budget and ignoring the dependence of filtration rate on length, yields a parabolic dependence for oyster growth rate on temperature of the same form

$$G \alpha bT - aT^2 \quad (16)$$

where a and b are the constants in equation 12 and T is temperature. If equation (16) is applied over the latitudinal range from Laguna Madre to Chesapeake Bay, then oyster growth rate and hence size should decrease at the southern and northern ends of the distribution. Maximum growth rate and largest adult size would be found near the center of this range. However, both the oyster and the hard clam (Ansell 1968) deviate from this expected distribution in that adult size remains constant over a wide latitudinal range that includes habitats from the northern Gulf of Mexico to north of Delaware Bay.

The observed rather than expected [as suggested by equation (16)] latitudinal distribution in size is also reproduced in the simulated oyster population distributions. This relationship between size and latitude arises through temperature effects on the allocation of net production to somatic and reproductive tissue growth and on filtration rate which determines the rate of food acquisition. The longer periods of low temperature in the spring and fall found at higher latitudes result in more time in which food is plentiful occurring at temperatures that favor somatic growth. As a result, decreased filtration rates at lower temperatures are balanced by an increase in food apportioned to somatic growth and size remains stable. Reproductive potential, however, declines in these populations.

Reduced size at lower latitudes is common in bivalves (e.g. Bauer 1992). Such a gradient in animal size can result from variations in temperature in one of two ways. First, an environment characterized by low food supplies and warm temperatures can produce large adult oysters despite increased reproduction because the total gain in energy from higher winter filtration rates results in a net accumulation of somatic tissue. The decline in size at low latitudes in the Gulf of Mexico suggests that this is not the normal condition. Alternatively, an environment characterized by moderate-to-high food supply and warm temperatures can produce smaller adult oysters because the greater allocation of net production to reproduction balances the positive effect of temperature on the rate of food acquisition. This is the more usual case.

Stunting, the presence of a relatively small adult size in a population, is generally considered to result from restricted food supply. The results of this modeling study suggest that, at least for oysters, temperature and reproductive effort are also important in restricting animal size. Hence, stunted populations can occur at the edge of the species' range where physiology directly limits size as well as in populations that fail to reach the size expected for their position within the latitudinal range.

The observed oyster sizes from around the Gulf of Mexico (Fig. 1) show two exceptions to the general trend of decreasing size at lower latitudes. It should be noted that the data presented in Figure 1 are in terms of length, rather than biomass, and so are subject to the aforementioned caveats concerning the plasticity of oyster growth form. First, the adult length observed at lower latitudes on both sides of the Gulf of Mexico is about 1 to 2 cm less than the average length observed in the northern Gulf. Such a length decrease is not easily produced in the simulated populations with a simple reduction in temperature and one biomass-length relationship. A 0.5 to 1 cm reduction in length is typical of the simulated populations. A temperature-dependent change in growth form modifying the size-to-biomass relationship may also be involved. Second, oysters from Moblie Bay through the Florida Panhandle area and in Tiger Pass on the Mississippi Delta are unusually small. This region characteristically has the coldest winter temperatures in the Gulf of Mexico (Collier 1954). However, the possibility that the colder temperatures reduce the growing season and thus limit adult size is not supported by the simulated populations. Even colder temperatures in Chesapeake Bay fail to reduce adult biomass. Either food supply is unusually meager in these two areas or mortality rates are unusually high. Thus, stunting may be of local (Tiger Pass) or regional (Florida Panhandle) extent. The effect of a change in growth form can be discounted in this case because the length-biomass relationship given in White et al. (1988) is adequate for at least some of these populations.

Butler (1953b) showed that oysters in Chesapeake Bay and the northern Gulf of Mexico reached about the same size in terms of length. The simulations summarized in Figure 14 generally show that Gulf of Mexico oysters slightly exceed Chesapeake Bay oysters in length when biomass is converted using a single length-biomass relationship. A latitudinal difference in growth form would explain this differential. Kent (1988) describes a wide range in growth forms from Chesapeake Bay, so that within-bay variations cannot be discounted. However, the relationship given in Paynter and DiMichele (1990) for a Chesapeake Bay population from Tolley Point Bar predicts oysters much longer for a given

weight and this prediction agrees with a biomass-length relationship obtained by Newell (University of Maryland, pers. comm.) from the Choptank River subestuary of the Chesapeake Bay. Lunz (1938) suggested that a primary influence of anthropogenic activities on oyster growth form was to decrease width and length, but with more of an effect on width. If true, this would explain a perceived variation between oyster size reported by Butler (1953b) and the more recent measurements reported by Paynter and DiMichele (1990) and Newell (University of Maryland, pers. comm.). Unfortunately, the observations reported in Butler (1953b) are not in terms of biomass. The same trend might explain the tendency in the simulated oyster populations from Chesapeake Bay to be slightly lower in weight and, therefore, length, than the northern Gulf of Mexico oysters (e.g. Fig. 11). The weight obtained from the simulated populations would result in a longer oyster in Chesapeake Bay using the conversions of Paynter and DiMichele (1990) and Newell (University of Maryland, pers. comm.).

The simulated oyster populations suggest an explanation for the concordance in year-to-year oscillations in oyster size throughout the Gulf of Mexico (Wilson et al. 1992). Climatic cycles, such as El Niño, change the Gulf-wide temperature and rainfall regime (Powell et al. 1992a). Size, through the direct effect of temperature on the allocation of net production to somatic and reproductive tissue or indirectly through variations in food supply, could be affected by climatic variations in temperature and rainfall. Furthermore, such climatic effects are likely introduced through variations in temperature during the colder part of the year. For example, the difference between a warm and cold winter could be sufficient to significantly alter adult size.

Reproduction

The reproductive processes included in the oyster population model are based upon simple empirical relationships; however, the simulated population distributions show trends typical of oyster populations throughout the east coast of the U.S. and the Gulf of Mexico. This suggests that reproductive effort in oysters is primarily a function of a genetically-determined temperature-dependent allocation of net production into somatic and reproductive tissue development and an environmentally determined scope for growth. This temperature dependency may be described by simple linear relationships such as those given by equations (12) and (13) which may reflect temperature-dependent reaction rates in protein synthesis or hormonal control. The mechanism underlying the temperature-dependent allocation of net production would appear to be an important unknown in the reproductive physiology of oysters.

Reproductive potential is the result of the same physiological and environmental conditions that govern adult size, i.e. the temperature- and season-dependent rate of food acquisition and the temperature-dependent allocation of net production into somatic growth and reproduction. However, small changes in either result in more pronounced changes in reproductive effort than in adult size. For example, the rate of food acquisition is higher in warmer months when most net production is allocated to reproduction. Hence, small changes in available food are magnified during this period. The effect of small variations in environmental conditions on oyster reproduction and spawning is discussed in detail by Hofmann et al. (1992).

The wide range of reproductive efforts produced from small changes in temperature or food supply suggests that comparisons of reproductive effort between oyster populations can only be made within the context of a complete environmental analysis of food supply, environmental conditions and a total energy budget for the animal. The wide range of reproductive efforts reported for bivalves in general (see Powell and Stanton 1985 for a review) probably results from these interactions. Thus, correlations between size and reproductive effort will be location and time specific, and general conclusions based upon such correlations may not be valid. For example, the relationship between temperature and reproduction given by Kaufman (1979) requires similar rates of food acquisition among populations to provide valid comparisons.

The assumption that populations of larger individuals should reproduce more is not always correct. For many situations, populations of smaller individuals may have a greater reproductive effort per unit of biomass. The simulated population distributions suggest that decreases in reproductive effort are related to increased size rather than to age. The apparent reproductive senility in these populations results from the differential scaling of filtration and respiration rate with body size, which reduces scope for growth at a given food supply in larger animals.

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Reprint 5

Correlation Between Bioassay-Derived P4501A1 Inductive Activity and Chemical Analysis of Clam (Laternula elliptica) Extracts from McMurdo Sound, Antarctica

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CORRELATION BETWEEN BIOASSAY-DERIVED P4501A1 INDUCTION ACTIVITY AND CHEMICAL ANALYSIS OF CLAM (*Laternula elliptica*) EXTRACTS FROM McMURDO SOUND, ANTARCTICA

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ABSTRACT

Variable levels of halogenated aromatic hydrocarbons were measured in clams (*Laternula elliptica*) collected from McMurdo Sound, Antarctica. Clams collected in and near Winter Quarters Bay contained high levels of organochlorine compounds, particularly polychlorinated biphenyls (PCBs). A strong gradient has been documented in Winter Quarters Bay that been linked to human activities at McMurdo Station. The activity of clam extracts as inducers of P4501A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity was determined using *in vitro* bioassays utilizing rat hepatoma H4IIE cells. The extracts which exhibited the highest induction activities were those derived from clams collected in contaminated areas. Additionally, there was an excellent linear correlation between induced EROD activity versus total PCB levels (r^2=0.96). The complimentary nature of both the analytical and bioanalytical data confirms the utility of the latter assay and provides a method for estimating the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxic equivalents in extracts from marine biota.

INTRODUCTION

Halogenated aromatic hydrocarbons (HAHs) are industrial compounds or combustion by-products which have been widely identified as environmental contaminants in almost every component of the global ecosystem (Tanabe, 1988; McFarland and Clarke, 1989; Safe, 1990, 1991; Rappe, 1993; Rappe *et al.*, 1993). The HAHs include the polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). These chemicals exhibit a number of common properties including their structural similarities, chemical stability, lipophilicity and toxicological effects. The problems associated with the environmental persistence and transport of HAHs and their preferential bioconcentration in the food chain is primarily due to their resistance to degradation and highly lipophilic properties.

PCBs, PCDDs and PCDFs have been identified as complex mixtures in diverse environmental samples and high resolution analytical procedures can give quantitative congener specific analysis of HAH mixtures (Mullin *et al.*, 1984; Tanabe, 1988; McFarland and Clarke, 1989; Schulz *et al.*, 1989; Duarte-Davidson *et al.*, 1991; Rappe, 1993; Rappe *et al.*, 1993). Risk assessment and risk management of these complex mixtures can be carried out using a toxic equivalency factor (TEF) approach in which all the toxic HAHs have been assigned a fractional potency relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (NATO/CCMS, 1988; Ahlborg, 1989; Safe, 1990; Ahlborg *et al.*, 1992). The TCDD or toxic equivalents (TEQ) of a mixture can be readily calculated from quantitative congener-specific analytical data (Safe, 1990).

In vitro bioassays have also been developed to determine the TEQ values of extracts from various environmental and industrial samples which exhibit "TCDD-like" activity (Bradlaw and Casterline, 1979; Trotter *et al.*, 1982; Casterline *et al.*, 1983; Zacharewski *et al.*, 1989; Ankley *et al.*, 1991, 1992, 1993; Tillitt *et al.*, 1991a, 1991b, 1992, 1993; Jones *et al.*, 1993). Since these compounds elicit similar toxic and biochemical responses via the aryl hydrocarbon (Ah) receptor signal transduction pathway (Safe, 1990), various Ah receptor-mediated responses including P4501A1 induction, antiestrogenicity and keratinization have been utilized to determine bioassay-derived TEQ values for any mixture (Bradlaw and Casterline, 1979; Trotter *et al.*, 1982; Casterline *et al.*, 1983; Gierthy *et al.*, 1984, 1993; Zacharewski *et al.*, 1989; Tillitt *et al.*, 1991a, 1991b, 1992, 1993; Ankley *et al.*, 1991, 1992, 1993; Krishnan *et al.*, 1992; Jones *et al.*, 1993; Krishnan and Safe, 1993). This approach is useful for biomonitoring extracts since it obviates the need for relatively expensive chemical analysis, detects all bioactive components in a mixture and their possible interactions with coextracted non-TCDD-like compounds. This approach is particularly useful for invertebrates because, although the presence of P4501A-monooxygenase enzymes has been confirmed in a number of invertebrates (Lee, 1982; James, 1989; Livingstone, 1991), there is no conclusive evidence showing they are inducible after exposure to aromatic hydrocarbons. Additionally, recent work by Hahn and coworkers (1992) did not detect the presence of the Ah receptor in nine invertebrate species. This suggests that invertebrates lack a functional Ah receptor, which is consistent with the failure to observe induced P4501A-dependent activity. McMurdo Sound, Antarctica, was selected for study because high concentrations of PCBs and

polynuclear aromatic hydrocarbons (PAHs) have been measured in sediments collected in Winters Quarter Bay and surrounding areas (Lenihan *et al.*, 1990; Risebrough *et al.*, 1990; Lenihan, 1992). This paper reports the results of a P4501A1-induction bioassay using rat hepatoma H4IIIE cells exposed to extracts from clams (*Laternula elliptica*) obtained from both highly contaminated and control sites in McMurdo Sound. Additionally, the bioassay results are compared to the results of chemical analyses of the same samples for organochlorine and aromatic hydrocarbon pollutants.

MATERIALS AND METHODS

Sampling. Clam samples, in pools of 9 to 15 individuals, were collected by divers from impacted and nonimpacted areas in McMurdo Sound, Antarctica. Contaminated clams were collected from two contaminated locations in the vicinity of the U.S. McMurdo Station, in Winter Quarters Bay and at the sewage outfall (WQB, Fig. 1). Clams collected from three sites located in remote areas of the McMurdo Sound were used as controls (Fig. 2).

Extraction and Cleanup. Approximately 5 to 15 g of wet tissue were used for the analysis of PAHs, PCBs and chlorinated pesticides. Fifty grams of anhydrous Na_2SO_4 and the appropriate amount of surrogates were added to each sample before extraction. The aromatic surrogate contained d_4-1,4-dichlorobenzene, d_8-naphthalene, d_{10}-acenaphthene, d_{10}-phenanthrene, d_{12}-chrysene, and d_{12}-perylene. The surrogate for PCBs and chlorinated pesticides contained 4,4'-dibromooctafluorobiphenyl, PCB 103 and PCB 198. The tissue samples were then extracted with methylene chloride (3 times × 100 ml) using a "Tissumizer" homogenizer. The combined extracts were concentrated to 10-15 ml in a flat-bottom flask equipped with a three ball Snyder condenser and transferred to Kuderna-Danish tubes. The tubes were heated in a water bath at 60°C to concentrate the extracts to a final volume of 1-2 ml in hexane.

The tissue extracts were initially cleansed by alumina (20 g, 5% deactivated with H_2O):silica (10 g, 1% deactivated with H_2O) column chromatography. The columns were eluted with 200 ml of 1:1 methylene chloride:pentane and the aluate was concentrated as described above. This fraction was further purified by high performance liquid chromatography to remove excess of lipid materials. The extracts were concentrated to a final volume of 0.5-a ml, hexane, for GC/MS and GC-ECD analyses. Extracts used in the bioassy were obtained as described above except that the surrogates were not added and the extracts were concentrated and dissolved in DMSO.

Instrumental Analysis. PAHs were analyzed by electron impact (70 eV) GC/MS in the selected ion mode (*i.e.* molecular ions) as previously described (Wade *et al.*, 1988). The GC/MS was calibrated and linearity was determined by injection of standards at five concentrations. Peak identity was confirmed by molecular ion, the ratio of the primary (base) ion to the secondary ion, and retention time. Instrument calibration was checked daily by reinjection of the original calibration mixture. The calibration check was maintained to within ± 10% on average for

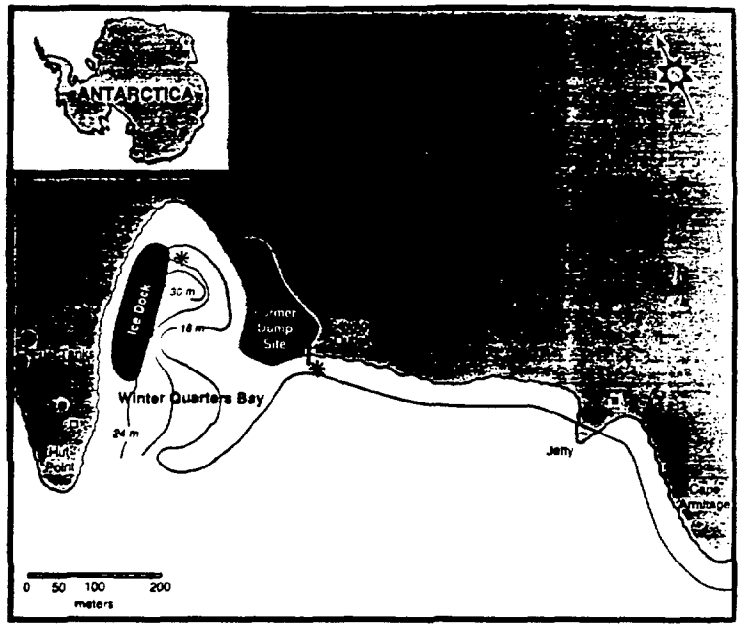

Figure 1. Location of Winter Quarters Bay and the sewage outfall in McMurdo Sound, Antarctica.

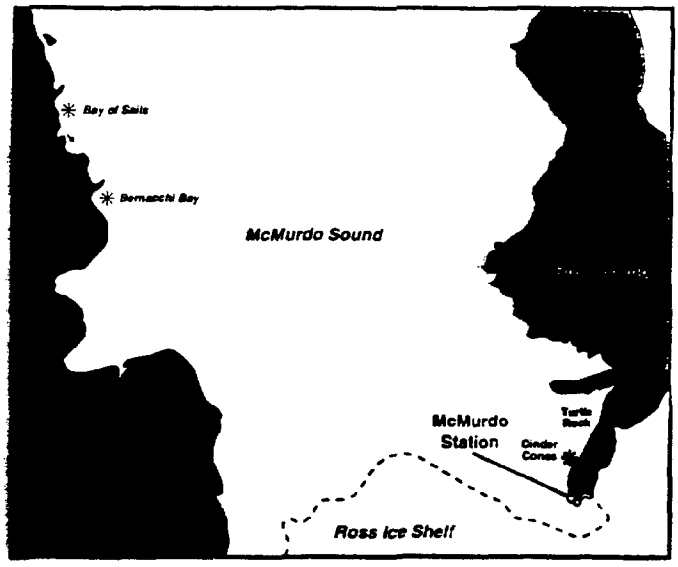

Figure 2. Locations of control sites in McMurdo Sound, Antarctica.

all analytes of interest. Quality assurance for each set of sample included a system blank and a matrix spike which were carried through the entire analytical scheme in a manner identical to the clam samples.

PCBs and chlorinated pesticides were analyzed by fused-silica capillary column GC-ECD (Ni^{63}) in splitless mode. Capillary columns, 30 meters long × 0.25 mm i.d. with 0.25 µm DB-5 film thickness, were temperature-programmed from 100 to 140°C at 5°C/min, from 140 to 250°C at 1.5°C/min, and from 250 to 300°C at 10°C/min 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 the carrier gas. Nitrogen or argon/methane (95:5) were used as make-up gases. The volume injected was 2 µl. The instruments were calibrated using authentic standards ar four different concentrations to compensate for the non-linear response of the electron capture detector. Tetrachloro-m-xylene (TCMX) was used as the GC internal standard to calculate the recoveries of the surrogates.

In Vitro Bioassay. H4IIE cells were grown as continuous cell lines in □-essential medium supplemented with 2.2 mg/ml tissue culture grade sodium bicarbonate, 5% fetal calf serum, and 10 ml/l antibiotic-antimycotic solution (Sigma). Stock cultures were grown in 150-cm^2 tissue culture flasks and incubated in a humidified mixture of 5% CO_2 and 95% air under atmospheric pressure. For enzyme assays, approximately 1×10^6 cells in 2 ml media/well were passaged to 6-well plates. Solutions of the clam extracts dissolved in dimethyl sulfoxide (DMSO) were added to the culture plates so that the final concentration of DMSO in the medium was < 0.25%. Cells were also treated with DMSO (solvent control) and different concentrations of TCDD to determine maximal induction activity. Cells were harvested by manual scraping from the culture plates, centrifuged at 1000 *g* for 6 min at 4°C, and resuspended in 100 µl of Tris-sucrose buffer (38 mM Tris-HCl, 0.2 M sucrose, pH 8.0). Aliquots (50 µl) of the cell suspension were incubated with 1.15 ml cofactor solution (containing 1 mg bovine serum albumin, 0.1 mg NADH, 0.1 mg NADPH, and 1.5 mg $MgSO_4$ in 0.1 M HEPES buffer, pH 7.5) in a 37°C water bath for 2 min. The reaction was started by adding 50 µl ethoxyresorufin solution (1 mg/40 ml) for a 6-min incubation and stopped by adding 2.5 ml methanol. Samples were centrifuged at 1000 *g* for 10 min. The supernatant was used for fluorescence measurement at an excitation wavelength of 550 nm, and an emission wavelength of 585 nm (Pohl and Fouts, 1980). Samples were run in triplicate and the results are expressed a means ± SD.

RESULTS AND DISCUSSION

Studies have shown that a strong organic contaminant gradient exists within Winter Quarters Bay that has been attributed to human activities associated with McMurdo Station (Risebrough *et al.*, 1990; Lenihan *et al.*, 1990; Lenihan, 1992). Contamination has been linked to a dump site, active until recent years; fuel storage tanks; shipping and construction activities; and station runoff. Additionally, the only sewer outfall for McMurdo Station is located at the mouth of Winter Quarters Bay where raw sewage is discharged. High concentrations of aromatic hydrocarbons and organochlorines were measured in sediments near the back of Winter Quarters Bay and decreased

with distance towards the mouth of the bay and with distance from the bay. Within Winter Quarters Bay, total purgeable hydrocarbons ranged from non-detectable to 4500 µg/g and total estimated PCBs ranged from 110 to 1400 ng/g (Risebrough *et al.*, 1990; Lenihan *et al.*, 1990). In contrast, at control locations, no purgeable hydrocarbons were detected and estimated total PCB levels varied from ≤ 0.01 to 0.8 ng/g. The concentrations of aromatic hydrocarbons and PCBs measured in Winter Quarters Bay sediments are considered high with respect to values reported for contaminated temperate locations and significant changes in the benthic community have been correlated with the contaminant gradient (Lenihan *et al.*, 1990; Lenihan, 1992).

The results in Table 1 summarize the quantitative analyses of organochlorine pesticides, total PCBs and PAHs in clam extracts from McMurdo Sound, Antarctica. The range of total hexachlorocyclohexanes (HCHs), chlordanes and DDT and related compounds varied from non-detectable to 2.83, non-detectable to 2.27, and 1.87 to 9.61 ng/g, respectively. Tissue chlordane and DDT levels were significantly higher in clams collected at sites in and near Winter Quarters Bay than at control locations. The highest levels of PCBs were measured ($\bar{x}$ = 409 ± 21 ng/g) in extracts from clam samples 7, 8, 9 and 10 which were collected from Winter Quarters Bay and the sewage outfall (Fig. 1). The PCB levels were significantly lower in samples collected at control locations in McMurdo Sound (Fig. 2). Total PAH levels in the clam extracts were above detection limits only from locations 2 and 10 and were not significantly different for control and contaminated sites.

The results in Table 2 summarize the induction of EROD activity in rat hepatoma H4IIE cells by aliquots of the clam extracts. Initial induction studies utilized 2 µl aliquots (run #1) for the induction studies and the results showed induced EROD activity in samples 6 through 9. In run #2, 5 µl aliquots were used and higher induced enzyme activities were observed in samples 6 through 9 whereas in samples 1 through 5 and 10, only low induction was detected. Dose-response induction by the extracts was not possible due to limited availability of the extracts. Sample 8A was a duplicate of 8 and there were no significant differences between the induced EROD activities in these samples (for run #2), thus confirming the reproducibility of the induction bioassay (Tillitt *et al.*, 1991b). TCDD-induced EROD activity was used as a positive control and 1 nM TCDD (0.644 ng/plate) was utilized as a 100% maximal induced response. Since the dose-response curve for induction of EROD activity by TCDD was nearly linear from 0 to 1 nM, the TCDD or toxic equivalents (TEQ) could be determine for the various extracts (see Table 2).

Previous studies have demonstrated that both ≥ 4-ring PAHs and several PCB congeners induce EROD activity in rat hepatoma H4IIE cells (Bradlaw and Casterline, 1979; Trotter *et al.*, 1982; Tillitt *et al.*, 1991b; Sawyer and Safe, 1982; Sawyer *et al.*, 1984; Piskorska-Pliszczynska *et al.*, 1986; Kamps and Safe, 1987); however, congener-specific chromatographic analysis of the "TCDD-like" coplanar and monoortho coplanar PCBs was not obtained in this study and TEQs could not be calculated. However, there was a linear correlation between total PCB levels and induction-derived TEQs (Fig. 3, $r^2 = 0.95$). The other organochlorine compounds present in high concentration in the extracts (Table 1) are not inducers of EROD activity. Thus, the high linear correlation between

Table 1. Organochlorine and PAH concentrations (ng/g dry weight) in *Laternula elliptica* extracts from McMurdo Sound, Antarctica.[a]

	Cinder Cones			Bernacchi Bay		Winter Quarters Bay				Bay of Sails
Sample No.	1	2	3	4	5	6	7	8	9	10
□-HCH	ND	ND	ND	ND	2.09 M	ND	2.19	1.96	2.25	ND
HCB	0.40	0.27	0.41	0.34	0.31	0.43	0.34	4.75	8.43	0.32
□-HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
□-HCH	ND	ND	ND	0.38	0.37	0.61	0.64	0.43	0.41	0.42
□-HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND	0.10	ND	ND	ND	ND	0.03
Hepta-epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Oxychlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
□-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
□-Chlordane	ND	0.36	0.25	ND	ND	ND	ND	0.39	ND	ND
trans-Nonachlor	ND	0.24	0.20	0.19	0.31	ND	ND	ND	0.14	0.12
cis-Nonachlor	ND	ND	ND	0.24	ND	1.53	1.61	1.88	1.75	ND
Aldrin	2.74	2.15	2.66	1.83	1.91	ND	1.66	1.64	1.88	ND
Dieldrin	0.77	0.71	0.79	0.66	ND	ND	ND	ND	ND	0.88 ND
Endrin	ND	1.03	1.07	ND	0.41	ND	ND	0.32	ND	ND
Mirex	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4'-DDE (O,P'-DDE)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4'-DDE (P,P'-DDE)	0.57	1.11	0.94	0.61	1.62	1.63	1.72	1.69	1.67	0.17
2,2'-DDD (O,P-DDD)	ND	ND	0.18	ND	ND	0.40	0.34	0.36	0.24	ND
4,4'-DDD (P,P'-DDD)	ND	ND	ND	0.80	0.36	1.75	1.89	1.83	1.78	ND
2,4'-DDT (O,P'-DDT)	0.65	0.42	0.85	1.58	2.11	3.19	3.03	3.65	3.00	1.09
4,4'-DDT (P,P'-DDT)	0.67	0.86	0.92	1.11	1.11	1.81	1.90	2.07	1.94	0.60
Total HCHs	ND	ND	ND	0.38	2.46	0.01	2.83	2.39	2.66	0.42
Total chlordanes	ND	0.59	0.45	0.43	0.41	1.53	1.61	2.27	1.88	0.15
Total DDT's	1.89	2.38	2.88	4.10	5.22	8.77	8.88	9.61	8.64	1.87
Total PCBs	22.2	19.2	17.5	19.0	19.1	383	414	433	404	5.1
Total PAHs	74.6 J	155.9	69.2 J	32.6 J	56.2 J	145.3 J	148.3 J	158.5 J	177.0	67.3 J
Total PAHs (≥ 4-rings)	16.7 J	8.9 J	12.0 J	5.0 J	6.6 J	1.63	21.2 J	31.7 J	77.3 J	7.1 J

[a] The analysis of clam extracts for organochlorine compounds and PAHs was carried out as described in the Materials and Methods section; ND = non-detectable.

J Below method detection limit. Data reported as ng/g dry weight. Samples 1,2 and 3 were collected at Cinder Cone; samples 4 and 5 were collected in Bernache Bay; samples 6 and 7 were collected near sewage outfall; samples 8 and 9 were collected in Winter Quarters Bay; and sample 10 was collected in Bay of Sails.

Table 2. Induction of EROD activity in rat hepatoma H4IIE cells by *Latermula elliptica* extracts from McMurdo Sound, Antarctica.[a]

		Run #1		Run #2	
Sample Number	Site	EROD Activity (pmol/min/mg)/g[b]	TEQ (ng)/g[f]	EROD Activity (pmol/min/mg)/g[b]	TEQ (ng)/g
1	Cinder Cones	ND[c]	ND	ND	ND
2	Cinder Cones	ND	ND	69.2 ± 39.9	0.06 ± 0.04
3	Cinder Cones	ND	ND	318.57 ± 47.9	0.25 ± 0.04
4	Bernache Bay	ND	ND	266.0 ± 45.4	0.21 ± 0.04
5	Bernache Bay	ND	ND	191.8 ± 44.6	0.16 ± 0.04
6	Sewage outfall	735.7 ± 107.1	0.98 ± 0.15	1840.0 ± 108.6	1.47 ± 0.09
7	Sewage outfall	694.3 ± 77.2	0.93 ± 0.10	1447.6 ± 37.7	1.16 ± 0.03
8	Winter Quarters Bay	1329.1 ± 60.6[d]	1.78 ± 0.08[d]	2078.0 ± 167.7[d]	1.66 ± 0.13[d]
9	Winter Quarters Bay	741.6 ± 213.8	0.99 ± 0.28	1558.5 ± 32.9	1.24 ± 0.03
10	Bay of Sails	ND	ND	201.4 ± 45.8	0.16 ± 0.04
11	Blank	ND[e]	ND	ND	ND
8A	Winter Quarters Bay	960.6 ± 186.2[d]	1.29 ± 0.25[d]	1755.6 ± 347.5[d]	1.40 ± 0.28[d]

[a] The clam extracts were dissolved in 50μl DMSO and either 2 or 5 µl aliquots (run 1 or 2) were used in the induction bioassay as described in the Materials and Methods. The results expressed as means ± SD for separate determinations for each sample.

[b] The results are expressed as the rate of ethoxyresorufin metabolized (pmol/min/mg)/g of dry extract.

[c] ND = non-detectable.

[d] A replicate of sample 8; no significant difference ($p < 0.05$) between the results for the two separate determination in run #2.

[e] A sample blank.

[f] TEQ (ng)/g = 0.644 ng × ($EROD_{sample}/EROD_{TCDD}$) × dilution factor/dry tissue weight (g).

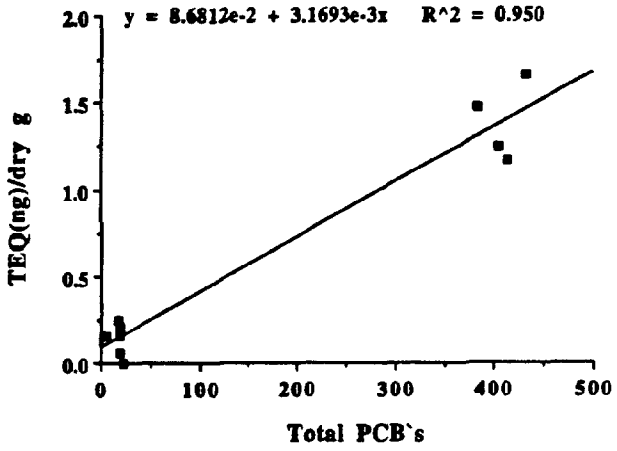

Figure 3. Correlation between induced EROD activity versus total PCB (▣) levels in *Laternula elliptica* extracts. The induction bioassay and results are derived from data in Table 2 and the analytical data are summarized in Table 1.

PCB levels versus induced EROD activity or TEQs (Fig. 3) coupled with the high concentrations of PCBs rela to the ≥ 4-ring PAHs (Table 1) indicate that the PCBs are the major P4501A1 inducers in the clam extracts.

These data demonstrate that the EROD induction bioassay on clam extracts from the Antarctic can be utili to detect uptake of PCBs from contaminated sediments in Winter Quarters Bay as evidenced by the correla between total PCB levels versus TEQs (or induced activity). Ongoing studies in our laboratories are investiga the application of this *in vitro* bioassay with diverse extracts of marine biota and sediments contaminated organochlorine pollutants and PAHs.

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