EPA/620/R-94/001
October 1993

LOUISIANIAN PROVINCE DEMONSTRATION REPORT EMAP - ESTUARIES - 1991

J. Kevin Summers[1],
John M. Macauley[1],
Virginia D. Engle[2],
Gary T. Brooks[2],
P. Thomas Heitmuller[2], and
A. Matt Adams[3]

U.S. Environmental Protection Agency
Environmental Research Laboratory
1 Sabine Island Drive
Gulf Breeze, FL 32561

U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL[1]
Technical Resources, Inc., Gulf Breeze, FL[2]
Computer Sciences Corporation, Gulf Breeze, FL[3]

DISCLAIMER

This report represents data from a single year of field operations of the Environmental Monitoring and Assessment Program (EMAP). Because the probability-based scientific design used by the EMAP necessitates multiple years of sampling, there may be significant levels of uncertainty associated with some of these data. This uncertainty will decrease as the full power of the approach is realized by the collection of data over several years. Similarly, temporal changes and trends cannot be reported, as these require multiple years of observation. Please note that this report contains data from research studies in only one biogeographic region (Louisianian Province) collected in a short index period (July-August) during a single year (1991). Appropriate precautions should be exercised when using this information for policy, regulatory or legislative purposes.

Mention of trade names or commercial products does not constitute endorsement or recommendation for us.

PREFACE

This document is a final draft of the report on the demonstration project completed by EMAP-E (Environmental Monitoring and Assessment Program - Estuaries) in the Louisianian Province in 1991. It is being distributed at this time for discussion and modification to finalize the summary of the non-base monitoring activities in 1991.

The appropriate citation for this report is:

Summers, J.K., J.M. Macauley, V.D. Engle, G.T. Brooks, P.T. Heitmuller, A.M. Adams, 1993. Louisianian Province Demonstration Report : EMAP - Estuaries - 1991. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Gulf Breeze, FL. EPA/600/R-93/xxx.

LOUISIANIAN PROVINCE
DEMONSTRATION REPORT
EMAP - ESTUARIES - 1991

Table of Contents

Table of Contents *(continued)*

EXECUTIVE SUMMARY

The Environmental Monitoring and Assessment Program (EMAP) is a comprehensive environmental monitoring network designed to:

- Estimate the current status and trends in the condition of the nation's ecological resources on a regional basis, with known statistical confidence;
- Seek associations among anthropogenic stress and ecological conditions; and
- Provide periodic statistical summaries and interpretive reports on ecological status and trends to resource managers and the public.

The first stage in implementing EMAP involves conducting demonstration projects for each Resource Group (ecosystem type; e.g., forests, estuaries, agroecosystems). Demonstration projects provide an opportunity to illustrate the kinds of assessments that can be accomplished using EMAP data (Summers et al. 1993) and to evaluate program design and indicator selection (this report). The 1991 Louisianian Demonstration represents the second demonstration project conducted by the EMAP-Estuaries Resource Group.

This report provides an evaluation of:

- Development of aggregate indicators like a benthic index of estuarine integrity;
- Testing of the sensitivity of all indicators by a direct comparison of their values at known locations of good and poor environmental quality;
- Efficacy of new, relatively untested indicators as representative of environmental condition (e.g., bile florescence, skeletal abnormalities, splenic macrophage aggregates);
- Appropriateness of the spatial scale used in sampling (i.e., dimensions of the sampling grid used in large estuarine sampling);
- Strengths of random and index sampling sites for small estuaries;
- Preliminary associations between response indicators and exposure indicators as well as among exposure indicators; and,
- Need for sample replication for benthic indicators in the Louisianian Province.

The 1991 Louisianian Province Demonstration involved sampling visits to 202 sites from the Rio Grande, TX to Anclote Anchorage, FL, as part of an overall probability-based sampling design to assess the status of the ecological condition of Louisianian Province estuaries.

A series of core and developmental indicators of overall, or portions of, estuarine condition was collected at each site. Additional research indicators were collected at selected sites in order to test their abilities to discriminate between known good and poor condition. All indicators represented either biotic integrity or some parameter of a healthy, diverse, sustainable biological community; pollutant exposure or some chemical aspect of the environment; habitat characterization; or, aesthetics relating to human use of estuarine resources.

This evaluation of indicators and design for the Louisianian Province is based on a single year of information; thus, it is subject to potential year-specific phenomena such as climate fluctuations (1991 was a high precipitation year), contaminant spills (a major oil spill occurred in Galveston Bay in late 1990), or year-class strengths (no deviations known). This assessment is preliminary and its findings should be confirmed by subsequent years of sampling in the Louisianian Province.

A companion report delineating a statistical summary of the 1991 results has been produced (Summers et al. 1993[1]) and should be used if the reader is interested in the ecological status of the estuaries of the Louisianian Province. The following conclusions have been drawn from the monitoring data collected from the Louisianian Province in 1991 with regard to indicators and design:

Response Indicator Development

- An index of benthic community structure has been developed that effectively discriminated between sites of known hypoxia and sediment contamination and reference sites. The strength and validity of this index will be assessed using the 1992 monitoring data.

Sensitivity of Indicators

- Benthic index values, benthic species diversity and number of species were sensitive indicators of ecological condition relating to hypoxia and sediment contamination.

- Number of finfish species and abundance/trawl were sensitive indicators of ecological condition in estuaries.

- Human use indicators and tissue contaminants in fish tissue were not indicative of ecological condition in estuaries where condition was defined as extent of hypoxia and sediment contamination.

- Total alkanes, pesticides and heavy metals were significantly associated with observed hypoxia and sediment contamination.

- Only a few PAHs and PCBs were associated with observed hypoxia and sediment contamination.

- Significant longitudinal gradients (East to West) in the Louisianian Province existed for the number of fish species/trawl, minimum dissolved oxygen concentration, Secchi depth, acid volatile sulfides, total organic carbon in sediments, total and

numerous specific alkanes, and several heavy metals.

Research Indicators

- Number of observed external fish pathologies/trawls were 2 to 3 times higher in regions of hypoxia and industrial contamination and a significant longitudinal gradient existed with western province fish having five times the pathologies observed in eastern province fish.

- The percent area occupied by splenic macrophage aggregates was 4-9 times greater in regions of hypoxia and sediment contamination than in reference areas for pinfish and Atlantic croaker.

- The rate of vertebral deformities was an order of magnitude higher in western province estuaries than in eastern ones with rates that were 3 to 7 times higher in areas of hypoxia and high industrial discharges.

- Selected blood chemistry compounds, including c-reactive proteins, were significantly higher in brown bullheads from heavily contaminated areas than in catfish from reference areas.

- Stable isotope and nutrient analysis in hypoxic areas indicated the high potential for eutrophic conditions resulting from algal production and decay.

Associations

- The benthic index was strongly associated with sediment contaminant levels and somewhat associated with dissolved oxygen concentrations, sediment toxicity, and habitat variation in Redox potential discontinuity depth and salinity.

Statistical Design

- Most response and exposure indicators showed no differences in distribution functions at the estuaries class level between index and randomly-placed sites.

- Significant spatial autocorrelation exists among many of the response and exposure indicators used in the 1991 Demonstration.

- No significant differences in the estimates of response and exposure indicators were observed, at the large estuarine class-level, between the base grid density and an enhanced density increasing the sample size by a factor of four; however local or estuary-specific estimates were significantly different.

---

[1] Summers, J.K., J.M. Macauley, P.T. Heitmuller, V.D. Engle, and G.T. Brooks. 1993. Statistical Summary: EMAP-Estuaries Louisianian Province-1991. U.S. Environmental Protection Agency, Office of Research and Development Environmental Research Laboratory, Gulf Breeze, FL. EPA/600/R-93/001.

SECTION 1

INTRODUCTION

The Environmental Monitoring and Assessment Program (EMAP) is a national program initiated by EPA's Office of Research and Development (ORD). EMAP was developed in response to the need for information about the degree to which existing pollution control programs and policies protect the nation's ecological resources. EMAP is an integrated federal program; ORD is coordinating the planning and implementation of EMAP with other federal agencies including the Agricultural Research Service (ARS), the Bureau of Land Management (BLM); the U.S. Fish and Wildlife Service (FWS), the Forest Service (FS), the U.S. Geological Survey (USGS), and the National Oceanic and Atmospheric Administration (NOAA). These other agencies and offices participate in the collection and analysis of EMAP data and will use it to guide their policy decisions, as appropriate.

EMAP-Estuaries (EMAP-E) represents one portion of EMAP's efforts in near coastal environments. These efforts are designed to provide a quantitative assessment of the regional extent of coastal environmental problems by measuring status and change in selected condition indicators. The results of this effort, with regard to the Gulf of Mexico, were documented in the 1991 Louisianian Province Statistical Summary (Summers et al. 1993). In addition to the statistical summary, the 1991 Demonstration was designed to assess the sensitivity of selected ecological indicators, test the efficacy of "new" indicators, and evaluate the appropriateness of several elements of the statistical design. This Demonstration Report represents the results of those evaluations.

1.1 OBJECTIVES OF THE 1991 LOUISIANIAN PROVINCE DEMONSTRATION

The Louisianian Province Demonstration was conducted in the summer of 1991 (July-August) to show the utility of probability-based regional monitoring programs for assessing the condition of estuarine resources. The sampling was conducted from 9 July through 30 August spanning 202 sites whose selection was based on a probabalistic design. The specifics of the planning activities, sampling design and indicator selection for the 1991 Louisianian Province Demonstration are documented in Summers et al.(1991). Specifics related to the conduct of the field sampling in 1991 can be found in Summers et al. (1992), while the summary of ecological status for the Louisianian Province can be found in

Summers et al. (1993).

The objectives of the 1991 Louisianian Province Demonstration were to:

1) demonstrate the value of regional monitoring using a statistically unbiased sampling design as a basis for assessing the condition of estuarine resources;

2) evaluate the ability of a selected suite of ecological and environmental indicators to discriminate among polluted and unpolluted sites over a regional scale;

3) obtain data on Louisianian Province specific variability in ecological parameters;

4) develop and refine analytical procedures for using regional-scale monitoring data to assess the ecological status of estuaries and apply these procedures to establish the baseline conditions in the Louisianian Province;

5) evaluate potential design constraints imposed by use of the unbiased sampling design; and

6) identify and resolve logistical problems associated with sampling estuarine resources in primarily shallow estuaries spanning over 1800 miles of coastline within a 4 to 6 week sampling period.

The field activities report (Summers et al. 1993b) addressed Objective #6 while the Statistical Summary (Summers et al. 1993a) addresses Objectives #1, #3, and #4. This report addresses Objectives #2 and #5.

1.2 PURPOSE AND ORGANIZATION OF THIS REPORT

The purpose of this report is to evaluate the utility of the indicators selected for use in the EMAP-Estuaries program for the Louisianian Province and to assess key elements of the sampling design with regard to scale and sample site location. In addition, the development of integrated indicators of estuarine condition is examined.

This report is organized in sections addressing the primary purposes of this report. Section 2 provides an evaluation of the development of integrated benthic and fish indices of estuarine condition. These composite indicators are discussed in detail with regard to the discriminant analyses performed to create them.

Section 3 discusses the sensitivity of selected indicators to differentiate between known good and poor ecological conditions. This analysis has been performed on all the indicators used in the 1991 Louisianian Province Demonstration.

Section 4 discusses the efficacy of research indicators tested at selected sites within the Demonstration. These indicators include fish blood chemistry, bile fluorescence, skeletal abnormalities, splenic macrophage aggregates, histopathology, and stable isotopes of carbon and nitrogen.

Section 5 examines statistical associations associated with several selected ecological indicators. These associations are the first steps in attempting to ascertain the probable general causes for observed

ecological degradation.

Section 6 examines the design issues evaluated in the 1991 Demonstration. These elements include spatial scale, index sampling, and spatial autocorrelation.

Section 7 summarizes the conclusions that can be drawn from these special elements of the 1991 Louisianian Province Demonstration.

Section 8 lists the literature cited in this report.

SECTION 2

INDICES OF ECOLOGICAL CONDITION

Response indicators are characteristics of the environment that provide quantitative evidence of the status of ecological resources and biological integrity of the sample site from which they are drawn (Messer 1990). Ecosystems with a high degree of biotic integrity (i.e., healthy ecosystems) are comprised of balanced populations of indigenous organisms with species compositions, diversity, and functional organizations comparable to natural habitats (Karr and Dudley 1981, Karr et al. 1986). Response indicators could include measurements of the kinds and abundances of biota present, the health of the individual organisms, and the sustainability of critical ecological processes. Numerous individual measures have been collected to characterize these portions of ecosystem condition; however, no single measurement can be made to depict overall estuarine health.

We have combined several of these individual measures into a single measure of estuarine condition with regard to: (1) benthic response indicators, and (2) fish response indicators. All composite elements of these indices were collected during the 1991 Louisianian Province Demonstration.

2.1 BENTHIC INDEX

Benthic organisms are invertebrates that live in the bottom sediments of aquatic habitats. In estuaries they are a major trophic link between primary producers and higher trophic levels, including fish, shellfish, birds, and other wildlife (Carriker 1967, Rhoads 1974). They are a particularly important source of food for juvenile fish and crabs (Chao and Musick 1977, Bell and Coull 1978, Holland et al. 1989). Estuarine benthos also have important roles in ecological processes that affect water quality and productivity. For example, the feeding and burrowing activities of macrobenthos affect sediment depositional patterns and chemical transformations (Carriker 1967, Rhoads 1974, Kemp and Boynton 1981). Benthic feeding activities can remove large amounts of particulate materials from shallow estuaries, which may improve water clarity (Cloern 1982, Officer et al. 1982, Holland et al. 1989).

The study of benthic communities in the Gulf of Mexico estuaries has historically followed two paths: (1) the identification of environmental factors which influence benthic community structure or (2) the evaluation of the health of benthic communities as an indication of environmental perturbations of either natural or anthropogenic origin. The conclusions of the former show that salinity and sediment type are among the most

important factors which determine benthic infaunal relationships in estuaries of the Gulf of Mexico (Flint and Kalke 1985; Gaston et al. 1988; Rabalais 1990; Rakocinski et al. 1991). The health or biological integrity of benthic communities has traditionally been characterized by measures of abundance, diversity, or the presence of pollution indicator species. These factors have been used as indicators of hypoxic conditions (Rosenberg 1977; Harper et al. 1981; Jurot et al. 1983), organic pollution (Cook and Brinkhurst 1973; Grizzle 1984; Reish 1986; Tsutsumi et al. 1991), and toxic contamination (Holland et al. 1973; Rygg 1986). It has been argued, however, that no single factor is sufficient to distinguish environmentally degraded from undegraded areas (Rosenberg 1977; Pearson and Rosenberg 1978; McManus and Pauly 1990).

The objective of this section is to detail the methodology used to develop a benthic index of estuarine integrity and to present the results of subsequent analyses using this preliminary index. The multivariate techniques of stepwise and canonical discriminant analysis are utilized to select and test a subset of parameters which describes the benthic community and discriminates between degraded and undegraded habitats. The components of the resultant benthic index are supported by the literature as individual indicators of environmental condition with some limitations if used alone.

2.1.1 SAMPLING METHODS

A total of 182 stations throughout the Gulf of Mexico were sampled as part of the Louisianian Province Demonstration during July-August 1991. Of these, 110 base sites were probabilistically located in large estuaries (> 250 km^2), small estuaries (< 250 km^2), and the tidal portion of the Mississippi River (from New Orleans to the delta). Fifty-two sites were systematically located in the areas of sediment deposition within small estuaries and the Mississippi River. In addition, 16 sites were specifically selected as indicator testing and evaluation (ITE) sites based on historically documented conditions of high or low concentrations of dissolved oxygen, high or low agricultural runoff of pesticides, and high or low levels of industrial contamination. Finally, the remaining four sites were randomly selected from existing base sites and these were revisited as a quality control measure and a measure of inter-index period variability.

At each of these sites, at least 3 replicate benthic samples were collected using a young-modified Van Veen grab that sampled a surface area of 400 cm^2. A small core (60cc) was taken from each grab for sediment characterization (i.e., total organic carbon and percent silt-clay). The remaining sample was sieved through a 0.5 mm screen, preserved in 10% formalin-rose bengal solution, and stored for at least 30 days prior to processing. In the laboratory, macrobenthic samples were transferred from formalin to an ethanol solution and sorted, identified to lowest practical taxonomic level, and counted.

2.1.2 ANALYSIS METHODS

The general approach for the development of a benthic index was originally described

in Weisberg et al. (1992) as part of the EMAP-E Demonstration in the Virginian Province (Cape Cod, MA to Cape Henry, VA). The first step in the development of a benthic index of environmental quality was to choose a test data set consisting of sites with known environmental conditions. Our original inclination was to use the a priori selected ITE sites which were located specifically due to their combinations of environmental conditions. Unfortunately, many of these sites were not characterized by the dissolved oxygen concentrations (i.e., hypoxic or not) or sediment contaminant levels (i.e., high or low) indicated by historical analysis or local expert judgements. Only 9 of the 16 ITE sites conformed to original expectations. These 9 sites were insufficient to conduct the benthic index analyses. The test data set consisted of these sites combined with a subset of the remaining 173 sites which represented either clearly undegraded or degraded environment conditions based on established criteria. Using data described in Summers et al. (1993b) for sediment contaminant concentrations, sediment toxicity, and dissolved oxygen levels, test sites were chosen to represent extremes within a range of environmental conditions that would adversely affect benthos.

Hypoxic conditions (dissolved oxygen concentrations < 2 ppm) can cause a reduction in abundance and number of benthic species (Harper et al. 1981; Gaston 1985). Although many benthic species are resistant to short periods of hypoxia (Rosenberg 1977), extended or recurrent periods of hypoxia or anoxia lead to mortality of the benthic community (Boesch 1985). The concentration of heavy metals in the sediment was chosen as evidence of toxic contamination because median effects threshold levels have been established for most heavy metals (Long and Morgan 1990) and heavy metals are lethal to many benthic species which have no method of detoxification (Bryant et al. 1984; McClusky et al. 1986). The results of sediment bioassasy using the amphipod, *Ampelisca abdita*, and the mysid, *Mysidopsis bahia*, were also used to determine if a test site was degraded. Sediment bioassays have been shown to be very effective in identifying toxic sediments in combination with sediment chemistry and physical sediment characterization (Chapman 1989).

2.1.2.1 DEVELOPMENT OF TEST DATA SET

Sites were classified as reference (undegraded) sites based on the absence of any natural or anthropogenic stress if: (1) the minimum dissolved oxygen value over a 24-hour period was greater than 3.0 ppm (Summers and Engle 1992), (2) sediment concentrations for any contaminant did not exceed the minimum effects concentration established by Long and Morgan (1990) (equals the concentration at which 10% of the collected data demonstrated adverse biological effects) and (3) the percent survival for *Ampelisca abdita* (10-day) or *Mysidopsis bahia* (96-hour) in acute sediment bioassays was indistinguishable from controls. Degraded sites were required to exhibit the cumulative impacts of low dissolved oxygen stress and contaminated sediment stress by using the following criteria: (1) the minimum dissolved oxygen concentration over a 24-hour period

Habitat	Reference Sites High Dissolved Oxygen and Low Contaminants	Degraded Sites Low Dissolved Oxygen and High Contaminants
Oligohaline (< 5 ppt)	Lake Calcasieu, La 29°59.35 93°20.08	Belle River, LA 29°50.25 91°09.05
		Amite River, LA 30°17.77 90°35.98
Mesohaline (5-18 ppt)	San Antonio Bay, TX 28°18.25 96°39.89	Houston Ship Channel, TX 29°44.06 95°08.13
	Bayou Grande, FL 30°22.21 87°16.23	Choctawhatchee River, FL 30°23.99 86°08.02
	Back of Biloxi Bay, MS 30°24.85 88°53.11	Tensaw River, AL 30°41.21 88°00.06
	South Bay, TX 26°03.05 97°10.96	Lake Pontchartrain, LA 30°02.71 90°10.03
		Mobile Bay, AL 30°37.00 88°00.00
Polyhaline (18-35 ppt)	Lake Pelto, LA 29°04.13 90°44.40	Perdido Bay, FL 30°20.55 87°27.50
	Crystal Bay, FL 28°54.73 82°44.24	Watsons Bayou, FL 30°08.59 85°37.96
	Grand Bay, AL 30°22.89 88°20.31	Mobile Bay, AL 30°26.18 88°03.99
	Pelican Bay, AL 30°13.99 88°05.68	
	Matagorda Bay, TX 28°35.60 96°25.35	
Marine (> 35 ppt)	Laguna Madre, TX 26°08.09 97°16.04	Garden Island Bay, LA 29°01.69 89°06.50
		Arroyo Colorado, TX 26°20.74 97°25.69

Table 2.1 Locations of test sites used to develop the benthic index of estuarine condition.

was < 2 ppm, (2) sediment concentrations for at least one sediment contaminant exceeded Long and Morgan's (1990) ER-M value for biological response (concentration at which 50% of collected data demonstrated adverse biological effects), and (3) acute sediment bioassays yielded a control-adjusted survival rate of < 80%.

Table 2.1 lists the locations of sites used in

the development of the benthic index. These sites were chosen not only to represent undegraded and degraded environmental conditions as described above but also to cover the range of salinities (0-42 ppt), sediment types (mud, muddy sand, sand) and biogeographical locations (east and west of the Mississippi delta) inhabited by benthos in Gulf of Mexico estuaries. Table 2.2 lists the candidate measures used to develop the benthic index. These measures were chosen to represent the major structural attributes of benthic assemblages. In order to ensure the values of abundance and proportion approximated a normal distribution, these values were adjusted using a $\log_{10}$(value+1) transformation for abundances and an arcsine transformation for proportions.

Measures of Biodiversity/Species Richness

Shannon-Wiener Diversity Index
Pielou's Evenness Index
Mean Number of Species
Mean Number of Polychaete Species

Measures of Abundance

Mean Benthic Abundance per site

Measures of Taxonomic Composition

Mean abundance of amphipods per site
Proportion of total benthic abundance as amphipods
Mean abundance of decapods per site
Proportion of total benthic abundance as decapods
Mean abundance of bivalves per site
Proportion of total benthic abundance as bivalves
Mean abundance of gastropods per site
Proportion of total benthic abundance as gastropods
Mean abundance of molluscs per site
Proportion of total benthic abundance as molluscs
Mean abundance of polychaetes per site
Proportion of total benthic abundance as polychaetes
Mean abundance of capitellid polychaetes per site
Proportion of total benthic abundance as capitellid polychaetes
Mean abundance of spionid polychaetes per site
Proportion of total benthic abundance as spionid polychaetes
Proportion of total polychaete abundance as spionid polychaetes
Mean abundance of tubificid oligochaetes per site
Proportion of total benthic abundance as tubificid oligochaetes

Table 2.2 List of candidate benthic measures used to develop the benthic index.

2.1.2.2 ADJUSTMENT FOR HABITAT GRADIENTS

Measures of estuarine benthic abundance and species richness have been linked to natural gradients in salinity and sediment characteristics (Jurot et al. 1983; Flint and Kalke 1985; Gaston et al. 1988; Rabalais 1990; Rakocinski et al. 1991). Because our initial purpose in developing a benthic index is to attribute spatial differences in benthic community structure to contaminant and low dissolved oxygen stress, natural differences due to salinity or sediment gradients would confound the analysis and reduce the effectiveness of an index. Pearson correlations were performed between all candidate measures and salinity, longitude of sampling site (as a measure of geographical gradient), percent silt/clay, and total organic carbon content of sediments. Although many of the correlations were statistically significant at $p < .05$ due to the large sample size, only three correlations accounted for at least 20% of the variation (Table 2.3). All three correlations involved measures of species richness or diversity with salinity. Unless the natural variation attributable to salinity is partitioned from the original data set, any developed index that used species richness or diversity as a component would include salinity variation as part of its definition of an algorithm separating anthropogenically degraded sites from undegraded sites (i.e., assigned status).

We used the method described in Weisberg et al. (1992) to adjust the candidate measures that were significantly related to the salinity gradient (i.e., Shannon-Wiener Diversity Index, mean number of species, and mean number of polychaete species) in order to remove variation due to that gradient. The method employed a two step process whereby: (1) the expected value of diversity or number of species at any given salinity is estimated, and (2) the percent deviation of each observed value from that expectation is calculated.

Habitat Variable	Var Variable	Prob > |R|	R	R^2
Mean Salinity	Mean # Species	.0001	.46	.21
Mean Salinity	Diversity-Grab 1	.0001	.47	.22
Mean Salinity	Diversity-Grab 2	.0006	.35	.12
Mean Salinity	Diversity-Grab 3	.0001	.45	.20
Mean Salinity	Amphipod Abundance	.0173	.24	.06
Mean Salinity	Decapod Abundance	.0003	.36	.13
Mean Salinity	Polychaete Abundance	.0001	.44	.19
Mean Salinity	Capitellid Abundance	.0003	.36	.13
Mean Salinity	Spionid Abundance	.0001	.38	.14
Mean Salinity	Tubificid Abundance	.0004	-.35	.12
Mean Salinity	% Molluscs	.0103	-.26	.07
Mean Salinity	% Gastropods	.0219	-.23	.05
Mean Salinity	% Polychaetes	.0001	.41	.17
Mean Salinity	% Spionid/Polychaetes	.0361	.21	.04
Mean Salinity	% Tubificids	.0006	-.34	.12
Longitude	Amphipod Abundance	.0347	-.21	.05
Longitude	% Capitellids	.0121	.25	.06
% Silt-Clay	Amphipod Abundance	.0001	-.42	.17
TOC	Diversity-Grab 2	.0027	-.33	.09

Table 2.3 Summary of significant correlations between habitat indicators and candidate benthic measures.

The expected value for diversity or number of species was estimated by first calculating the 90th percentile of observed diversity or species richness values for overlapping intervals of 5 ppt salinity (e.g., salinity intervals of 0-5 ppt, 1-6 ppt, 2-7 ppt, ..., 39-42 ppt). A third order polynomial was then fit through the 90th percentile

values and the midpoints of the salinity intervals. We used the data from the randomly-selected base stations to obtain as good a fit as possible.

The polynomials calculated for each of the three variables are shown in Table 2.4. The explained variation for these relationships ranged between 0.84-0.87. This process assumes that the 90th percentile represents the number of species that would occur at undegraded reference sites. This assumption proved to be reasonable because calculated expected values did not differ from those observed for the undegraded reference sites; however, the observed number of species at degraded sites consistently fell

Expected Diversity =

$0.754 + (0.008\ S) + (0.0016\ S^2) - 0.003\ S^3$

Expected Number of Benthic Species =

$13.908 - 1.115\ S + 0.1244\ S^2 - 0.0019\ S^3$

Expected Number of Polychaete Species =

$4.751 - 0.2262\ S + 0.0435\ S^2 - 0.0007\ S^3$

S = Salinity (ppt)

Table 2.4 Polynomials used to adjust benthic parameters significantly related to salinity.

far below the regression line defining the 90th percentile for expected number of species (Fig. 2.1a,b,c).

The salinity-adjusted candidate measures (proportion of expected diversity, proportion of expected number of species, and proportion of expected mean number of polychaete species) were calculated by dividing the observed value by the expected value for each measure. These values were substituted for the original, unadjusted variables in the list of candidate

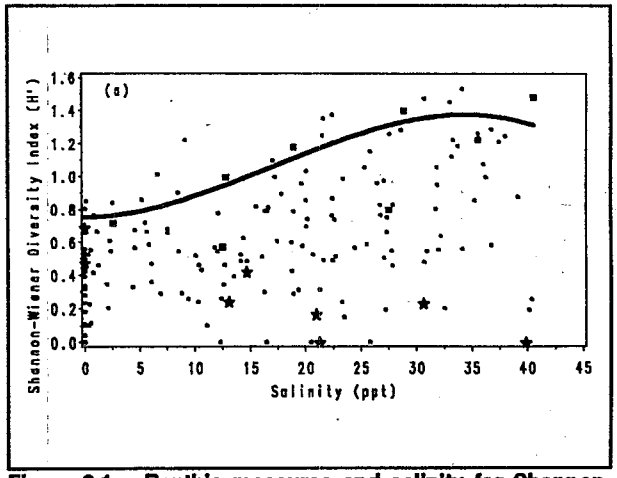

Figure 2.1a Benthic measures and salinity for Shannon-Wiener diversity index. (■ = reference sites, ★ = degraded sites, ● = base sites).

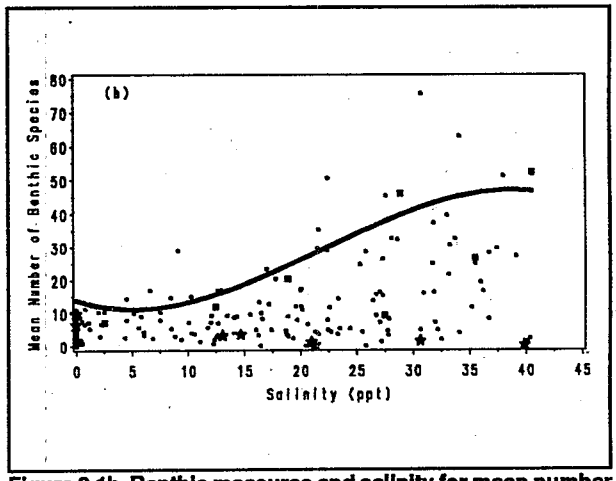

Figure 2.1b Benthic measures and salinity for mean number of species. (■ = reference sites, ★ = degraded sites, ● = base sites).

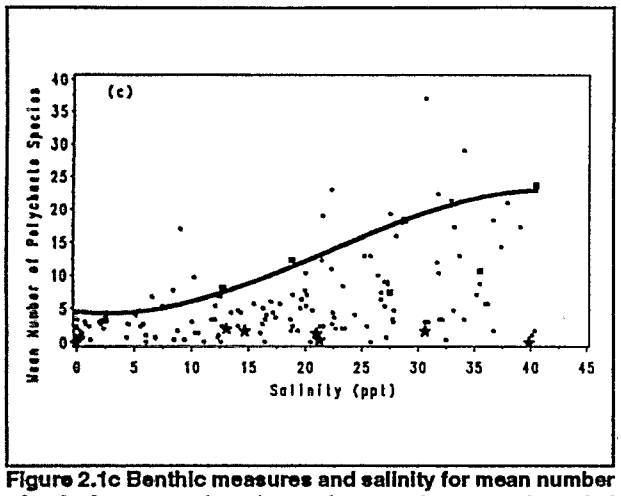

Figure 2.1c Benthic measures and salinity for mean number of polychaete species. (■ = reference sites, ★ = degraded sites, ● = base sites).

measures (Table 2.2). These new measures were no longer significantly correlated with salinity.

2.1.2.3 DISCRIMINANT ANALYSIS METHODS

Once the test data set consisting of values for the candidate measures at both reference and degraded sites was established, stepwise and canonical discriminant analyses were applied. Stepwise discriminant analysis selects a subset of the candidate measures which best discriminates between the reference and degraded sites. Applying canonical discriminant analysis to this subset of variables yields a linear combination of the quantitative variables that shows the most substantial difference between degraded and undegraded sites (Williams 1983; SAS Institute 1989). This analysis also produces estimates of classification efficiency whereby sites that have been misclassified as reference or degraded are enumerated. The coefficients derived from canonical discriminant analysis are then standardized to a mean of 0 and a standard deviation of 1 in order to calculate a discriminant score. Because discriminant scores may result in a distribution among negative and positive scores, they are then normalized to a range of 0 to 10 using the test data set in order to be more easily understood and graphically represented.

2.1.3 DISCRIMINANT ANALYSIS RESULTS

The first stepwise discriminant analysis suggested that only two benthic measures were required to discriminate between degraded and undegraded sites: (1) proportion of expected number of polychaete species and (2) mean abundance of decapods (Table 2.5). However, the polychaete species-decapod model resulted in an 18% misclassification rate of reference sites (i.e., false positives) and a 17% misclassification rate of degraded sites (i.e., false negatives). A total of 55% of the total variance was explained by this model.

Evaluation of the results of the stepwise discriminant analysis suggested that several of the stations in the test data set may be misclassified as reference or degraded (i.e., the conditions were not sufficient to represent extremes of the degradation gradient). The original classification of sites, following the established criteria, had some locations with acceptable dissolved oxygen concentrations near the degraded criteria

Analysis Sequence	Candidate Measures Selected by Analysis	Percent False Positives	Percent False Negatives	Canonical r^2
Analysis I-All Stations	1) Proportion of expected number of polychaete species 2) Mean abundance of decapods	18.2	16.7	.55
Analysis II-Analysis I with Misclassified Stations Removed	1) Proportion of expected number of polychaete species	12.5	0.0	.81
Analysis III-Analysis II with Excluded Proportion of Expected Number of Polychaete Species	1) Proportion of expected diversity, 2) Percent of total abundance as tubificids 3) Percent of total abundance as bivalves 4) Proportion of expected number of species 5) Percent of total abundance as capitellids 6) Mean abundance of capitellids 7) Mean abundance of tubificids 8) Mean abundance of bivalves	0.0	0.0	.99
Analysis IV-Analysis II with all but the first three variables removed	1) Proportion of expected diversity 2) Percent of total abundance as tubificids 3) Percent of total abundance as bivalves	0.0	0.0	.90

Table 2.5 Sequence of stepwise and canonical discriminant analyses conducted for combining candidate benthic measures into an index.

(e.g., 2-3 ppm) or with low *Ampelisca* survival (75-85%) in the sediment toxicity tests although no corresponding high sediment contaminant concentrations were observed. Four sites, classified as degraded sites, experienced low *Ampelisca* mortalities or low contaminant levels that exceeded the criterion by only a small margin for one of the criteria. Because these 7 sites did not conform to all three levels of the criteria established and were either degraded or undegraded in terms of dissolved oxygen concentrations, sediment contaminants, and sediment toxicity, simultaneously, they were considered misclassified and removed from the analysis (i.e., they were misclassified to begin with).

The revised data set was re-evaluated using stepwise discriminant analysis. The results of this second analysis suggested that only one candidate measure was required to discriminate between degraded and reference sites: proportion of expected number of polychaete species (Table 2.5). Use of this variable alone accounted for 81% of the variability observed in the test

data set. While a viable model, we decided that a model based solely on the presence or absence of a single indicator group such as polychaetes would severely limit the effectiveness of discriminating among reference and degraded sites (Pearson and Rosenberg 1978). Once the model was applied to a continuous gradient of effects rather than the extremes portrayed by the test data set, reliance on a single indicator could result in poor discriminant power. Because proportion of expected polychaete species was significantly correlated with the proportion of expected diversity ($r^2 = 0.58$, $p < .05$) and diversity has some historical support as an indicator of estuarine integrity, the discriminant analysis was repeated eliminating the expected number of polychaetes as a potential variable. This new model resulted in an eight parameter model (Table 2.5) with an overall $r^2 = 0.99$ which included proportion of expected diversity as the primary contributor. Subsequent regression analysis revealed that all but the first three variables entering the stepwise discriminant analysis exhibited significant collinearity and contributed little to the overall model r-square (i.e., < 1-2% for each of the five minor variables). Eliminating these five variables resulted in a model using only the three remaining variables (i.e., proportion of expected diversity, proportion of total benthic abundance as tubificid oligochaetes, and proportion of total benthic abundance as bivalves) accounting for 90% of the observed variation in the test data set with no misclassifications. Inspection of the discriminant scores for this final model showed all reference sites to have values >0 (0.3-4.3) and all degraded sites to have values < 0 (-3.8 to -1.8). This distribution of discriminant scores provided a clear demarcation between undegraded and degraded sites.

These indicators were first standardized and then combined to make a composite benthic index using the following algorithm:

Score = (2.38 x D)-(1.67 x T)+(0.67 x B)

where:

D = Proportion of expected diversity (Shannon-Weiner) at observed salinity
T = Proportion of total benthic abundance as tubificids
B = Proportion of total benthic abundance as bivalves

The final development of the benthic index involved calculating the discriminant scores for all sample sites and normalizing the calculated scores to a scale of 0 to 10. This normalization step was used to ease interpretability and graphical display with

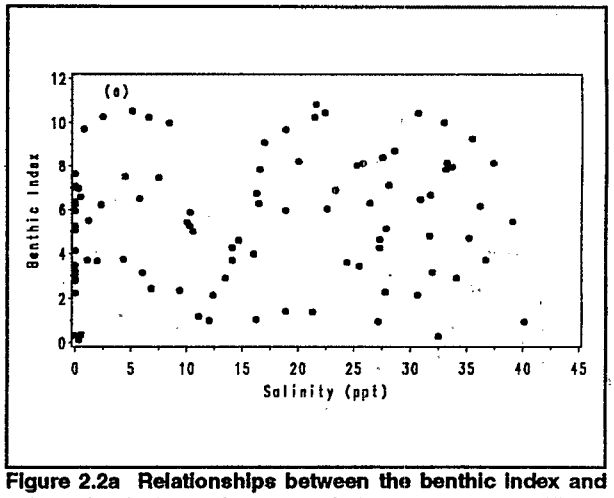

Figure 2.2a Relationships between the benthic index and salinity for the base sites. Correlations were not significant at $p < .05$.

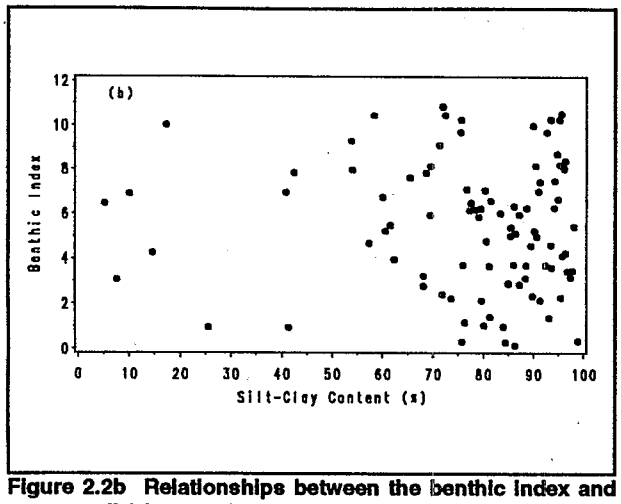

Figure 2.2b Relationships between the benthic index and percent silt/clay for the base sites. Correlations were not significant at $p < .05$.

the original range of -3.75 to 4.32 being normalized to 0 to 10 with the break point between degraded and undegraded sites being at 4.1 (i.e., corresponding to roughly 0.0 in the discriminant score).

One final check was made to assure that the benthic index was not related to habitat parameters (e.g., salinity, silt-clay content), and the index was not significantly related to these parameters (Fig. 2.2a,b). This final step was important because the benthic index was designed to be an indicator of degradation experienced at a site (whether anthropogenic or climatic) rather than inherent differences in benthic community structure due to salinity and sediment variations.

2.1.4 APPLICATION OF BENTHIC INDEX TO LOUISIANIAN PROVINCE

As was described in the Statistical Summary (Summers et al. 1993b), the application of the benthic index to the 182 sites from which benthic data were collected in the Louisianian Province showed that 31% ±10% (estimate ± 95% confidence interval) of the sediments in the Gulf of Mexico estuaries contained benthic community structures similar to those seen in areas of environmental stress (Fig. 2.3). The percentage of area degraded (i.e., 4.1 benthic index) varied among the estuarine classes with 25% ±12% of the benthic communities in large estuaries; 41%±15% in small estuaries, and 80%±25% in the Mississippi River being degraded (Fig. 2.4).

Although Alabama, Mississippi, and Texas estuaries showed the greatest proportion of degraded benthic communities among the Gulf States (Fig. 2.4), the largest area of degraded benthos was found in Louisiana and Texas (6200 km^2).

2.2 FISH INDEX

Fish have several advantages as potential indicators of estuarine condition. Because fish have long life-spans and dominate the upper end of the food web, their responses integrate many short-term and small-scale environmental perturbations. They are known to respond to most of the major environmental problems of concern in estuaries (NOAA 1988), including eutrophication, habitat modification, and the presence of pathogenic or toxic contaminants. For example, eutrophication

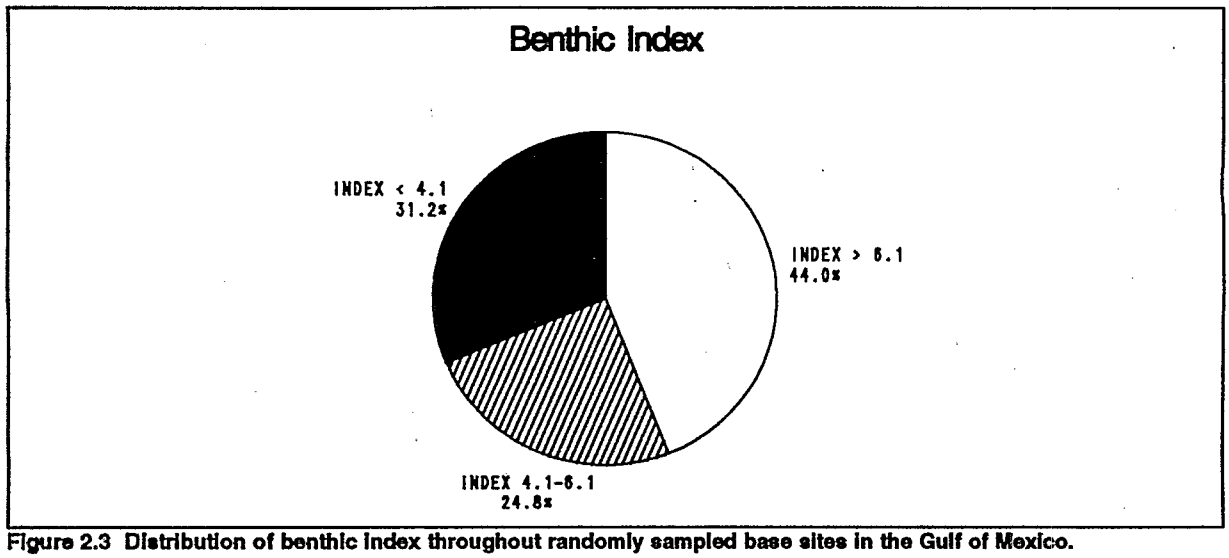

Figure 2.3 Distribution of benthic index throughout randomly sampled base sites in the Gulf of Mexico.

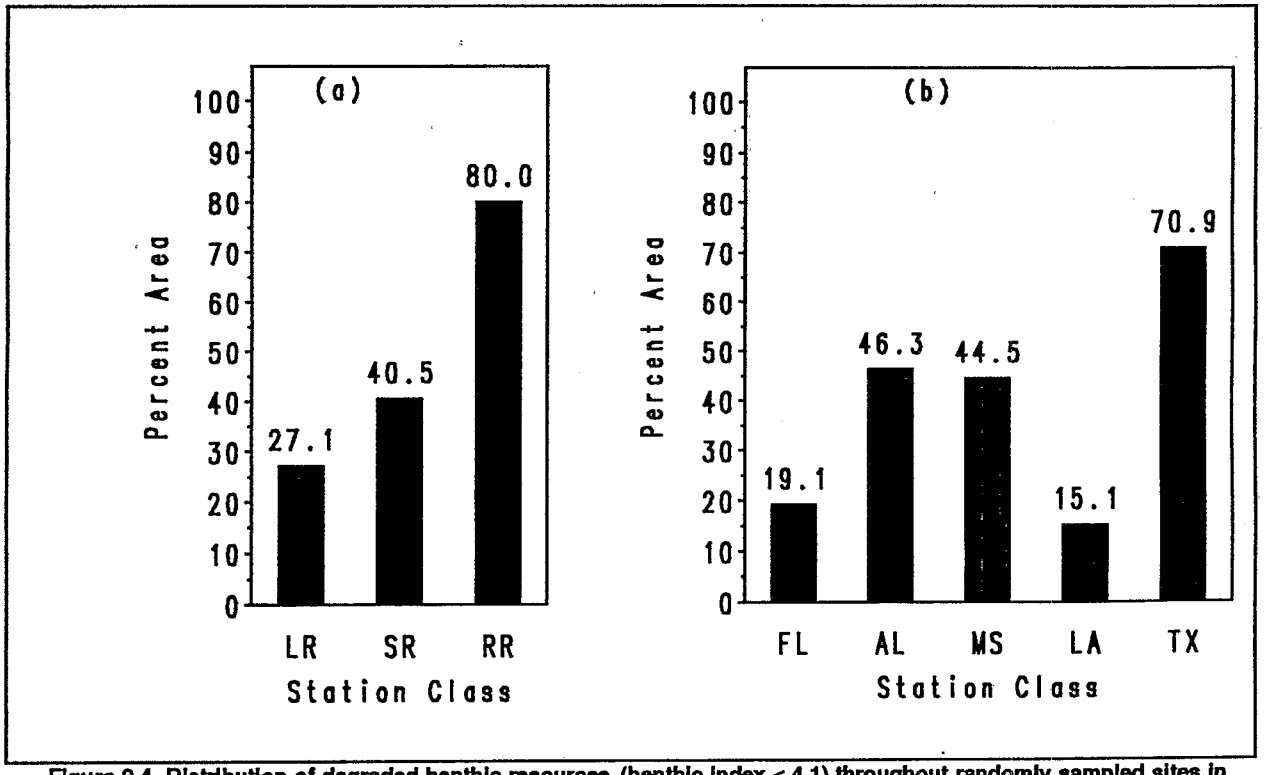

Figure 2.4 Distribution of degraded benthic resources (benthic index < 4.1) throughout randomly sampled sites in the Gulf of Mexico a) by estuary class and b) by state. (LR=large estuary, SR=small estuary, RR=Mississippi River)

can affect fish adversely by diminishing dissolved oxygen concentrations to below critical levels for growth, survival, or structural development. Habitat modification, such as the loss of submerged aquatic vegetation, has been linked to decreased fish productivity through loss of important nursery areas. Toxic and pathogenic contaminants can decrease fish growth, reproduction, or survival and can make fish unsafe for human consumption.

Fish are valuable indicators because of their importance in determining public perception of estuarine quality. Reports of fishery closures due to chemical and viral contamination alarm the public. In addition, the public has an economic interest in estuarine and near coastal fisheries. More than seven billion dollars are spent annually in this country on saltwater recreational fishing, the vast majority of which occurs in estuaries or within three miles of the coast. Combining recreational and commercial fishing, estuaries and near coastal waters account for 70% of U.S. landings (NOAA 1987).

The use of indices of overall health of the fish assemblage occurring at a site has gained great favor in freshwater environments, where the Index of Biotic Integrity (IBI)(Karr 1981) has become the standard measure in several states for defining environmental quality (Plafkin et al. 1989). The IBI incorporates measures for the individual, population, and assemblage level. To date, a validated index of estuarine environmental quality based on information about fish assemblages that is applicable over broad geographic areas has not been developed. The major difficulty with accomplishing this is that the mobility and migratory behavior of estuarine fish may limit our ability to delineate a response to environmental conditions at the site of collection.

2.2.1 SAMPLING METHODS

A total of 182 stations throughout the Gulf of Mexico were sampled as part of the Louisianian Province Demonstration during July-August 1991. These locations are the same as those described above for benthic sampling.

At each of these sites, a single fish sample was collected using a 16-ft otter trawl pulled at approximately 1m/s for 10 minutes. If the first trawl resulted in no or few fish, a second trawl was taken. The contents of the fish trawls were identified to species, enumerated, and examined for external pathological disorders (e.g., lesions, swellings, scoliosis). A subset (up to 30 fish) of each species in the catch was measured for length to the nearest 0.1 millimeter. All fish displaying external pathologies were forwarded to a histopathology laboratory and up to ten individuals of selected target species were forwarded for contaminant analysis of edible fillets.

2.2.2 ANALYSIS METHODS

The general approach for the development of a fish index was originally described by Weisberg et al. (1992) as part of the EMAP-E Demonstration in the Virginian Province. However, the fish index

developed in the Virginian Province was determined to be inadequate to characterize the differences between degraded and undegraded sites in the Virginian Province. The development of a fish index in the Louisianian Province from its 1991 Demonstration data uses the same test data set developed for the benthic index. This data set is based on occurrence of hypoxia, elevated levels of sediment contaminants, and sediment toxicity. Table 2.1 listed the locations of the sites used in the test data set. These sites represent undegraded and degraded environmental conditions, a variety of habitats (e.g., salinities, depth zones, open water vs. nearshore). Table 2.6 shows the list of candidate measures used to develop the fish index. These measures were chosen to represent the major ecological attributes of fish assemblages and health characteristics of individual fish. In order to ensure that values of abundance and proportion approximated a normal distribution, these values were adjusted using a $\log_{10}$(value+1) transformation for abundances and an arcsine transformation for proportions.

Species Richness and Diversity
Abundance
Shannon-Wiener Index
Number of Species
Number of Species to comprise 90% of the Catch
Community Composition
% Engraulidae (Anchovies)
% Clupeidae (Herrings)
% Ariidae (Catfish)
% Penaeidae (Shrimp)
% Carangidae (Jacks)
% Sciaenidae (Drums)
% Sparidae (Porgies)
% Bothidae (Flounders)
% Tetraodontidae (Puffers)
Presence of Endangered Species
Trophic Dynamics
% Top Carnivores
% Planktivores
% Benthic Invertivores
% Planktonic Invertivores
Health of Individual Organisms
Number of Gross External Pathologies in Catch

Table 2.6 Candidate variables for the development of fish index of estuarine condition.

2.2.2.1 ADJUSTMENT FOR HABITAT GRADIENTS

Because our purpose in the construction of the fish index is to attribute spatial differences in fish community structure to contaminant and low dissolved oxygen stress, natural differences due to habitat types or gradients would confound development of the index. Pearson correlations were performed between all candidate measures and bottom salinity, longitude of the site, total organic carbon content of the sediment, and percent silt-clay content of the sediments. Many correlations were significant (Table 2.7) but none of the correlations accounted for more than 20% of the observed variability in the test data set.

Because no correlations accounted for more than 25% of the observed variability in the test data set, no adjustments for natural environmental gradients were made to the test data set.

Habitat Variable	Number of Significant Correlations ($p < .05$)	Number of Correlations ($R^2 > .10$)	Number of Correlations ($R^2 > .20$)
Bottom Salinity	6	2	0
Longitude of Site	6	0	0
Total Organic Carbon	3	0	0
Percent Silt Clay	5	2	0
Bottom Dissolved Oxygen	0	0	0
Water Dept	3	1	0
Habitat Type	4	3	0
ALL	27	8	0
Percent of All Correlations (133)	20%	6%	0%

Table 2.7 Results of Pearson correlation analyses between variables used in fish index development and habitat variables.

2.2.2.2 DISCRIMINANT ANALYSIS METHODS

Stepwise and canonical discriminant analysis was applied to the test data set in the same manner as with the benthic index. The discriminant results were evaluated for classification error and the discriminant scores were normalized to a scale of 0 to 10.

2.2.2.3 DISCRIMINANT ANALYSIS RESULTS

The stepwise discriminant analysis showed that 11 fish community measures could be included to account for 97% of the variability observed in the test data set. However, 7 of the variables accounted for < 1% of that total variability even though 6 of the 7 variables were significant (Table 2.8). Thus, only four variables (number of species, proportion of total catch as shrimp, number of species comprising 90% of catch, and proportion of total catch as puffers) accounted for 95% of the variability between degraded and undegraded sites (Table 2.9). However, number of species alone accounts for 78% of the differences between degraded and reference sites. The combination of number of species with proportion of total catch as shrimp accounts for 88% of these difference. There were no misclassifications within the test data set.

Discriminant scores ranged from -3.7 to 6.1 with all reference sites having scores > 0 and all degraded sites having scores < 0. This distribution provided a clear demarcation between degraded and undegraded sites. These indicators were first standardized and then combined to make a composite fish index using the following algorithm:

$$\textbf{Score} = (2.28 \times SP) + (0.94 \times S)$$

where:

SP = Number of species comprising the catch

S = Proportion of total catch as shrimps.

Analysis Number	Variables Included	Squared Canonical Correlation
#1 - All Variables	1) Number of Species 2) % Shrimp 3) Number of Species Comprising 90% Catch 4) % Puffers 5) % Anchovies 6) % Carangids 7) % Catfish 8) % Sciaenids 9) Number of External Pathologies/Trawl 10) Benthic Index 11) % Sparids	0.999
#2 - Analysis #1 but Including Only Variables Contributing > .02 to Squared Canonical Correlation	1) Number of Species 2) % Shrimp 3) Number of Species Comprising 90% Catch 4) % Puffers	0.945
#3 - Analysis #1 but Including Only Variables Contributing > .05 to Squared Canonical Correlation	1) Number of Species 2) % Shrimp	0.876

Table 2.8 Results of discriminant analyses in the development of a fish index.

The final development of the fish index involved calculating the discriminant scores for all sample sites and normalizing the calculated scores.

2.2.2.4 APPLICATION OF THE FISH INDEX TO LOUISIANIAN PROVINCE

The application of the fish index to the 182 sites from which the fish data was collected in the Louisianian Province showed that 66% of the estuarine waters of the Gulf of Mexico had fish communities with low number of species as characterized by trawls (Fig. 2.5). Inspection of the subpopulation estimates for large estuaries, large tidal rivers and small estuaries showed that 69%, 78%, and 60% of the fish communities in each of these classes, respectively, were degraded (Fig. 2.6). Inspection of the data, as a preliminary validation, suggests that the index is relatively weak when applied overall to the Louisianian Province data set because apparently "healthy" sites were categorized as degraded because relatively few species were collected. For example, a trawl at a site could produce 200-300 fish but of only one or two species (e.g., pinfish, menhaden, catfish). This site would be assessed as degraded and yet be highly productive.

Even though the analysis accounted for significant portions of the variability between degraded and undegraded sites in the test data set, there is sufficient evidence to suggest that its overall application results in errors, particularly with regard to trawls in open, large estuarine sites. This poor agreement between the observed conditions and the fish index in large estuarine sites suggested that either: (1) separate indices are required for different habitats (i.e., open water versus small shallow estuarine systems) or (2) that a multi-species compositional index like the IBI might

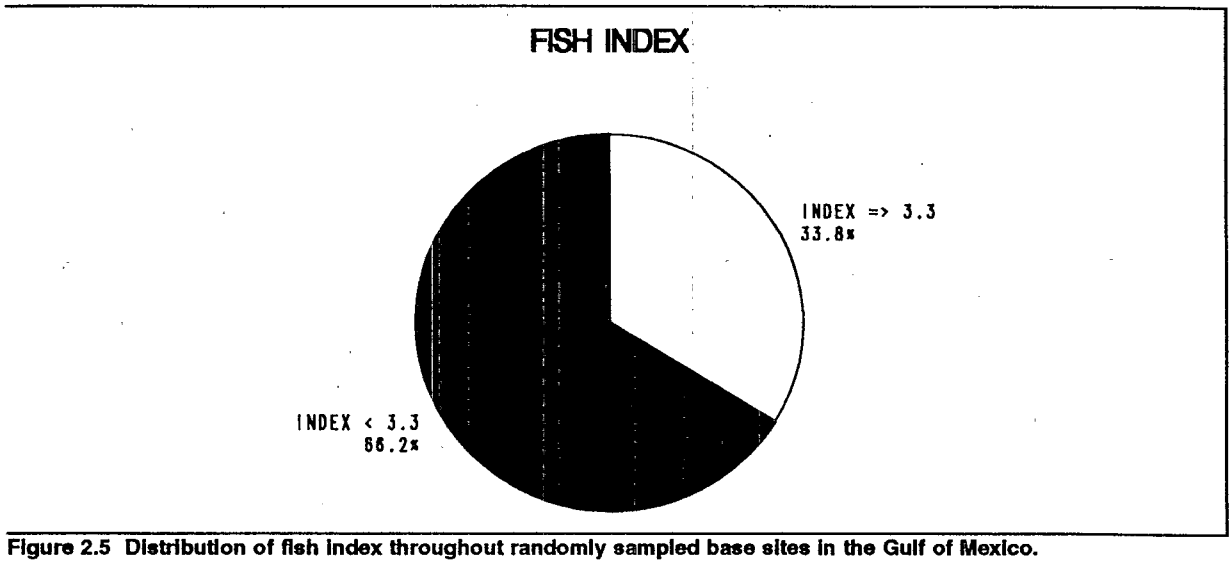

Figure 2.5 Distribution of fish index throughout randomly sampled base sites in the Gulf of Mexico.

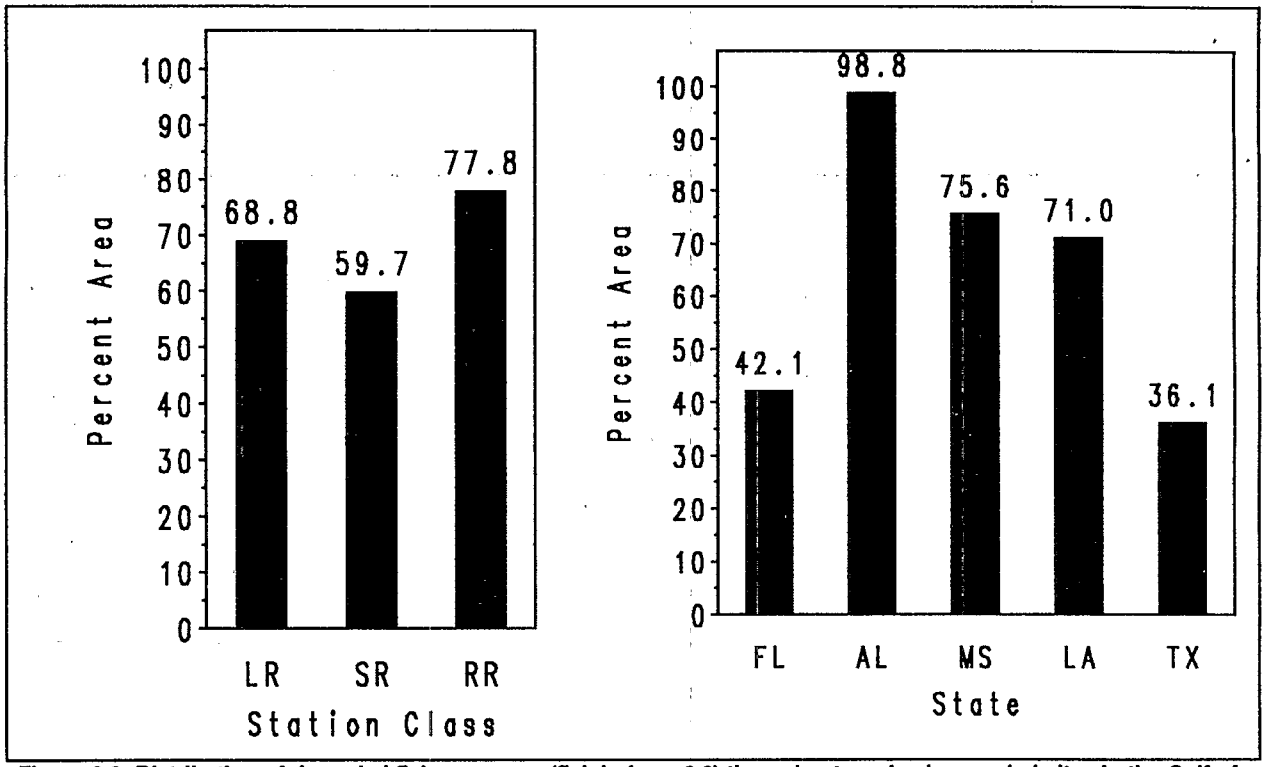

Figure 2.9 Distribution of degraded fish resources (fish index < 3.3) throughout randomly sampled sites in the Gulf of Mexico (a) by estuary class and (b) by state (LR=large estuary, SR=small estuary, RR=Mississippi River.

categorize sites more correctly. Relatively few degraded sites were located in large estuarine resources; thus, there may be too few sites within individual estuarine classifications that are degraded to permit a valid analysis. The collection of additional sites in 1992 will be combined with the 1991 data in an effort to develop fish indices for individual habitat types.

SECTION 3

SENSITIVITY OF INDICATORS

One of the most difficult activities within the development of EMAP demonstrations and the eventual implementation of estuarine monitoring is the selection of indicators. Although EMAP has developed a rigorous tiered selection process (Knapp et al. 1990), the true utility of individual indicators cannot be determined until they are tested at the geographic and temporal scales EMAP require. In addition, numerous indicators may be redundant in that they reflect similar portions of the variability observed in the environment.

One of the major objectives of EMAP in its early demonstrations is to evaluate the tiered selection process for indicators and to assess the utility of "known" ecological indicators. "Known" in this sense refers to the belief by many ecologists that the indicators in question can differentiate, to some degree, between good and poor ecological conditions. The indicators used in the 1991 Louisianian Province Demonstration (Table 3.1) were selected through a rigorous process. Numerous ecologists discussed, at workshops and through individual contacts, the pros and cons of using these indicators, as well as those indicators discarded, to ascertain environmental condition. The selection process focused on the perceived ability of the indicators to differentiate between good and poor ecological conditions, the directness of their interpretation, their logistical feasibility in terms of implementation in the field from small vessels with no laboratory facilities nearby, and their relationship to the endpoints of concern for EMAP-E (i.e., estuarine condition based on ecological integrity and aesthetic value).

In order to provide an assessment of the ability of the selected indicators to differentiate between good and poor sites, we have analyzed all indicators with reference to their ability to differentiate between a subset of the 1991 monitoring sites representing extreme values of contaminants and hypoxia described in Section 2. The sites comprising this data set are shown in Table 3.2. A good site is characterized by relatively high dissolved oxygen conditions throughout the day (i.e., > 4 ppm at all times), low contaminant concentrations in sediments (alkanes, PAHs, PCBs, pesticides, or metals), no sediment toxicity as shown by *Ampelisca* bioassays, and, in some cases, the existence of healthy seagrass beds. A bad site is characterized by hypoxic conditions (DO < 2 ppm) at least 20% of the time and sediment contaminant concentrations greater than the ERL-L levels (i.e., those concentrations producing significant ecological effects in 10% of studies reviewed) described by Long and Morgan (1990)(Table 3.3), and known high levels of industrial point source discharges or heavy agricultural applications of pesticides within the estuary's watershed.

Indicator	Category	Indicator
Biotic Condition	Core	Benthic Community Composition
		Benthic Abundance
		Benthic Biomass
		Benthic Index
	Developmental	Fish Community Composition
		Fish Abundance
		Fish Lengths
		Fish Index
		Fish Pathology
		Tissue Contaminants
		Continuous Dissolved Oxygen Concentration
		Relative Abundance of Large Bivalves
	Research	Histopathology of Fish
		Skeletal Abnormalities
		Blood Chemistry
		Bile Florescence
		Stable Isotope Ratios
		Splenic Macrophage Aggregates
		Liver Contaminant Concentration
		Whole Body Contaminant Concentration
Abiotic Condition	Core	Salinity
		Temperature
		pH
		Water Depth
		Redox Potential Discontinuity Layer Depth
		Percent Silt-Clay
		Percent Total Organic Carbon
	Developmental	Marine Debris
		Percent Light Transmittance
		Secchi Depth
		Sediment Contaminants
		Sediment Toxicity
		Instantaneous Dissolved Oxygen Concentration

Table 3.1 Ecological Indicators (measured and calculated) used in the 1991 Louisianian Province Demonstration.

3.1 ECOLOGICAL INDICATORS

EMAP-E focuses on response indicators to characterize the ecological status of the estuarine resources of the Louisianian Province. Biotic condition indicators are ecological characteristics that integrate the responses of living resources to specific or multiple pollutants and other stresses.

Abiotic condition indicators are ecological characteristics that can be linked, conceptually, to decreases in estuarine condition. Within EMAP-E these indicators include: (1) measures of attributes of the benthic and fish communities as they relate to biotic condition, (2) continuous dissolved oxygen concentrations as they relate to in the eutrophication process, and

GOOD ECOLOGICAL CONDITION (REFERENCE SITES)				
Location	**Latitude**		**Longitude**	
Calcasieu Lake Canal, LA	29	59.38'	93	20.03'
Laguna Madre, TX	26	8.00'	97	16.00'
San Antonio Bay, TX	28	18.30'	96	39.90'
Matagorda Bay, TX	28	35.58'	96	25.46'
Crystal Bay, FL	28	53.24'	82	44.41'
Grand Bay, AL	30	22.89'	88	20.33'
South Bay, TX	26	2.98'	97	10.98'
Bayou Grande, FL	30	22.14'	87	16.23'
BAD ECOLOGICAL CONDITION (AFFECTED SITES)				
Perdido Bay, FL/AL	30	27.08'	87	22.60'
Watsons Bayou, FL	30	8.59'	85	38.00'
Choctawhatchee River, FL	30	24.00'	86	8.00'
Arroyo Colorado, TX	26	20.00'	97	25.76'
Mobile Bay, AL	30	26.17'	88	3.99'
Belle River, LA	29	50.25'	91	9.05'
Tensaw River, AL	30	41.35'	88	0.00'
Amite River, LA	30	17.84'	90	33.60'

Table 3.2 Sites comprising the test data set for assessing the sensitivity of ecological indicators. Criteria for good and bad condition are described in text.

(3) the presence of marine debris, water clarity and contaminant concentrations in edible fish/shellfish tissue as they relate to human uses of estuaries. Only core and developmental response indicators will be assessed here; research response indicators will be discussed in Section 4.

3.1.1 BENTHIC COMMUNITY INDICATORS

Of the benthic indicators collected during the 1991 Louisianian Province Demonstration, only mean number of species in a grab, the proportion of total abundance as bivalves, the biodiversity associated with a grab (Shannon-Weiner Index), and the benthic index described in Section 2 consistently differentiated between "good" (reference)and "bad" (affected) sites (Table 3.4). Mean benthic abundance; proportion of total abundance as amphipods, decapods, polychaetes, or tubificid oligochaetes; or the abundance of large bivalves did not significantly discriminate among the good and bad sites. However, mean abundance and proportion of total abundance as amphipods did show higher values at good sites than at bad sites although the variability associated with these variables was rather high. In addition, the proportion of total abundance as polychaetes, decapods, and tubificid oligochaetes showed higher values at bad sites than at good sites but again high variability resulted in an inability to discriminate between the sites.

Mean number of benthic species at good sites were generally seven times higher at good sites than at bad sites (Table 3.5) and accounted for 45% of the variability between these sites. Calculation of the Shannon-Weiner Diversity Index for each benthic grab produced values at good sites that were five times greater than at bad sites and diversity accounted for 67% of the variability observed among these sites (Table 3.5). These two indicators are strongly colinear; thus only biodiversity enters into the construction of the benthic index. The proportion of total benthic

CHEMICAL ANALYTE	CRITERION
Trace Elements (ppm)	
Aluminum	NA
Antimony	2
Arsenic	33
Cadmium	5
Chromium	80
Copper	70
Iron	NA
Lead	35
Manganese	NA
Mercury	0.15
Nickel	30
Silver	1
Tin	NA
Zinc	120
Butyltins (ppb)	
Monobutyltin	NA
Dibutyltin	NA
Tributyltin	NA
Polycyclic Chlorinated Biphyenyls (ppb)	
Total PCBs	50
PCB Congeners	NA
Chlorinated Pesticides (ppb)	
DDT	1
DDE	2
DDD	2
Total DDT	3
Aldrin	NA
alpha-BHC	NA
beta-BHC	NA
delta-BHC	NA
alpha-Chlordane	0.5
gamma-Chlordane	0.5
Dieldrin	0.02
Endrin	0.02
Hexachlorobenzene	NA
Heptachlor Epoxide	NA
Lindane	NA
Mirex	NA
cis-Nonachlor	NA
trans-Nonachlor	NA
Oxychlordane	NA
Alkanes and Isoprenoids	
C10-C34	NA
Pristane	NA
Phytane	NA

CHEMICAL ANALYTE	CRITERION
Polynuclear Aromatic Hydrocarbons (ppb)	
Acenaphthene	150
Acenaphthylene	NA
Benzo(a)anthracene	230
Benzo(a)pyrene	400
Benzo(b)fluoranthene	NA
Benzo(e)pyrene	NA
Benzo(g,h,i)perylene	NA
Biphenyl	NA
Chrysene	400
C1-Chrysene	NA
C2-Chrysene	NA
C3-Chrysene	NA
Dibenzo(a,h)anathracene	60
Dibenzothio	NA
C1-Dibenzothio	NA
C2-Dibenzothio	NA
C3-Dibenzothio	NA
Fluoranthene	600
Fluorene	35
C1-Fluorene	NA
C2-Fluorene	NA
C3-Fluorene	NA
Naphthalene	340
C1-Naphthalene	NA
C2-Naphthalene	NA
C3-Naphthalene	NA
C4-Naphthalene	NA
Perylene	NA
Phenanthrene	225
C1-Phenanthrene	NA
C2-Phenanthrene	NA
C3-Phenanthrene	NA
C4-Phenanthrene	NA
Pyrene	350
(i)1,2,3,c-,d-pyrene	NA
1-methylnaphthalene	NA
1-methylphenanthrene	NA
2-methylnaphthalene	65
2,3,5-trimethylnaphthalene	NA
2,6-dimethylnaphthalene	NA
Total PAHs	4000

Table 3.3 Criteria values used to characterize degraded sediments (from Long and Morgan 1990). NA = Not Available.

Indicator	Mean		Significance
	Good	Bad	
Mean Number of Species	23.8	3.4	**
Mean Abundance	95.0	60.0	
Percent Amphipods	4.3	0.0	
Percent Decapods	4.4	12.5	
Percent Bivalves	19.0	2.4	*
Percent Polychaetes	50.5	56.5	
Percent Tubificids	0.4	7.8	
Abundance - Large Bivalves	0.0	0.0	
Biodiversity	1.0	0.3	***
Benthic Index	8.3	3.9	***

---
* p<0.05
** p<0.01
***p<0.001

Table 3.4 Results of sensitivity tests for benthic community indicators. Good refers to ecological reference sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

abundance as bivalves was nine times greater a good sites than at bad sites accounting for 26% of the variability.

These two indicators (diversity and percent bivalves) combine with percent tubificids (not significant as a univariate indicator) to construct a benthic index that accounts for 89% of the variability observed between good and bad sites.

Indicator	Pr > |F|	R^2
Mean Number of Species	.0048	0.45
Percent Bivalves	.0416	0.26
Biodiversity	.0001	0.67
Benthic Index	.0001	0.89

Table 3.5 Significance levels and R^2 associated with benthic community indicators that successfully discriminated between site types.

3.1.2 FISH COMMUNITY INDICATORS

Of the fish community indicators collected during the 1991 Demonstration, the number of fish species in a trawl, the abundance of fish in a trawl, and the fish index described in Section 2 could significantly differentiate between good and bad sites (Table 3.6). None of the proportional taxonomic groups could successfully differentiate between good and bad sites.

The number of fish species in a trawl was 3 times greater at good sites than bad sites accounting for 47% of the variability among

Indicator	Mean		Significance
	Good	Bad	
Number of Species	9.5	3.8	**
Abundance	63.9	20.6	*
Percent Catfish	10.3	8.6	
Percent Puffers	0.6	0.0	
Percent Sciaenids	16.5	25.4	
Percent Clupeids	3.2	11.1	
Percent Bothids	0.5	4.2	
Fish Index	5.9	1.0	*

---
* p<.05
** p<.01
***p<.001

Table 3.6 Results of sensitivity tests for fish community indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

Indicator	Pr > |F|	R^2
Number of Species	.007	0.47
Abundance	.021	0.35
Fish Index	.017	0.53

Table 3.7 Significance levels and R^2 associated with fish community indicators that successfully discriminated between site types.

sites (Table 3.7). Fish abundance was also about 3 times higher at good sites but only accounted for 35% of the observed variability. Although there were some problems associated with the implementation of the fish index as described in Section 2, the index was about 6 times greater at good sites and accounted for 53% of the observed variation.

3.1.3 DISSOLVED OXYGEN INDICATORS

Continuous monitors were deployed at all sites during the 1991 Demonstration for a 24-hour period that recorded dissolved oxygen concentrations and percent saturation every 15 minutes. Earlier studies (Summers and Engle 1992) showed that selected data collected from these continuous records can be used to assess the dissolved oxygen conditions at a site for the July-August index period. These characteristics were minimum dissolved oxygen concentration and the percentages of time between 6:00 PM and 6:00 AM that concentrations were below 2 ppm and below 5 ppm. In addition, instantaneous measures of dissolved oxygen concentration at the time of sampling (i.e., generally between 7:00 AM and 5:00 PM) were taken every meter from surface to bottom.

Indicator	Mean		Significance
	Good	Bad	
Minimum Concentration	5.5	0.5	***
Percent of Time with:			
Concentration < 2ppm	0.0	81.8	***
Concentration < 5ppm	12.0	100.0	***
Instantaneous Bottom Concentration	5.9	2.3	***

---
* p<.05
** p<.01
***p<.001

Table 3.8 Results of sensitivity tests for dissolved oxygen indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

All four of these dissolved oxygen measures could differentiate with varying levels of significance between good and poor sites (Table 3.8). Although the instantaneous dissolved oxygen measures were significantly higher at good sites (factor of 2), the instantaneous measures did not account for nearly all the variability as did the continuous measures. Minimum dissolved oxygen concentrations were five times lower at bad sites and accounted for 92% of the variability in the test data set

Indicator	Pr > |F|	R^2
Minimum Concentration	<.001	0.92
Percent of Time with:		
Concentration < 2 ppm	<.001	0.81
Concentration < 5 ppm	<.001	0.81
Instantaneous Bottom Concentration	<.001	0.69

Table 3.9 Significance levels and R^2 associated with dissolved oxygen indicators that successfully discriminated between site types.

(Table 3.9). The percentage of time that dissolved oxygen concentrations were below 2 ppm was about 80 times longer at poor sites than at good sites and accounted for 81% of the variation while the percentage of time less than 5 ppm was eight times more frequent at bad sites accounting for 81% of the variability. Thus, while instantaneous measures of dissolved oxygen can differentiate between clearly poor sites and reference areas, continuous measures are necessary to identify areas that have cyclic conditions characterized by low dissolved oxygen conditions at night (Summers et al. 1993).

3.1.4. HUMAN USE INDICATORS

Of the human use indicators measured in the 1991 Louisianian Province Demonstration, none could significantly differentiate between the good and bad sites (Table 3.10). Even though these indicators could not differentiate between the sites, all human use variables showed the expected distribution of values. Marine

Indicator	Mean	
	Good	Bad
Percent Occurrence of Marine Debris	29.0	50.9
Percent Surface Light Reaching 1 meter	24.1	19.1
Secchi Depth (m)	1.8	1.1
Tissue Contaminants:		
DDD	8.9	104.7
DDE	0.0	1.2
DDT	2.7	5.7
Aldrin	0.0	0.0
Chlordane	0.6	0.2
Dieldrin	0.6	1.3
Endosulfan	0.3	0.9
Endrin	0.5	0.3
Heptachlor	1.8	0.0
Heptachlor Epoxide	0.3	0.9
Hexachlorobenzene	434.5	36.1
Lindane	0.0	0.0
Toxaphene	0.0	0.0
Trans-Nonachlor	0.8	0.0
Total PCBs	64.6	52.5
Aluminum	8.0	3.2
Arsenic	0.7	0.9
Cadmium	0.0	0.1
Copper	4.8	0.6
Lead	0.0	0.0
Mercury	0.0	0.0
Nickel	0.7	0.6
Selenium	0.6	0.4
Silver	0.1	0.1
Tin	1.4	1.0
Zinc	54.0	56.2

---
* p<.05
** p<.01
***p<.001

Table 3.10 Results of sensitivity tests for human use aesthetic indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

debris occurred twice as frequently at bad sites than good sites. Water clarity, measured as the proportion of surface PAR (photosynthetically active radiation) reaching a depth of one meter and Secchi depth, was about 25% better at good sites than bad sites. Fish contaminant concentrations in edible fish tissues in Atlantic croaker, marine catfish, and shrimp were an average of seven times higher at bad sites than at good sites. For two contaminants, DDE (an intermediate DDT breakdown product) and heptachlor, no residues were found at any good sites while an average of 1.2 ppb and 1.8 ppb occurred at the bad sites, respectively. The high degree of variability in these human use indicators makes them poor discriminators of ecological condition. However these indicators represent important factors to human uses of estuaries that vary in the expected manner (e.g., better water clarity and lower tissue concentrations at good sites).

3.2 HABITAT INDICATORS

A number of habitat indicators were measured during the 1991 Demonstration, including indicators of water column and sediment characteristics. These indicators were selected as possible indirect measures of ecological condition or as possible covariates in the evaluation of response indicators. We tested the sensitivity of these indicators in order to evaluate their correlative strength with a response indicator. For example, degree of stratification (bottom salinity-surface salinity), may be related to dissolved oxygen concentration and show this relationship consistently at good and poor sites.

3.2.1 WATER COLUMN HABITAT INDICATORS

Five water column habitat indicators were measured during the 1991 demonstration. These were instantaneous water temperature, salinity, pH, and dissolved oxygen and the degree of stratification. Degree of stratification was measured as the simple difference between bottom and surface salinity rather than as sigma-T as no surface to bottom temperature differences occurred.

As expected, instantaneous bottom dissolved oxygen concentrations (as described in Section 3.1.3) differentiated between good and bad sites (Table 3.11). Average instantaneous dissolved oxygen concentrations on the bottom were 2.3 ppm at bad sites and 5.9 ppm at good sites accounting for 69% of the variation in good and bad sites. The only other water column habitat indicator that could differentiate between test data sites was stratification. Degree of stratification was different between site types with average conditions showing well mixed water columns at good sites (0.45 ppt difference) and stratified conditions at bad sites (9.3 ppt difference) accounting for 42% of observed variability.

Water temperature showed virtually the same means at good and bad sites; 29.9 C and 29.3 C, respectively. Similarly, ranges of bottom salinities in the test data set were almost identical with 2.6-40.5 ppt at good sites and 0.0-39.9 ppt at bad sites. The mean values of bottom pH were identical at

Indicator	Mean		Significance
	Good	Bad	
Bottom Water Temperature	29.9	29.3	
Bottom Salinity	22.4	17.6	
Bottom pH	7.9	7.9	
Percent of Surface Light Reaching 1 meter	24.1	19.2	
Stratification (Bottom-Surface Salinity)	0.5	9.3	**
Bottom Instantaneous Dissolved Oxygen	5.9	2.3	***

---
* p<.05
** p<.01
***p<.001

Table 3.11 Results of sensitivity tests for water column habitat indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

7.9 for good and bad sites.

3.2.2 SEDIMENT CHARACTERISTICS

As with the water column habitat indicators, sediment characteristics are primarily measured for their potential use in evaluating replicate differences as indirect measures of condition and in benthic community data and sediment contaminant concentrations at a site. As part of the 1991 Demonstration, percentage of total organic carbon, percent silt-clay content, concentration of acid volatile sulfides, and the depth of the redox potential discontinuity layer were measured for each sediment grab or composite. Only the percent total organic carbon (TOC) in the sediment could successfully discriminate between good and bad sites (Table 3.12). Good sites had an average TOC percentage of 0.9% while bad sites showed heavily enriched conditions with 3.2% TOC values. TOC content of the sediments only accounted for 25% of the observed variation in the test data set. Acid volatile sulfides in sediments were three times higher at bad sites than good sites although this difference was not significant.

3.3 EXPOSURE INDICATORS

Several measures of the magnitude and extent of pollution exposure were collected at each site during the 1991 Louisianian Province Demonstration in order to ascertain some preliminary links between observed estuarine degradation and observed pollution exposure. While this may be the primary purpose of this data, we examined the ability of these indicators to discriminate between good and bad sites because most historical monitoring data in the Louisianian Province is of this type. If an exposure indicator could be found that differentiated between site types then some potential to examine trends backward in time could exist. The exposure indicators examined were dissolved oxygen concentrations; sediment toxicity as measured by bioassay; sediment concentrations of 27 alkanes and isoprenoids, 44 polynuclear aromatic hydrocarbons (PAHs), 20 polycyclic

Indicator	Mean		Significance
	Good	Bad	
Acid Volatile Sulfides	1.0	3.1	
Percent Total Organic Carbon	0.9	3.2	*
Percent Silt-Clay	73.1	72.7	
Mean Depth of Redox Potential Discontinuity Layer (mm)	43.4	51.9	

---
* p<.05
** p<.01
***p<.001

Table 3.12 Results of sensitivity tests for sediment habitat indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

chlorinated biphenyls (PCBs), 3 butyltins, 21 chlorinated pesticides, and 14 heavy metals.

3.3.1 DISSOLVED OXYGEN CONCENTRATIONS

All measures, instantaneous and continuous, of dissolved oxygen concentration differentiated between good and bad sites (see Table 3.8). These data, discussed earlier, provide information at all three levels of indicators: response (as a eutrophication endpoint), habitat (as a barrier to fish movement), and exposure (as a factor for benthic mortality, growth, and reproduction).

3.3.2 SEDIMENT TOXICITY

Sediment bioassays are the most direct measure for estimating the potential for contaminant-induced effects in biological communities. Direct measures of sediment contaminant concentrations can be misleading because many chemicals are bound tightly to sediment particles, are chemically-complexed, or the contaminant resulting in toxicity may be present but not analyzed for. Two types of sediment toxicity bioassays were run using sediment from the 1991 Demonstration sites. These were a 10-day *Ampelisca* (amphipod) test and a 3-day *Mysidopsis* (mysid) test.

Neither bioassay could successfully discriminate between the site categories in the test data set although the results of both bioassays tended towards expected directions (Table 3.13). Average amphipod corrected survival rates of 98% and 83% for good and bad sites, respectively, were not significantly different because the survival rates at bad sites ranged from 11-104%. While the range of survival rates at good sites was significantly narrower (86-109%), the paucity of low survival sites resulted in an inability to differentiate between good and bad sites. Without further poor survival sites, this comparison may be misleading. Like the amphipod bioassay, the mysid test also failed to discriminate between good and bad sites with average survival rates of 96% and 81%, respectively. The ranges of survival rates were similar to that observed for amphipod with good sites having a rather

Indicator	Mean Good	Mean Bad	Significance
10-day Ampelisca Test Survival Rate	97.8	82.7	
3-day Mysid Test Survival Rate	96.2	80.7	

---
* p<.05
** p<.01
***p<.001

Table 3.13 Results of sensitivity tests for sediment toxicity exposure indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

narrow range (90-103%) and bad sites displaying a wide range (0-111%). As with amphipod, the mysid test appears to be varying in the appropriate direction to distinguish between good and bad sites but without additional low survival sites in the test data set, this indicator will not clearly discriminate between site categories and the likelihood of assessing a site as a false negative exists.

3.3.3 SEDIMENT CONTAMINANTS

A composite sediment sample from each location in the 1991 Louisianian Province Demonstration was analyzed for the contaminants listed in Table 3.14. The contaminants can be categorized into five groups: alkanes and isoprenoids, PAHs, PCBs, pesticides, and heavy metals. All of the constituents of each group was tested for indicator sensitivity as well as a combined measure (e.g., total alkanes). In addition, both measured concentrations (observed) and aluminum-corrected concentrations of heavy metals were evaluated.

Fifteen alkanes and total alkanes and isoprenoids successfully discriminated between good and bad sites (Table 3.15). While both types of sites (good and bad) had mean alkanes concentrations below the degraded criterion determined in Summers et al. (1993b) of 7000 ppb, the average increase in individual alkane concentrations from good to bad sites was a factor of 12.5 and was a factor of 9.4 for total alkanes.

Four of the 44 measured PAHs proved to be good discriminators of ecological condition: benzo(b)fluoranthene, benzo(e)pyrene, benzo(g,h,i)perylene, and (i)1,2,3,c-,d-pyrene (Table 3.16). Although the concentrations of PAHs in the sediments in the test data set were generally below criteria levels, the average difference between good and bad sites for these four PAHs was a factor of 8.9. Total PAHs showed no significant difference between good and bad sites.

Although all concentrations were low, 2 of the 20 PCB congeners showed significant differences between good and bad sites (Table 3.17): PCB 128 and 187. PCB 187 is a composite of three PCBs that coelute (PCB 187, 182, and 159). While the concentrations of PCBs 128 and 187 were negligible at good sites (0.02 ± and 0.01 ± ppb, respectively), the concentrations of these contaminants at bad sites were significantly higher (0.19 ± 0.06 ppb for PCB 128 and 0.30 ± 0.12 ppb for PCB 187.

Polynuclear Aromatic Hydrocarbons (ppb)

Acenaphthene
Acenaphthylene
Anthracene
Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(e)pyrene
Benzo(g,h,i)perylene
Biphenyl
Chrysene
C1-Chrysene
C2-Chrysene
C3-Chrysene
C4-Chrysene
Dibenzo(a,h)anathracene
Dibenzothio
C1-Dibenzothio
C2-Dibenzothio
C3-Dibenzothio
Fluoranthene
Fluorene
C1-Fluorene
C2-Fluorene
C3-Fluorene
Naphthalene
C1-Naphthalene
C2-Naphthalene
C3-Naphthalene
C4-Naphthalene
Perylene
Phenanthrene
C1-Phenanthrene
C2-Phenanthrene
C3-Phenanthrene
C4-Phenanthrene
Pyrene
(i)1,2,3,c-,d-pyrene
1-methylnaphthalene
2-methylnaphthalene.
1-methylphenanthrene
2,3,5-trimethylnaphthalene
2,6-dimethylnaphthalene
Total PAHs

Trace Elements (ppm)

Aluminum
Antimony
Arsenic
Cadmium
Chrimium
Copper
Lead
Manganese
Mercury
Nickel
Selenium
Silver
Tin
Zinc

PCBs

Total PCBs
20 Congeners

Alkanes

Total Alkanes and Isoprenoids
C10-C34
Phytane
Pristane

Butyltins

Monobutyltin
Dibutyltin
Tributyltin

Pesticides

DDD
DDE
DDT
Aldrin
BHC
Chlordane
Dieldrin
Endosulfan
Endrin
Heptachlor
Heptachlor Epoxide
Hexachlorobenzene
Mirex
Toxaphene
Trans-Nonachlor

Table 3.14 List of contaminants analyzed from sediments during the 1991 Louisianian Province Demonstration.

Indicator	Mean		Significance	R^2
	Good	Bad		
Total Alkanes	607.3	5715.5	**	0.40
C10	4.2	14.3	*	0.26
C11	6.2	8.4		
C12	6.5	9.1		
C13	3.7	7.0		
C14	9.9	10.5		
C15	24.3	55.9		
C16	19.8	18.0		
C17	58.1	265.5	**	0.39
Phytane	32.0	54.1		
C18	17.5	20.0		
Pristane	38.9	37.7		
C19	25.0	48.1		
C20	19.6	31.5		
C21	28.1	70.8		
C22	12.4	41.3	*	0.36
C23	21.7	152.3	**	0.40
C24	13.6	88.9	**	0.44
C25	31.9	271.1	**	0.42
C26	10.9	120.3	**	0.47
C27	36.5	477.1	**	0.41
C28	13.3	183.3	**	0.46
C29	60.6	1291.0	*	0.33
C30	16.4	230.8	**	0.36
C31	59.9	1335.8	*	0.34
C32	14.1	220.6	**	0.40
C33	20.4	585.7	**	0.37
C34	1.7	66.4	*	0.34

---

* p<.05
** p<.01
***p<.001

Table 3.15 Results of sensitivity tests for sediment alkane exposure indicators. Good refers to ecological sites with minimal impacted and bad sites refers to ecological sites impacted by hypoxia and contaminants.

Indicator	Mean		Significance	R^2
	Good	Bad		
Acenaphthene	0.3	1.5		
Acenaphthylene	0.5	3.0		
Anthracene	0.7	6.1		
Benzo(a)anthracene	1.7	20.7		
Benzo(a)pyrene	1.9	19.5		
Benzo(b)fluoranthene	2.6	33.6	*	0.33
Benzo(e)pyrene	2.1	20.0	*	0.27
Benzo(g,h,i)perylene	2.2	12.2	*	0.38
Benzo(k)fluoranthene	1.5	15.1		
Biphenyl				
Chrysene	2.4	23.6		
C1-Chrysene	3.1	19.1		
C2-Chrysene	4.3	15.1		
C3-Chrysene	2.5	5.5		
C4-Chrysene	2.9	6.0		
Dibenzo(a,h)anathracene	0.5	2.6		
Dibenzothio	9.1	1.3		
C1-Dibenzothio	9.0	4.1		
C2-Dibenzothio	12.0	11.3		
C3-Dibenzothio	9.0	15.2		
Fluoranthene	3.8	35.4		
Fluorene	1.0	2.5		
C1-Fluorene	4.2	3.8		
C2-Fluorene	11.2	10.1		
C3-Fluorene	16.8	17.1		
Naphthalene	1.4	15.7		
C1-Naphthalene	2.5	19.5		
C2-Naphthalene	4.2	11.9		
C3-Naphthalene	12.2	15.5		
C4-Naphthalene	18.9	16.2		
Perylene	1.4	58.5		
C1-Phenanthrene	13.2	15.1		
C2-Phenanthrene	15.3	22.2		
C3-Phenanthrene	11.6	20.7		
C4-Phenanthrene	9.1	21.1		
Pyrene	5.9	41.0		
(i)1,2,3,c-,d-pyrene	1.7	12.8	*	0.37
1-methylnaphthalene	1.0	4.3		
1-methylphenanthrene	3.3	3.5		
2-methylnaphthalene	1.5	6.2		
2,3,5-trimethylnaphthalene	3.9	3.3		
2,6-dimethylnaphthalene	1.8	4.3		

* p<.05
** p<.01
***p<.001

Table 3.16 Results of sensitivity tests for sediment polynuclear aromatic hydrocarbon indicator. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

Indicator	Mean Good	Mean Bad	Significance	R^2
PCB Congener #				
8	0.20	0.11		
18	0.01	0.02		
28	0.01	0.14		
44	0.01	0.21		
52	0.04	0.48		
66	0.00	0.14		
101	0.04	0.74		
105	0.00	0.29		
110 & 77	0.07	1.35		
118,108, & 149	0.03	0.40		
126	0.00	0.08		
128	0.02	0.19	*	0.36
138	0.36	0.85		
153	0.02	0.65		
170	0.11	0.47		
180	0.04	0.29		
187,182, & 159	0.01	0.30	*	0.29
195	0.01	0.03		
205	0.02	0.03		
209	0.03	0.02		

---
* p<.05
** p<.01
***p<.001

Table 3.17 Results of sensitivity tests for sediment polycyclic chlorinated biphenyl exposure indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

The concentration of tributyltin showed no pattern related to site type (Table 3.18). In fact, observed tributyltin concentrations were slightly higher at good sites than those seen at bad sites. Five of the 21 pesticides tested showed significant differences between the site categories in the test data set: 2,4-DDD, 4,4-DDE, total DDT, total chlordane, and total BHC (lindane)(Table 3.19). Total DDT concentrations in sediments between good and bad sites differed by a factor of 125, while the DDT degradation products DDE and DDD differed by factors of 58 and 170, respectively. Although the mean concentrations of total chlordanes at good and bad sites was low (< 1ppb), the mean concentrations at bad sites was 93 times higher than at good sites. Similarly, the average concentrations of total BHC at bad sites was only 0.03 ppb but this concentration was three times greater than concentrations observed at good sites. Four pesticides were not observed at any of the test data sites (beta-BHC, alpha- and beta-endosulfan, and toxaphene);

therefore, the analysis does not test the ability of these pesticides to differentiate among site types.

Heavy metal concentrations were evaluated from two perspectives: (1) the difference between predicted and observed anthropogenic concentrations corrected using aluminum, and (2) the ratio between observed concentrations and criteria levels. Differences between predicted anthropogenic concentrations and observed aluminum-adjusted concentrations for eight of the fifteen metals differentiated between good and bad sites (Table 3.20). Metal concentrations at good sites averaged 2.5 ppm below the predicted anthropogenic levels while concentrations at bad sites averaged 7.2 ppm above that level. When compared to criteria level concentrations, six of the tested metals described above were also successful discriminators of site type (cadmium, chromium, nickel, lead, tin, and zinc)(Table 3.21). Three metals (arsenic, silver, and copper) could differentiate between good and bad sites only when compared to a criterion level. Metals at bad sites occurred at higher ratio (observed: criterion levels) than at good sites by an average factor of 3.3. Total metals were 16 times higher at bad sites than at good sites.

3.4 CONFOUNDING FACTORS AFFECTING SENSITIVITY ANALYSES

We have described the results of crude sensitivity analyses on all of the core and developmental indicators used in the 1991 Louisianian Province Demonstration. In general, many of the indicators used could differentiate between sites with ecological status at extreme ends of the condition gradient. Only habitat indicators (as expected) and PAH indicators could not differentiate effectively between opposite ends of the ecological condition gradient.

Indicator	Mean		Significance	R^2
	Good	Bad		
Tributyltin	3.1	2.8		

---
* p<.05
** p<.01
***p<.001

Table 3.18 Results of sensitivity tests for sediment tributyltin indicator. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

Indicator	Mean		Significance	R^2
	Good	Bad		
2,4'DDD	0.00	0.17	*	0.25
4,4'DDD	0.01	1.28		
2,4'DDE	0.00	1.96		
4,4'DDE	0.06	3.38	**	0.44
2,4'DDT	0.01	0.03		
4,4'DDT	0.00	2.05		
Aldrin	0.00	0.02		
alpha-BHC	0.00	0.01		
beta-BHC	0.00	0.00		
delta-BHC	0.00	0.01		
gamma-BHC	0.00	0.01		
alpha-Chlordane	0.00	0.29		
gamma-Chlordane	0.00	0.29		
Dieldrin	0.01	0.08		
Endosulfan I	0.00	0.00		
Endosulfan II	0.00	0.00		
Endrin	0.00	0.03		
Hexachlorobenzene	0.12	0.03		
Heptachlor	0.00	0.01		
Heptachlor Epoxide	0.00	0.00		
Mirex	0.00	0.01		
cis-Nonachlor	0.00	0.08		
trans-Nonachlor	0.00	0.13		
Oxychlordane	0.00	0.00		
Toxaphene	0.00	0.00		
Total DDT	0.07	8.87	**	0.35
Total BHC	0.01	0.03	*	0.29
Total Chlordane	0.01	0.81	*	0.26

---

* p<.05
** p<.01
***p<.001

Table 3.19 Results of sensitivity tests for pesticide indicators. Good refers to ecological sites with minimal impacts and bad sites refers to ecological sites impacted by hypoxia and contaminants.

Indicator	Mean		Significance	R^2
	Good	Bad		
Antimony	0.18	0.28		
Arsenic	-0.59	4.02		
Cadmium	-0.03	0.12	**	0.41
Chromium	-7.46	13.57	*	0.30
Copper	-1.39	4.20		
Iron	-0.22	0.48	*	0.25
Manganese	-63.69	5.63		
Mercury	0.11	0.31		
Nickel	-2.81	3.97	*	0.28
Lead	-1.61	5.70	*	0.35
Silver	-0.02	0.06		
Tin	-0.15	0.56	*	0.48
Zinc	-7.72	32.83	**	0.37
Average Number of Metals Exceeding Criterion Value	0.13	2.13	**	0.45

*p <.05 ** p<.01 ***p<.001

Table 3.20 Results of sensitivity tests for the difference between sediment heavy metal indicator (OBS) and the predicted concentration based of background aluminum level. Good refers to ecological sites with minimal impacts and bad sites refers to ecolog

Indicator	Mean		Significance	R^2
	Good	Bad		
Antimony	0.34	0.48		
Arsenic	0.12	0.35	*	0.29
Cadmium	0.01	0.06	*	0.50
Chromium	0.30	0.85	*	0.40
Copper	0.08	0.25	**	0.46
Mercury	1.03	2.50		
Nickel	0.24	0.80	**	0.39
Lead	0.27	0.73	***	0.54
Silver	0.08	0.21	*	0.29
Tin	0.28	0.74	**	0.45
Zinc	0.23	0.90	**	0.51
Average Number of Metals Exceeding 95% Confidence Interval	0.13	1.00		

* p< .05 ** p< .01 ***p< .001

Table 3.21 Results of sensitivity tests for sediment heavy metal indicator (OBS) a proportion of critical value (CV). Good refers to ecological sites with minimal impacts and bad

3.4.1 GEOGRAPHICAL GRADIENTS

One possible confounding factor that could affect this assessment would be the existence of a significant longitudinal gradient in the indicators. The good and bad sites in the test data set are comprised of about 50% of locations in each class being east of the Mississippi delta. Therefore, if significant differences exist due to site position relative to the Mississippi delta (i.e., east or west), the inability to discriminate between site types might be due to an existing east-west gradient. In this case, sufficient good and bad sites would have to be located east and west of the Mississippi delta and the sensitivity recomputed. In the event that a east-west gradient existed and the indicator could still differentiate between good and bad sites, the observed sensitivity to site types might be even stronger.

Table 3.22 displays a summary of all indicators by indicator class and shows those that portray strong east-west gradients coupled with their ability to differentiate between site types. An asterisk in the longitudinal gradient column alone suggests that the indicator should be retested after being subsetted by region. Unfortunately, sufficient bad sites do not exist east of the delta to permit this re-analysis. Possibly, the re-analysis can be completed by combining the 1991 and 1992 data sets. Few indicators showed strong east-west gradients and an inability to assess site differences. One response indicator (proportion of total benthic abundance as polychaetes) showed significantly higher mean proportion of polychaetes the benthic community in eastern areas (77%) than in western areas (28%) with the gradient explaining 40% of the observed variability. Two other response indicators (number of fish species and minimum dissolved oxygen concentration) showed strong longitudinal gradients, but they also easily differentiated between site types. The average number of fish species per trawl was two times greater in the west than in the east. Similarly, minimum dissolved oxygen concentrations were, on the average, 2.7 times lower in estuaries east of the Mississippi River than those to the west.

One sediment habitat indicator (concentration of acid volatile sulfides) showed concentrations four times higher in eastern than in western sediments and an inability to differentiate between good and bad sites. This AVS gradient may correspond to similar gradients seen for arsenic, chromium, lead, selenium, tin, and zinc; all of which showed significantly higher concentrations east of the Mississippi delta than to the west. A gradient exists for total organic carbon content of sediments in which estuarine sediments east of the delta have 4 times the organic carbon content of sediments to the west. The existence of this gradient suggests that the discriminatory strength of TOC would be stronger if the effects of the east-west gradient were removed.

Only two exposure indicators (both sediment contaminants) showed strong longitudinal gradients without the ability to differentiate between site types. One pesticide (gamma-BHC) was not found in the west and found at only a few locations in the east. This apparent gradient probably represents the low number of

sites where this pesticide is found rather than a true longitudinal gradient. However, the same cannot be said for the one PAH (i.e., perylene) which showed a strong east-west gradient. Perylene, though generally low in concentration, showed concentrations about 4 times higher in the east than in the west. No PAHs other than perylene showed a significant east-west gradient. Nearly all the alkanes showed strong east-west gradients with eastern sites averaging 3 to 5 times higher concentrations than those in the west; however the alkanes already show the capacity to differentiate between good and bad sites. Most heavy metals also showed strong east-west gradients with higher concentrations in the east half of the Louisianian Province. Like alkanes, heavy metals are a good indicator of ecological condition so that correction for a east-west gradient should only increase that differentiating power.

In summary, longitudinal gradients (whether natural or anthropogenic) do not appear to affect significantly the ability of the selected indicators to differentiate between extreme ecological conditions.

3.4.2 SOURCE ASSOCIATIONS

While the test data set has been constructed to reflect extreme conditions (i.e., hypoxia, high sediment contaminant concentrations, and sediment toxicity), some indicators may be related to only one of these criteria. Therefore, its ability to separate good and bad sites will only occur along that gradient and its ability to differentiate among several contaminant gradients would be weakened. We evaluated these relationships by testing the indicators abilities to differentiate along three individual exposure gradients (Table 3.23):

- Dissolved oxygen conditions (DO) categorized as having significant versus minor sources of organic material effluents in the vicinity of the site,
- Sediment contaminant conditions due to point sources as categorized as having numerous versus few industrial outfalls near the site (IND), and
- Sediment contaminant conditions due to non-point sources as categorized as having high versus low pesticide applications in the counties of the watershed (AGRO).

No response indicators showed an inability to differentiate ecological conditions due to the overriding effect of a point source or non-point source gradients. Only one biotic condition indicator (proportion of total catch as marine catfish) showed a strong gradient associated with hypoxia being five times lower under hypoxic conditions without any relationships to industrial or agricultural contaminants. Only four other biotic condition variables showed relationships to a dissolved oxygen gradient (fish index, and three dissolved oxygen indicators). The fish index values are 4 times greater under high dissolved oxygen conditions than under hypoxic conditions. As expected, minimum dissolved oxygen concentrations are lower and the percentage of time dissolved oxygen is less than 2 ppm or 5 ppm are higher under hypoxic conditions.

Only one habitat indicator (bottom pH) showed a strong relationship to the dissolved oxygen gradient with lower pHs co-occurring with hypoxic conditions but no significant relationship to industrial or agricultural contaminant sources.
As might be expected, seven PAHs, total PAHs, three alkanes, and two metals showed significant relationships to industrial point sources. In all these cases, areas receiving high industrial discharges were characterized by significantly higher sediment contaminants than sites receiving low discharges. Nine PAHs, total PAHs, total DDT, and one alkane showed similar relationships to dissolved oxygen gradients portraying higher concentrations under hypoxic conditions. Only manganese showed lower concentrations under hypoxic conditions.

In summary, with the possible exception of selected PAHs, region-wide gradients were associated with the location of industrial discharges and areas of high agricultural loadings. However, regional gradients of dissolved oxygen may mask the ability of some industrial contaminants (e.g., PAHs) to differentiate between sites receiving high industrial contaminant discharges and those receiving relatively few discharges.

Indicator	Discriminatory Power	Longitudinal Gradient		Significance
		East	West	
Response Indicators				
Number of Benthic Species	*	6.0	112.8	
Benthic Abundance		44.3	42.9	
Percent Amphipods		5.7	6.6	
Percent Bivalves	*	3.4	14.1	
Percent Polychaetes		76.9	28.5	*
Percent Tubificids		0.3	0.0	
Abundance of Large Bivalves		1.9	0.0	
Biodiversity	*	0.4	0.6	
Benthic Index	*	5.7	6.6	
Number of Fish Species	*	4.4	8.9	*
Fish Abundance	*	47.6	100.0	
Percent Catfish		7.5	11.0	
Percent Puffers		0.0	0.3	
Percent Sciaenids		26.8	25.7	
Percent Clupeids		15.3	12.7	
Percent Bothids		0.0	0.0	
Fish Index	*	2.9	5.4	
Minimum Dissolved Oxygen	*	1.5	4.0	*
Percent of Time < 2ppm	*	31.9	14.4	
Percent of Time < 5ppm	*	60.1	49.0	
Instantaneous Bottom Dissolved Oxygen	*	4.7	4.9	
Marine Debris		0.2	0.2	
Percent Light Transmittance		23.5	12.8	
Secchi Depth		1.3	0.6	*
Tissue Contaminants				
DDD		26.2	45.2	
DDE		4.6	1.1	
DDT		13.0	7.7	
Aldrin		0.6	0.2	
Chlordane		4.4	1.4	
Dieldrin		1.4	1.8	
Endosulfan		0.6	2.5	
Endrin		0.9	0.3	
Heptachlor		1.2	0.0	
Heptachlor Epoxide		0.6	2.9	
Hexachlorobenzene		15.2	294.2	
Lindane		2.2	0.0	
Mirex		4.7	7.7	
Toxaphene		83.3	362.1	
Trans-Nonachlor		4.1	1.2	
Total PCBs		60.9	36.7	
Aluminum		24.1	4.7	
Arsenic		0.3	0.6	
Cadmium		0.0	0.1	
Copper		0.6	0.3	
Lead		0.0	0.0	
Mercury		0.1	0.1	
Nickel		0.7	0.6	

Table 3.22 Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province for discriminatory power (* = significant at p < .05) and the co-occurrence of a significant longitudinal east-west gradient (*).

Indicator	Discriminatory Power	Longitudinal Gradient		Significance
		East	West	
Response Indicators				
Selenium		0.6	0.6	
Silver		0.2	0.1	
Tin		1.8	1.2	
Zinc				
Habitat Indicators				
Water Temperature		29.2	30.3	
Salinity		18.1	19.3	
pH		7.7	7.8	
Light Transmittance		23.5	12.8	
Secchi Depth		1.3	0.6	*
Stratifiation	*	6.1	5.9	
Instantaneous Bottom DO	*	4.7	4.9	
Acid Volatile Sulfides		4.2	1.0	*
Percent Organic Carbon	*	3.5	0.9	*
Percent Silt-Clay		81.3	80.7	
RPD Depth		45.1	29.7	
Exposure Indicators				
Ampelisca Bioassay		98.1	95.4	
Mysid Bioassay		101.9	99.0	
Alkanes				
Total	*	5006.4	1100.3	*
C10	*	19.9	3.4	
C11		16.7	9.6	
C12		7.2	6.1	
C13		8.4	8.9	
C14		11.6	18.9	
C15		48.7	48.2	
C16		17.4	41.7	
C17	*	177.4	123.1	
Phytane		29.4	136.3	
C18		19.0	59.9	
C19		34.0	82.0	
C20		26.6	45.4	
C21		78.3	55.2	
C22	*	46.8	15.9	*
C23	*	132.5	18.7	*
C24	*	92.6	9.5	*
C25	*	249.5	24.5	*
C26	*	119.4	11.4	*
C27	*	400.8	36.1	*
C28	*	184.0	14.1	*
C29	*	1190.7	64.3	*
C30	*	211.5	23.4	*
C31	*	1162.3	66.1	*
C32	*	181.5	24.1	*
C33	*	443.5	28.7	*
C34	*	53.2	2.3	*

Table 3.22(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province for discriminatory power (* = significant at $p < .05$) and the co-occurrence of a significant longitudinal east-west gradient (*).

Indicator	Discriminatory Power	Longitudinal Gradient		Significance
		East	West	
Exposure Indicators				
PAHs				
Acenaphthene		1.6	0.7	
Acenaphthylene		3.0	1.2	
Anthracene		6.7	2.4	
Benzo(a)anthracene		23.0	6.3	
Benzo(a)pyrene		22.4	8.2	
Benzo(b)fluoranthene	*	32.3	9.5	
Benzo(e)pyrene	*	20.8	7.6	
Benzo(g,h,i)perylene	*	13.0	9.2	
Benzo(k)fluoranthene		20.0	5.9	
Biphenyl				
Chrysene		26.1	9.2	
C1-Chrysene		21.7	9.4	
C2-Chrysene		17.3	13.0	
C3-Chrysene		5.7	8.5	
C4-Chrysene		6.2	5.8	
Dibenzo(a,h)anathracene		2.9	1.6	
Dibenzothio		2.0	4.3	
C1-Dibenzothio		5.2	20.6	
C2-Dibenzothio		11.8	38.9	
C3-Dibenzothio		15.2	27.2	
Fluoranthene		44.2	14.1	
Fluorene		3.1	2.6	
C1-Fluorene		5.2	15.3	
C2-Fluorene		11.6	55.1	
C3-Fluorene		17.8	62.4	
Naphthalene		20.5	2.1	
C1-Naphthalene		20.5	2.1	
C2-Naphthalene		23.6	6.8	
C3-Naphthalene		27.0	40.7	
C4-Naphthalene		25.3	90.8	
Perylene		46.7	11.4	*
C1-Phenanthrene		19.5	59.9	
C2-Phenanthrene		26.5	72.2	
C3-Phenanthrene		22.8	42.9	
C4-Phenanthrene		23.5	22.1	
Pyrene		45.8	20.0	
(l)1,2,3,c-,d-pyrene	*	14.3	6.5	
1-methylnaphthalene		10.6	1.4	
1-methylphenanthrene		4.8	12.2	
2-methylnaphthalene		15.2	2.2	
2,3,5-trimethylnaphthalene		6.6	13.5	
2,6-dimethylnaphthalene		8.2	2.5	
Tributyltin		2.9	2.1	

Table 3.22(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province for discriminatory power (* = significant at p < .05) and the co-occurrence of a significant longitudinal east-west gradient (*).

Indicator	Discriminatory Power	Longitudinal Gradient		Significance
		East	West	
Exposure Indicators				
PCBs				
Total		44.8	19.2	
Congener 8		0.4	0.1	
Congener 18		0.1	0.0	
Congener 28		0.4	0.0	
Congener 44		0.4	0.0	
Congener 52		1.1	0.1	
Congener 66		0.7	0.0	
Congener 101		2.2	0.2	
Congener 105		0.9	0.1	
Congener 110/77		3.3	0.2	
Congener 118/108/149		1.6	0.1	
Congener 126		0.0	0.0	
Congener 128	*	0.4	0.0	
Congener 138		2.1	0.3	
Congener 153		1.6	0.1	
Congener 170		0.4	0.3	
Congener 180		0.3	0.1	
Congener 187/182/159	*	0.3	0.0	
Congener 195		0.0	0.0	
Congener 206		0.0	0.0	
Congener 209		0.0	1.2	
Pesticides				
2,4' DDD	*	0.2	0.1	
4,4' DDD		1.2	0.3	
2,4' DDE		0.9	0.0	
4,4' DDE	*	2.6	1.0	
2,4' DDT		0.0	0.0	
4,4' DDT		0.3	0.0	
Aldrin		0.0	0.0	
alpha-BHC		0.0	0.0	
beta-BHC		0.0	0.0	
delta-BHC		0.0	0.0	
gamma-BHC		0.1	0.0	*
alpha-Chlordane		0.1	0.1	
gamma-Chlordane		0.1	0.0	
Dieldrin		0.1	0.1	
Endosulfan I		0.0	0.0	
Endosulfan II		0.0	0.0	
Endrin		0.0	0.0	
Hexachlorobenzene		0.0	1.6	
Heptachlor		0.0	0.0	
Heptachlor Epoxide		0.0	0.5	
Mirex		0.0	0.0	
cis-Nonachlor		0.0	0.1	
trans-Nonachlor		0.1	0.1	
Oxychlordane		0.0	0.0	

Table 3.22(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province for discriminatory power (* = significant at p < .05) and the co-occurrence of a significant longitudinal east-west gradient (*).

Indicator	Discriminatory Power	Longitudinal Gradient		Significance
		East	West	
Exposure Indicators				
Pesticides				
Toxaphene		0.0	0.0	
Total DDT	*	5.2	1.4	
Total BHC	*	0.0	0.0	
Total Chlordane	*	0.4	0.7	
Heavy Metals(OBS-P)[1]				
Antimony		0.2	0.1	
Arsenic		7.44	0.3	
Cadmium	*	0.0	0.0	
Chromium	*	23.6	-4.4	*
Copper		3.8	-0.1	
Iron	*	1.0	-0.2	*
Lead	*	6.0	-1.5	*
Manganese		-111.3	-81.0	
Mercury		0.3	0.2	
Nickel	*	1.7	-1.1	
Selenium		0.4	0.0	*
Silver		0.0	0.0	
Tin	*	0.6	-0.1	*
Zinc	*	15.5	-9.1	
Heavy Metals (%CV)[2]				
Antimony		45.6	35.0	
Arsenic	*	45.5	20.2	
Cadmium	*	3.6	2.7	
Chromium	*	97.2	50.0	
Copper	*	24.2	14.0	
Lead	*	74.3	40.8	
Mercury		227.5	168.3	
Nickel	*	72.2	46.3	
Silver	*	18.7	11.2	
Tin	*	73.3	40.8	
Zinc	*	75.8	37.6	

[1] Observed - Predicted concentrations based on aluminum regression (positive value denoted anthropogenic sources)
[2] Percentage of criterion value

Table 3.22(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province for discriminatory power (* = significant at p < .05) and the co-occurrence of a significant longitudinal east-west gradient (*).

Indicator	Discriminatory Power	Source Gradients		
		DO	IND	AGRO
Response Indicators				
Number of Benthic Species	*			
Benthic Abundance				
Percent Amphipods				
Percent Decapods				
Percent Bivalves	*			
Percent Polychaetes				
Percent Tubificids				
Abundance of Large Bivalves				
Biodiversity	*			
Benthic Index	*			
Number of Fish Species	*			
Fish Abundance	*			
Percent Catfish		+		
Percent Puffers				
Percent Sciaenids				
Percent Clupeids				
Percent Bothids				
Fish Index	*	+		
Minimum Dissolved Oxygen	*	+		
Percent of Time < 2 ppm	*	+		
Percent of Time < 5	*	+		
Instantaneous Bottom				
Dissolved Oxygen	*			
Marine Debris				
Percent Light Transmittance				
Secchi Depth				
Tissue Contaminants				
DDD				
DDE				
DDT			+	
Aldrin				
Chlordane			+	
Dieldrin				
Endosulfan			+	
Endrin				
Heptachlor				
Heptachlor Epoxide				
Hexachlorobenzene		+		
Lindane				
Mirex				
Toxaphene				
Trans-Nonachlor			+	
Total PCBs				
Aluminum				
Arsenic				
Cadmium				

Table 3.23 Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province significant discriminatory power (*) and the co-occurrence of significant source gradients (+).

Indicator	Discriminatory Power	Source Gradients		
		DO	IND	AGRO
Response Indicators				
Copper				
Lead				
Mercury				
Nickel				
Selenium				
Silver				
Tin				
Zinc				
Habitat Indicators				
Water Temperature				
Salinity				
pH		+		
Light Transmittance				
Secchi Depth				
Stratification	*			
Instantaneous Bottom DO	*			
Acid Volatile Sulfides				
Percent Organic Carbon	*			
Percent Silt-Clay				
RPD Depth				
Exposure Indicators				
Ampelisca Bioassay				
Mysid Bioassay		+		
Alkanes				
Total	*			
C10	*			
C11				
C12			+	
C13			+	
C14			+	
C15				
C16				
C17	*	+		
Phytane				
C18				
Pristane				
C19				
C20				
C21				
C22	*			
C23	*			
C24	*			
C25	*			
C26	*			
C27	*			
C28	*			
C29	*			
C30	*			

Table 3.23(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province significant discriminatory power (*) and the co-occurrence of significant source gradients (+).

Indicator	Discriminatory Power	Source Gradients		
		DO	IND	AGRO
Exposure Indicators				
C31	*			
C32	*			
C33	*			
C34	*			
PAHs				
Acenaphthene		+		
Acenaphthylene				
Anthracene				
Benzo(a)anthracene				
Benzo(a)pyrene				
Benzo(b)fluoranthene	*			
Benzo(e)pyrene	*			
Benzo(g,h,i)perylene	*	+		+
Benzo(k)fluoranthene				
Biphenyl				
C1-Chrysene	*			+
C2-Chrysene	*			+
C3-Chrysene	*			+
C4-Chrysene	*			
Dibenzo(a,h)anathracene		+	+	+
Dibenzothio			+	
C1-Dibenzothio				
C2-Dibenzothio				
C3-Dibenzothio			+	
Fluoranthene				
Fluorene			+	
C1-Fluorene				
C2-Fluorene				
C3-Fluorene				
Naphthalene				
C1-Naphthalene				
C2-Naphthalene	•			
C3-Naphthalene				
C4-Naphthalene				
Perylene				
Phenanthrene			+	
C1-Phenanthrene				
C2-Phenanthrene				
C3-Phenanthrene				
C4-Phenanthrene				
Pyrene			+	
(i)1,2,3,c-,d-pyrene	•	+		+
1-methylnaphthalene				
1-methylphenanthrene				
2-methylnaphthalene				
2,3,5-trimethylnaphthalene				
2,6-dimethylnaphthalene				
Total PAH			+	
Tributyltin				

Table 3.23(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province significant discriminatory power (*) and the co-occurrence of significant source gradients (+).

Indicator	Discriminatory Power	Source Gradients DO	IND	AGRO
Exposure Indicators				
PCBs				
Total				
Congener 8				
Congener 18				
Congener 28				
Congener 44				
Congener 52				
Congener 66				
Congener 101				
Congener 105				
Congener 110/77				
Congener 118/108/149				
Congener 126				
Congener 128	*			
Congener 138				
Congener 153				
Congener 170				
Congener 180				
Congener 187/182/159	*			
Congener 195				
Congener 206				
Congener 209				
Pesticides				
2,4' DDD	*			
4,4' DDD				
2,4' DDE				
4,4' DDE	*			
2,4' DDT				
4,4' DDT	*			
Aldrin				
alpha-BHC				
beta-BHC				
delta-BHC				
gamma-BHC				
alpha-Chlordane				
gamma-Chlordane				
Dieldrin				
Endosulfan I				
Endosulfan II				
Endrin				
Hexachlorobenzene				
Heptachlor				
Heptachlor Epoxide				
Mirex				
cis-Nonachlor				
trans-Nonachlor				
Oxychlordane				
Toxaphene				
Total DDT	*		+	
Total BHC	*			
Total Chlordane	*			

Table 3.23(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province significant discriminatory power (*) and the co-occurrence of significant source gradients (+).

Indicator	Discriminatory Power	Source Gradients		
		DO	IND	AGRO
Exposure Indicators (Cont)				
Heavy Metals (OBS-P)[1]				
Antimony				
Arsenic				
Cadmium	*			
Chromium	*			
Copper			+	
Iron	*			
Lead	*			
Manganese			+	
Mercury				
Nickel	*			
Selenium				
Silver				
Tin	*			
Zinc	*			
Heavy Metals (%CV)[1]				
Antimony				
Arsenic	*			
Cadmium	*			
Chromium	*			
Copper	*			
Lead	*			
Mercury				
Nickel	*			
Silver	*			
Tin	*			
Zinc	*			

[1] Observed - Predicted concentrations based on aluminum regression (positive value denoted anthropogenic sources)

[1] Percentage of criterion value

Table 3.23(cont) Summary of results of sensitivity tests for EMAP-E indicators in the Louisianian Province significant discriminatory power (*) and the co-occurrence of significant source gradients (+).

SECTION 4

RESEARCH INDICATORS

A key element of the EMAP indicator strategy is the identification and testing of new indicators that may:

- Address an ecological issue not presently examined by one of the present indicators,
- Replace a present indicator with a more sensitive or direct indicator, or
- Allow a regional interpretation of the data because the new indicator does not respond to natural gradients that confound regional evaluations (e.g., salinity, longitudinal).

Six research indicators were identified for indicator testing and logistical evaluation during the 1991 Louisianian Province Demonstration. Indicator testing refers to a determination of the scientific credibility of the indicator and its ability to differentiate between impacted and reference sites at population rather than suborganismal levels. Logistical evaluation refers to the ability of EMAP-E crews to collect the samples required for the indicator in a manner consistent with the EMAP field protocols. A research indicator must be compatible with both types of testing in order to be considered for further implementation within EMAP as a developmental indicator. The indicators examined in the 1991 Demonstration were:

- Histopathology of target fish (primarily catfish, croaker, pinfish, seatrout, and spot),
- Frequency and Density of Splenic Macrophage Aggregates in Atlantic croaker and pinfish,
- Frequency and type of vertebral abnormality
- Blood chemistry of fish
- Bile florescence in fish
- Stable carbon and nitrogen isotopes.

Information for these research indicators was not collected at the base EMAP sites but rather through a special study that augmented the 1991 Demonstration.

4.1 INDICATOR TESTING AND EVALUATION (ITE) SAMPLING DESIGN

Sufficient information is not available to verify the reliability of these research indicators for the estuaries of the Louisianian Province. Therefore, a study to

Site	Designation	Latitude	Longitude
Eastern Subregion			
Apalachicola Bay, FL	Reference	29 40.00'	84 56.65'
Watsons Bayou, FL	Contaminated[1]	30 8.50'	85 38.00'
Choctawhatchee River, FL	Hypoxia Contaminated[2]	30 24.00'	86 8.00'
Escambia Bay, FL	Contaminated[3]	30 31.70'	87 10.00'
Perdido Bay, AL/FL	Hypoxia	30 27.08'	87 22.60'
Wolf Bay, AL	Contaminated[2]	30 19.71'	87 35.72'
Mobile Bay, AL	Hypoxia Contaminated[3]	30 37.00'	88 0.00'
Bayou Casotte, MS	Hypoxia Contaminated[1]	30 20.00'	88 30.71'
Western Subregion			
Calcasieu Lake Canal, LA	Hypoxia	29 59.38	93 20.03'
Galveston Bay, TX	Contaminated[1]	29 31.66'	94 56.90'
Houston Ship Channel, TX	Hypoxia Contaminated[1]	29 43.96'	95 8.04'
Brazos River, TX	Hypoxia Contaminated[3]	28 57.79'	95 22.83'
Lavaca Bay, TX	Contaminated[3]	28 38.30'	96 32.41'
San Antonio Bay, TX	Reference	28 18.30'	96 39.90'
Laguna Madre, TX	Contaminated[2]	26 8.00'	97 16.00'
Arroyo Colorado, TX	Hypoxia Contaminated[2]	26 20.33	97 25.76'

---

[1] Sediment contamination due to industrial sources
[2] Sediment contamination due to agricultural runoff
[3] Sediment contamination due to industrial and agricultural sources

Table 4.1 Locations of indicator testing and evaluation (ITE) sites used in the 1991 Louisianian Province Demonstration. Designation refers to level of hypoxia and sediment contamination.

determine the reliability of these indicators to discriminate between polluted and unpolluted environments was conducted. This study also, in some instances, provides for the first field testing of these indicators and the first assessments of the accuracy and precision of these indicators to discriminate ecological conditions on large geographical scales. Samples for the ITE study were collected at 16 locations (8 east and 8 west of the Mississippi River Delta)(Table 4.1). These 16 sites were selected, based on historical information, to represent combinations of environmental situations related to dissolved oxygen and sediment contaminant (industrial and agricultural) conditions. By combining extreme conditions, several ITE sites were located throughout the estuaries of the Gulf of Mexico. For example, conditions of hypoxia without significant industrial discharges or agricultural runoff were

examined in Perdido Bay, FL for regions east of the delta. Hypoxic, highly industrially contaminated conditions receiving little agricultural runoff in the western Gulf were sampled in the Houston Ship Channel, TX. These research indicators were collected in conjunction with the standard EMAP-E indicators (Section 3) at these sites during the period July 7-August 31, 1991.

4.2 HISTOPATHOLOGY OF TARGET SPECIES

While gross fish pathology is a response indicator used in EMAP-E for environmental condition, it may not provide insight into the potential cause of the pathology. In addition, the existence of external pathologies may or may not be related to the occurrence of internal pathologies of the liver, spleen, or gills. To address this concern, EMAP-E performed detailed histological examinations of all fish observed to have external pathologies (base and ITE sites) and randomly-selected individuals of target and non-target species at ITE sites. All individuals that "failed" the gross pathology examination and up to 25 randomly selected individuals of the target species (Table 4.2) that "passed" this examination at the ITE sites were subjected to an extensive histopathological examination. In addition, up to 10 randomly selected individuals of non-target species collected from the ITE sites were examined. Histopathology will be maintained at the research indicator level until it can be shown to discriminate clearly between polluted and unpolluted sites.

Brown shrimp	(*Penaeus aztecus*)
Atlantic croaker	(*Micropogonias undulatus*)
White shrimp	(*Penaeus setiferus*)
Hardhead catfish	(*Arius felis*)
Blue Crab	(*Callinectes sapidus*)
Spot	(*Leiostomus xanthurus*)
Pinfish	(*Lagodon rhomboides*)
Southern flounder	(*Paralichtys lethostigma*)
Gafftopsail catfish	(*Bagre marinus*)

Table 4.2. Target species collected at ITE sites during the 1991 Louisianian Province Demonstration.

Representative tissue samples were taken from specimens and processed for histological analysis. Tissue samples were dehydrated in an ethanol gradient, cleared in a xylene solution, infiltrated, and embedded in paraffin. Sections were cut at 6um on a rotary microtome, stained with Harris' hematoxylin and eosin and examined microscopically. The results of this microscopic examination were used to characterize the types of external/internal pathologies and to create a baseline for these features for the Louisianian Province. Based on these findings, a determination of whether or not to continue with histopathology as a developmental indicator will be made.

Table 4.3 lists the histopathologic conditions observed in fish collected at the ITE sites. These conditions are listed in terms of parasitic infection and specific histopathologic condition. Sample numbers beginning with the prefix "RF" refer to the reference fish collected (i.e., "passed" the field examination for gross pathologies) and "FP" refers to those fish collected with obvious external pathologies. With the exception of Atlantic croaker collected from the Houston Ship Channel, the accuracy of the field gross pathology tests were

Table 4.3 Results of histopathology of fish from 1991 Louisianian Province Demonstration.

Site	Species	n	Gross Pathologies Detected	Histopathological Conditions: Parasites: Unidentified pathogen/parasite	Protozoans	Helminths	Crustaceans	Epitheliocystis	Telangiectasis	Hyperplasia	Inflammation	Bacteremia/ pancreatitis	Necrosis	Vacuolation/fatty degeneration	Megalocytosis	Karyomegaly
Perdido Bay, AL	*Anchoa mitchilli*	10	0													
	Brevoortia patronus	3	0													
	Chloroscombrus chrysurus	2	1			1							1			
Escambia Bay, FL	*Carangidae sp.*	2	0		1						1					
	Lepisosteus oculatus	4	0								4					
Watsons Bayou, FL	*Lagodon rhomboides*	42	2	2	12		31	13	12	4	15	1		2		
	Leiostomus xanthurus	4	0		2		3	1	1							
	Trachinotus falcatus	1	N						1							
Bayou Casotte, MS	*Harengula pensacolae*	9	N	3		2	1	4	1							
Choctawhatchee River, FL	*Brevoortia patronus*	15	1	1							2					
	Leiostomus xanthurus	21	1		2		6	3			7					
	Lepisosteus oculatus	2	0						2	2						
	Anchoa mitchilli	17	0													
	Caranx hippos	1	0													
Wolf Bay, AL	*Bagre marinus*	21	2	1		2	1			1	4		4	1	1	
	Brevoortia patronus	10	0								4					
	Lagodon rhomboides	21	2		1		18				3	1	2			
	Leiostomus xanthurus	20	0		3	1	3				1		2			
Apalachicola Bay, FL	*Caranx latus*	1	N		1					1	1					
	Chloroscombrus chrysurus	2	N		1											
	Dorosoma petenense	7	N						1							
	Micropogonias undulatus	4	N			1			2							

Table 4.3 (*Cont.*) Results of histopathology of fish from 1991 Louisianian Province Demonstration.

Site	Species	n	Gross Pathologies Detected	Histopathological Conditions Parasites												
				Unidentified pathogen/parasite	Prototozoans	Helminths	Crustaceans	Epitheliocystis	Telangiectasis	Hyperplasia	Inflammation	Bacteremia/ pancreatitis	Necrosis	Vacuolation/fatty degeneration	Megalocytosis	Karyomegaly
Mobile Bay, FL	*Anchoa mitchilli*	2	0													
	Brevoortia patronus	7	0													
Calcasieu Lake, LA	*Caranx hippos*	2	0		2											
	Chaetodipterus faber	7	1			2		1		1						
	Dorosoma petenense	6	0	1	2	4		4	2	1	3					
	Trachinotus falcatus	4	0		1			1	3		1					
	Trichiurus lepturus	3	0	1	2	2		3			1				2	2
	Selene vomer	2	0	1	1						1					
Houston Ship Channel, TX	*Micropogonias undulatus*	18	15		3	6		1								
Galveston Bay, TX	*Micropogonias undulatus*	7	0		6	5	1	1	5		1					
Laguna Madre, TX	*Lagodon rhomboides*	25	0		1	7		1	1							
	Micropogonias undulatus	10	1	1		2	3		3		1					
San Antonio Bay, TX	*Bagre marinus*	1	0		1		1									
	Micropogonias undulatus	11	0		5		2	1	8	1						
Lavaca Bay, TX	*Bagre marinus*	19	0		4											
	Micropogonias undulatus	14	0		3	3	1	2	4		1					
Brazos River, TX	*Leiostomus xanthurus*	10	1	1	2		3		4	1						
	Micropogonias undulatus	8	0			3	1		7		1					

acceptable (i.e., accuracy rate = 97%). Atlantic croaker collected from the Houston Ship Channel all showed significant levels of fin erosion. However, because this condition was common to all croaker collected at this site, the field crews had no pattern of reference to compare with for these fish and thus erroneously categorized them as without gross defects.

ANOVA testing of the ITE pathology data showed that while the number of pathologies per trawl at contaminated sites was greater than those at reference sites (0.83 and 0.33, respectively), these differences were not significant given the variation in the estimates. No significant longitudinal patterns were detected although the number of pathologies per trawl in the western subregion (1.3) was more than twice that observed in the eastern subregion (0.5). Although the number of pathologies found under hypoxic conditions (1.3) was three times those found in reference areas (0.4), this difference was not significant. The incidence of pathologies in fish collected from areas of sediment contamination were twice that seen at uncontaminated sites (1.2 versus 0.6 at industrial sites); however, this difference was also not significant. The high degree of variability might make use of pathology a difficult and insensitive indicator for EMAP. However, the fact that incidence of pathology appeared to vary along the expected gradients for dissolved oxygen and sediment contamination suggests that the indicator might be sensitive but that the significance of this sensitivity will not be observed without increased sample sizes. This situation was more evident when examining rate of pathology (# of pathologies/100 fish), which corrects for the variability in catch size. Rate of pathology was three times more frequent in areas with sediments contaminated by industrial discharges (2.2%) than in uncontaminated areas (0.6%). However, this difference was not statistically significant. Although pathology rate varied as expected along the exposure gradients, the only significant difference observed from the ITE collections was a significant east-west gradient with western sites having five times (2.4%) the pathology rate as eastern sites (0.5%). The levels of pathology rate seen at uncontaminated sites agrees well with the overall background rates of pathology seen in the Louisianian Province (0.6%)(Summers et al. 1993). Because histopathologic condition varies in the appropriate directions (i.e., increases in poor environments and decreases at reference sites) and in a consistent manner, histopathology will be retained in the 1992 EMAP sampling in the Louisianian Province as a developmental indicator and examined at a broader geographic scale. If the variability in this measure continues to mask its ability to differentiate between impacted and unimpacted sites based on this broader test, the pathology indicator will be reviewed for removal from the EMAP indicators.

4.3 SPLENIC MACROPHAGE AGGREGATES

Pigment-bearing macrophages are a prominent feature of fish spleen, kidney, and liver (Agius 1980) and in advanced teleosts they form discrete aggregations called macrophage aggregates

Indicator	Gradient	Mean
Number of Pathologies	Site Type	
	Good	0.33
	Bad	0.83
	Longitudinal	
	East	0.56
	West	1.26
	Dissolved Oxygen	
	Hypoxic	1.26
	High	0.43
	Contamination (I)	
	High	1.21
	Low	0.60
	Contamination (A)	
	High	0.52
	Low	1.17
Frequencies of Pathologies	Site Type	
	Good	0.64
	Bad	0.89
	Longitudinal*	
	East	0.50
	West	2.41
	Dissolved Oxygen	
	Hypoxic	1.51
	High	1.11
	Contamination (I)	
	High	2.16
	Low	0.69
	Contamination (A)	
	High	0.75
	Low	1.85

Table 4.4 ANOVA results of number and frequency of pathologies using the ITE data set. (I = Industrial Sources; A = Agricultural Runoff). (* = $p < 0.05$).

(MAs)(Wolke et al. 1985). Suggested functions for these aggregates include the centralization of foreign materials and cellular debris for destruction, detoxification, and/or reuse (Ferguson 1976; Ellis et al. 1976). It has been demonstrated that MAs' occurrence may vary depending on the size, nutritional state, or health of a particular fish (Agius 1979, 1980; Agius and Roberts 1981; Wolke et al. 1985) with the number and size of MAs increasing with age, starvation, and/or disease. Recent studies suggest that MAs may be sensitive histological indicators of fish health and environmental quality. By comparing the MA number and percent area occupied by MAs among fish of the same age and species from various sites of known environmental condition, it may be possible to determine their relative conditions at those sites.

Data on MAs were collected from 6 µm histological sections of spleen from selected fish species of similar size. Sections were stained with Harris' hematoxylin and eosin or Perl's prussian blue method (Luna 1968). Occurrence of MAs are assessed by two methods. First, during initial histological evaluation, the occurrence and intensity of MAs were rated using a scale of 0 to 4, with 0 being no MAs present, 1 indicating minimal occurrence, and 2 through 4 indicating light, moderate, and heavy MA intensity, respectively. Secondly, the MA number and individual MA area were estimated from three random fields per spleen using computer image analysis (MicroComp™ Integrated Image Analysis System Particle Analysis). These data, identified by individual and site, were compiled and analyzed (i.e., blind to the conditions at any

site). These data were analyzed for differences in number of MAs per mm^2, average MA area (μm^2), and percent area occupied by MAs (Table 4.5). Analyses were completed on a combination of all data (i.e., all species combined) and by selected species.

Because of the small number of sites from which ITE samples were collected, the analyses were completed on a data set that combined fish species. The percentage of area occupied by macrophage aggegrates was significantly different between sites with good ecological condition (high dissolved oxygen, low contaminants) and poor ecological conditions (Table 4.6) regardless of species. Areas experiencing hypoxic condition, showed significantly higher proportions of the spleen having macrophage aggregates than under unstressed dissolved oxygen conditions. However, contaminated sites, whether from industrial discharges or agricultural runoff did not show any pattern associated with macrophage aggregate concentrations. This lack of a pattern associated with contaminated sediments may be due to the highly significant dissolved oxygen gradient ($p < 0.001$) which might overshadow any gradient due to contaminants because the data set includes contaminated sites that are hypoxic as well as having high dissolved oxygen conditions. In order to ascertain whether the dissolved oxygen gradient is confounding the identification of a contaminant gradient, the interaction of hypoxia and contaminant level was examined. Although the percentage of area occupied by macrophage aggegrates was higher under conditions of contaminated sediments under high levels of dissolved oxygen (1.3%) than in high DO-uncontaminated areas (1.0%), the difference was not significant. The macrophage aggregate also showed a significant longitudinal gradient with eastern sites (4.0%) covering about four times the area of the spleen than western sites (1.2%).

Another possible confounding factor might be that all species of fish collected were analyzed as a combined data set. Sufficient data existed so that analysis could be performed on the species level for only two species: pinfish (*Lagodon rhomboides*) and Atlantic croaker (*Micropogonias undulatus*). In pinfish, macrophage aggregates covered a significantly higher proportion of the spleen in the fish from sites with poor environmental condition (6.0%) than in fish from reference areas (1.5%)(Table 4.7). Macrophage aggregates were more dense in fish from eastern sites (4.7%) than those from western sites (1.5%). Macrophage aggregate density in pinfish was significantly higher in areas experiencing hypoxia (6.0%) and in areas receiving high industrial discharges (6.0%) as compared to reference sites (1.8%).

Macrophage aggregate densities in Atlantic croaker (*Micropongias undulatus*) were significantly higher at sites characterized by poor environmental conditions but were not significantly higher at any of the other site combinations described above although sites with bad environmental conditions, sites in the eastern subregion, hypoxic sites, and sites with high industrial discharges had higher macrophage aggregate densities than their counterpart sites (Table 4.7).

Table 4.5 Results of splenic macrophage aggregate analysis for 1991 Louisianian Province Demonstration.

Site	Species	Splenic Macrophage Aggregates								
		Histologically determined intensity						Computer image analysis		
		n	0	1	2	3	4	MAs/ mm^2	Avg MA area in μm^2	% area occupied
Perdido Bay, AL	*Brevoortia patronus*	2			1	1		24.4	429.6	1.025
Escambia Bay, FL	*Carangidae sp.*	1			1			14.2	945.6	1.346
Watsons Bayou, FL	*Lagodon rhomboides*	19		1	9	6	3	41.7	1531.0	6.433
		23			8	12	3	44.6	1367.5	5.582
	Leiostomus xanthurus	3			2		1	28.8	594.4	1.642
		1					1	37.6	1997.6	7.515
	Trachinotus falcatus	1		1				5.1	357.2	0.182
Choctawhatchee River, FL	*Brevoortia patronus*	2				1	1	60.0	1838.0	10.486
		9			4	4	1	47.2	1284.2	5.929
	Leiostomus xanthurus	6				5	1	39.3	2367.2	8.819
		12			1	4	7	39.1	2408.1	9.473
	Lepisosteus oculatus	1	1					0.0	----	----
		1	1					0.0	----	----
	Caranx hippos	1			1			12.2	540.0	0.659
Wolf Bay, AL	*Bagre marinus*	21			14	7		18.4	1851.9	3.639
	Lagodon rhomboides	16			10	6		27.3	751.7	2.013
	Leiostomus xanthurus	20		12	8			7.6	301.0	0.221
Apalachicola Bay, FL	*Micropogonias undulatus*	1			1			23.4	662.14	1.549
Mobile Bay, AL	*Brevoortia patronus*	7		1	5	1		23.7	545.1	1.254
Calcasieu Lake, LA	*Trachinotus falcatus*	1			1			7.9	239.2	1.900

Table 4.5(*cont.*) Results of splenic macrophage aggregate analysis for 1991 Louisianian Province Demonstration.		Splenic Macrophage Aggregates								
Site	Species	Histologically determined intensity						Computer image analysis		
		n	0	1	2	3	4	MAs/ mm^2	Avg MA area in μm^2	% area occupied
Galveston Bay, TX	*Micropogonias undulatus*	2		1	1			10.2	723.1	0.757
Laguna Madre, TX	*Lagodon rhomboides*	1			1			20.9	739.5	1.546
	Micropogonias undulatus	9		3	6			13.8	536.1	0.731
San Antonio Bay, TX	*Bagre marinus*	1		1				3.1	554.0	0.169
	Micropogonias undulatus	8		3	5			9.5	641.2	0.590
Lavaca Bay, TX	*Bagre marinus*	19		14	5			5.3	286.7	0.147
	Micropogonias undulatus	3		1	2			27.9	621.3	1.931
Brazos River, TX	*Leiostomus xanthurus*	6		2	1	2	1	20.5	975.5	1.923
	Micropogonias undulatus	1				1		32.5	733.1	2.385

Indicator	Gradient	Mean
Percent Area Occupied by Macrophage Aggregates	Site Type*	
	Good	0.99
	Bad	5.25
	Longitudinal*	
	East	3.99
	West	1.21
	Dissolved Oxygen*	
	Hypoxic	4.35
	High	1.22
	Contamination (I)	
	High	2.59
	Low	3.25
	Contamination (A)	
	High	3.28
	Low	2.49

Table 4.6 Results of the analysis of percentage of area occupied in the spleen by macrophage aggregates. (I=Industrial, A=Agricultural). (* = $p < 0.05$).

Indicator	Gradient	Mean Pinfish	Mean Croaker
Percent Area Occupied by Macrophage Aggregates	Site Type*PC		
	Good	1.55%	0.66%
	Bad	6.01%	5.98%
	Longitudinal		
	East	4.68%	1.54%
	West	1.55%	1.28%
	Dissolved Oxygen*P		
	Hypoxic	6.01%	2.38%
	High	1.78%	1.11%
	Contamination (I)*P		
	High	6.01%	1.69%
	Low	1.78%	0.96%
	Contamination (A)*P		
	High	1.78%	1.68%
	Low	6.01%	0.97%

Table 4.7 Results of the analysis of percentage of area occupied in the spleen by macrophage aggregates for pinfish (P) and Atlantic croaker (C). (I=Industrial, A=Agricultural). (* = $p < 0.05$).

Because of the ability of macrophage aggegrates to discriminate between sites of known good and poor environmental condition for at least one target species, this indicator will be elevated to developmental status in 1992 and macrophage aggregate information will be developed for selected target species throughout the Province.

4.4 VERTEBRAL ABNORMALITIES

Measurement of skeletal deformities in fish has been proposed as a means of monitoring pollution effects in marine environments (Bengtsson 1979; Bengtsson and Bengtsson 1983). Likewise, measurements of biochemical composition and mechanical properties of vertebrae have been shown to be indicators of bone development in fish exposed to contaminants in the laboratory (Hamilton et al. 1981; Mayer et al. 1977), and in the field (Mayer et al. 1988; Mehrle et al. 1982). Skeletal abnormalities in fourhorn sculpin (*Myoxocephalus quadricornis*) have been used to monitor the impacts of ore smelter and pulp mill effluents in the Baltic Sea (Bengtsson et al. 1985).

Effects of organic and inorganic contaminants on bone integrity are similar in that vertebral anomalies are produced, although they may develop through different modes of action (Mayer et al. 1978). This similarity makes the use of

Site	Designation	Percentage of Skeletal Deformities
Apalachicola Bay, FL	Reference	7.1
Watsons Bayou, FL	Contaminated[1]	4.7
Choctawhatchee River, FL	Hypoxia Contaminated[2]	4.4
Escambia Bay, FL	Contaminated[3]	0.0
Perdido Bay, AL/FL	Hypoxia	0.0
Wolf Bay, AL	Contaminated[2]	0.0
Mobile Bay, AL	Hypoxia Contaminated[3]	0.0
Bayou Casotte, MS	Hypoxia Contaminated[1]	0.0
Western Subregion		
Calcasieu Lake Canal, LA	Hypoxia	0.0
Galveston Bay, TX	Contaminated[1]	25.0
Houston Ship Channel, TX	Hypoxia Contaminated[1]	36.8
Brazos River, TX	Hypoxia Contaminated[3]	0.0
Lavaca Bay, TX	Contaminated[3]	44.1
San Antonio Bay, TX	Reference	35.3
Laguna Madre, TX	Contaminated[2]	10.7
Arroyo Colorado, TX	Hypoxia Contaminated[2]	NA[4]

[1] Sediment contamination due to industrial sources
[2] Sediment contamination due to agricultural runoff
[3] Sediment contamination due to industrial and agricultural sources
[4] No fish caught during trawling due to extreme hypoxia

Table 4.8 Condition of the vertebral column in fishes examined from ITE sites in the 1991 Louisianian Province Demonstration.

biochemical composition and mechanical properties, as well as vertebral deformities, conducive to assessing the abuse and effects of an array of contaminants on fish health (Mayer et al. 1988).

All preserved fishes collected from ITE sites during the 1991 Louisianian Province Demonstration were x-rayed laterally with a Hewlett Packard™ Faxitron Series X-ray System set at 50kVp for 20 to 50 seconds, depending on the size of the specimen. Kodak™ Industrex M-2 film was used for all radiographs and they were developed for 5 minutes in Kodak™ D-19 developer. Vertebral anomalies were determined from the x-rays by light box and confirmed by low-power light microscopy. Deformities were classified according to Bengtsson and Bengtsson (1983).

The observed incidences of vertebral deformities at the ITE sites are shown in Table 4.8. In general, vertebral deformities were found only at sites with contaminated sediments. The exception to this observation is the reference site in San Antonio Bay, TX. All the vertebral deformities found at this site were Atlantic croaker with anterior curvature and only 12 specimens were examined. While San Antonio Bay sediments were confirmed by contaminant analysis as having low concentrations in the upper 2 cm of sediments, deeper sediments show that San Antonio Bay has had some sediment contamination in the past. A significant longitudinal gradient was

observed with western sites having a frequency of vertebral deformities that was eleven times greater (17.9%) than those observed in the east (Table 4.9). With a longitudinal gradient this strong, it would be expected to be difficult to ascertain any relationships from a mixed dataset. Even with the strong longitudinal gradients, skeletal deformities were seven times more prevalent in hypoxic areas and four times more prevalent in areas receiving high industrial discharges although the gradients were not significant due to the high variability induced by a dataset including the longitudinal gradient (Table 4.9). Regardless of the longitudinal gradient, vertebral deformities in pinfish (*Lagodon rhomboides*) and Atlantic croaker (*Micropogonias undulatus*) were significantly higher under hypoxic and high industrial discharge conditions.

Indicator	Gradient	Mean
Percentage of Fish with Vertebral Deformities	Site Type	
	Good	8.46
	Bad	1.19
	Longitudinal*	
	East	1.64
	West	17.93
	Dissolved Oxygen*	
	Hypoxic	16.44
	High	2.32
	Contamination (I)	
	High	16.31
	Low	4.76
	Contamination (A)	
	High	7.77
	Low	10.70

Table 4.9 Results of the analysis of percentage of vertebral deformities from ITE sites during the 1991 Louisianian Province Demonstration. (I=Industrial, A=Agricultural). (* = p < 0.05).

4.5 BLOOD CHEMISTRY

In both human and veterinary medicine, clinical chemistry measurements and routine hematology are used to assess the health of individuals. Altered values in serum enzymes or other proteins, electrolytes, or blood cells of fish can be indicative of tissue damage, tumors, or impaired immunological functions. Blood was collected from all fish greater than 200 mm in total length at ITE sites. Blood, removed by vacutainer, was stored on ice, shipped as soon as possible to the laboratory, and analyzed using a Beckman™ Synchron CX-5.

Unfortunately, only 7 of the 16 ITE sites visited provided fish of sufficient size to extract blood. Further analysis of these samples was not attempted and, based on logistical problems in providing fish of adequate size, no samples will be collected in 1992 for blood chemistry. However, the funding provided for these analyses were re-channelled into an evaluation of blood chemistry in fish outside the Louisianian Province for future applications in EMAP-Estuaries.

Brown bullheads (*Ictalurus nebulosus*) were collected from 4 freshwater sites: Old Woman Creek, OH (reference); Niagara River, NY (moderately contaminated); Buffalo River, NY (moderately contaminated); and, Black River, OH (heavily contaminated). Table 4.10 shows the percentage of bullheads having excessive concentrations of the serum constituents. Excessive concentrations were determined as any concentrations

Serum Constituent	Site[1]			
	OWC	NR	BR	BLR
Aspartate aminotransferase	0.0	4.0	14.0	66.0
Alkaline phosphatase	0.0	0.0	2.9	11.0
Lactate dehydrogenose	0.0	37.0	9.0	25.0
Alanine aminotransferase	0.0	59.0	26.0	78.0
Total protein	0.0	0.0	10.0	67.0
Creatinine	0.0	0.0	0.0	20.0
Blood urea nitrogen	0.0	97.0	66.0	50.0
Triglycerides	0.0	97.0	38.0	62.0
C-Reactive Proteins	0.0	100.0	---	100.0

[1]OWC = Old Woman Creek, OH
NR = Niagra River, NY
BR = Buffalo River, NY
BLR = Black River, OH

Table 4.10 Percentage of brown bullheads exhibiting excessive serum chemistry concentrations.

greater than the upper 95% confidence limit observed at the reference site. Clear gradients from reference through heavily contaminated sites were observed for AST, ALP, TP, CREA, and ALT. In addition, C-reactive protein levels, a protein associated with liver tumors, were significantly higher in bullheads taken from the Black (1300 ± 329) and the Niagara (1027 ± 384) River than concentrations observed in bullheads from the reference site (442 ± 20).

These data suggest that blood chemistry could provide a strong indicator along a gradient of sediment contamination. This discriminatory power needs to be demonstrated with estuarine species from the Louisianian Province. Bullheads are observed in many oligohaline estuaries in the Virginian Province. Therefore, if the logistical problems of collecting the appropriate fish can be solved in the Louisianian Province, the potential of serum chemistry to provide useful indicators should be re-examined.

4.6 BILE FLORESCENCE

Organisms exposed to petroleum compounds often accumulate polynuclear aromatic hydrocarbons (PAHs). Tissue analysis for PAHs often show only trace concentrations, even after high-level exposures because enzymatic-mediated metabolism can rapidly reduce concentrations. The exposure of fish to PAHs can be assessed by measuring the concentration of metabolites in bile. The relative concentration of individual PAH metabolites of benzo(a)pyrene, phenanthrene, and naphthalene in bile were determined using PHLC with florescence detection. Bile was extracted from all fish greater than 200 mm in total length collected at ITE sites.

As with blood chemistry, only 7 of the 16 ITE sites visited provided fish of sufficient size to extract blood. These samples were analyzed, but small sample sizes made it difficult to find significant differences. Based on logistical problems in providing fish of adequate size, no samples will be collected in 1992 for bile florescence. However, the results of the analysis on fish from the 7 ITE sites are reviewed below.

The concentration of benzo(a)pyrene in bile from fish at sites of poor environmental condition (570 ppb) was nearly twice that found at reference sites (325 ppb) but this difference was not significant due to the large variability observed in individual fish (Table 4.11). Fish in the western subregion showed nearly ten times as much benzo(a)pyrene in bile (1113 ppb) as those eastern sites (196 ppb) but, again, this difference was not significant due to high variability. The pattern of contaminant concentrations in bile appeared to follow expected relationships with higher concentrations found in hypoxic and heavily industrialized areas.

Concentrations of naphthalene and phenanthrene in bile of fish collected at ITE sites showed similar patterns to that of benzo(a)pyrene with the exception that observed concentrations at good and bad sites were about equal (Table 4.11).

4.7 STABLE ISOTOPES RATIOS

Stable isotopes, often in combination with elemental analyses, have been used traditionally to distinguish terrestrial and marine sources of organic matter in estuarine systems (Coffin et al. 1992).

Indicator	Gradient	Mean Concentration[1]		
		Benzo	Phen	Naph
Concentrations of PAHs in Bile	Site Type			
	Good	570	16000	57500
	Bad	325	13700	51250
	Longitudinal			
	East	196	NA[2]	NA
	West	1113		
	Dissolved Oxygen			
	Hypoxic	1080	55800	185833
	High	500	22811	71111
	Contamination (I)			
	High	1080	50367	159444
	Low	500	14467	53333
	Contamination (A)			
	High	736	27342	92500
	Low	1216	70667	215000

---

[1] Benzo = Benzo(a)pyrene;
Phen = Phenanthrene;
Naph = Naphthalene

[2] Insufficient data to perform test

Table 4.11 Results of the analysis of concentration of selected PAHs (ng/g) in the bile of fish collected at ITE sites. (I=Industrial, A=Agricultural). (* = $p < 0.05$).

In particular, isotopic analyses of carbon ($\delta^{13}C$) differentiate C_3 and C_4 terrestrial plants from algal sources of organic matter (Fry and Sherr 1984, Coffin et al. 1992). Stable nitrogen isotopes ($\delta^{15}N$) combined with $\delta^{13}C$ can trace terrestrial, marine, and in some cases, anthropogenic contributions in estuarine systems (Fogel and Cifuentes 1992).

During the 1991 Louisianian Province Demonstration, samples for stable isotope analysis were collected from the 16 ITE sites. The objective was to identify estuaries that combined significant anthropogenic inputs with net heterotrophic activity, suggesting the existence of, or potential for, oxygen depletion and eutrophication. To accomplish this objective, $\delta^{13}C$ and $\delta^{15}N$ was measured in suspended particulate matter, humic acids, and bacterial bioassays.

Particulate and dissolved suspended matter were obtained from a depth of 1 m at all 16 locations. Suspended particulate matter (SPM) samples were collected by pushing water through 47 mm GF/F filters (heated to 450 C for 2 hr) using a Masterflex^R peristaltic pump. Humic acids (HA) were collected by a modification of the method of Fox (1983). First, 1 L of water was pre-filtered through a 47 mm GF/F filter as described for SPM samples. The filtrate was then acidified to pH 2 with 8N H_2SO_4 to precipitate humic acids. Other macromolecules, such as proteins and mucopolysaccharides, will also precipitate at low pH (Thurman 1985). This precipitate was captured on a 47 mm GF/F filter.

Approximately 30 ml of unfiltered water was collected in Quorpak^R bottles and preserved with 2% $HgCl_2$ for isotopic analysis of dissolved inorganic carbon. These samples were refrigerated at 4 C prior to isolation of CO_2 for isotopic analysis by the *in vacuo* acidification and purging technique described in Grossman (1984). All samples, including the zeolite with the exchanged ammonium, were analyzed isotopically by a modified Dumas combustion that converts organic carbon and organic nitrogen to CO_2 and N_2, respectively, for mass spectral analysis (Macko 1981).

Uniformly high nutrient concentrations were only observed in the Houston Ship Channel, with values similar to or higher than those reported for more "polluted" estuaries (e.g., Delaware Estuary, Sharp et al. 1982)(Table 4.12). Sites selected for high agricultural runoff show high nutrient concentrations. Brazos River, Choctawhatchee River, and Escambia Bay had significant nitrate+nitrite (NO_3) and ammonium (NH_4) concentrations, but had low PO_4). Wolf Bay had high NH_4 content, but no NO_3. In contrast the reference sites had low NH_4. Those sites with high agricultural runoff but low nutrients showed high chlorophyll a levels. Arroyo Colorado had the greatest algal biomass of 37.5 ug/l chlorophyll a. Wolf Bay, Mobile Bay, Lavaca Bay, and Galveston Bay showed high chlorophyll a concentrations.

The range of carbon isotopes for SPM measured at the ITE sites, -30.7 to -17.3 ppt (Table 4.13), was similar to that reported for other estuaries that have been extensively studied (Cifuentes et al. 1988; Fogel et al. 1992). Without further analysis, it would appear that we

Station	Abbr.	Date	Salinity (ppt)	Chlora (µg/l)	C:N	PO4 (µM)	NO3 (µM)	NH4 (µM)	SIOH4 (µM)	DON (µM)	DOC (µM)
Southern Laguna Madre	SLM	8/7/91	36.0	2.86	7.1	0.10	0.24	0.09	20.50	47.6	177.5
San Antonio Bay	SAB	8/4/91	12.5	14.69	7.4	3.04	0.24	0.19	160.60	158.8	1232.5
Bayou Casotte	BC	8/9/91	26.4	11.27	5.9	0.58	0.24	0.19	32.80	11.0	185.0
Watson's Bayou	WBY	7/16/91	23.6	2.52	5.2	0.10	0.24	0.19	33.60	17.5	352.5
Lavaca Bay	LB	7/13/91	12.0	6.53	4.7	0.68	0.24	0.19	132.00	113.2	365.8
Wolf Bay	WB	8/14/91	15.9	16.32	6.7	0.10	0.24	4.73	98.30	22.4	369.2
Arroyo Colorado	AC	8/5/91	15.5	37.54	5.3	4.47	0.24	0.72	191.80	87.8	505.0
Brazos River	BR	7/12/91	9.5	6.73	6.0	1.78	38.64	6.24	93.80	67.3	254.2
Perdido Bay	PB	8/14/91	10.6	4.34	5.3	0.05	0.24	0.28	90.20	18.2	351.7
Apalachicola Bay	AB	7/15/91	10.0	LOST	4.8	0.02	2.22	0.13	86.90	16.8	1027.5
Galveston Bay	GB	7/10/91	12.0	14.48	4.6	6.34	0.24	0.19	119.70	72.4	394.2
Lake Calcasieu	LC	7/15/91	2.5	14.08	5.4	0.63	4.59	0.19	89.30	62.8	1070.0
Choctawhatchee Bay	CB	7/17/91	0.0	3.21	4.8	0.31	10.13	1.14	83.60	14.6	403.3
Houston Ship Channel	HSC	7/11/91	7.5	10.61	6.8	11.17	137.60	10.03	140.20	131.5	730.0
Mobile Bay	MB	8/24/91	7.4	7.63	4.0	0.04	0.63	0.19	62.30	15.0	225.8
Escambia Bay	EB	7/18/91	18.0	3.79	5.8	0.10	8.55	2.46	135.20	11.5	640.8

B.D. = Below Detection

Table 4.12 Pigment, elemental, nutrient, and dissolved organic data from ITE sites during the 1991 Louisianian Province Demonstration.

Station	Date	$\delta^{13}C$ DIC	$\delta^{13}C$ SPM	$\delta^{13}C$ HA	$\delta^{13}C$ BA	$\Delta^{13}C$	$\delta^{15}N$ NH4	$\delta^{15}N$ NO3	$\delta^{15}N$ SPM	$\delta^{15}N$ HA	$\delta^{15}N$ BA
Southern Laguna Madre	8/7/91	0.1	-17.3	-22.4	-23.4	-17.4	B.D.	B.D.	8.6	14.8	10.7
San Antonio Bay	8/4/91	-2.6	-21.0	-22.4	-22.9	-18.4	B.D.	B.D.	7.4	6.5	8.3
Bayou Casotte	8/9/91	-2.6	-21.5	-22.9	-22.6	-18.9	B.D.	B.D.	8.7	11.3	8.4
Watson's Bayou	7/16/91	-2.1	-22.1	-23.6	-24.2	-20.0	B.D.	B.D.	5.8	12.6	14.5
Lavaca Bay	7/13/91	-4.2	-22.8	-22.3	-24.0	-18.6	B.D.	B.D.	8.2	12.4	8.0
Wolf Bay	8/14/91	-2.4	-23.9	-24.5	-23.8	-21.5	7.2	B.D.	10.6	14.2	15.0
Arroyo Colorado	8/5/91	-5.9	-24.5	-24.4	-24.2	-18.6	B.D.	B.D.	13.5	9.5	12.6
Brazos River	7/12/91	-8.7	-25.1	-24.5	-24.5	-16.4	10.0	36.6	6.5	7.5	14.9
Perdido River	8/14/91	-3.0	-25.8	LOST	-25.2	-22.8	B.D.	B.D.	10.0	LOST	16.0
Apalachicola Bay	7/15/91	-4.6	-26.9	-24.9	-24.2	-22.3	B.D.	2.1	8.5	13.1	11.1
Galveston Bay	7/10/91	-6.3	-27.3	-24.3	-23.7	-21.0	B.D.	B.D.	12.4	9.2	15.3
Lake Calcasieu	7/15/91	-9.5	-27.3	-25.4	-24.7	-17.8	B.D.	-2.4	17.3	4.5	9.5
Choctawhatchee Bay	7/17/91	-11.1	-27.4	-26.1	-24.1	-16.3	B.D.	3.3	7.1	10.5	14.4
Houston Ship Channel	7/11/91	-10.4	-27.6	-26.4	-25.5	-17.2	32.6	7.8	15.0	12.7	28.5
Mobile Bay	8/24/91	-11.1	-28.1	-25.9	-24.2	-17.0	B.D.	B.D.	8.9	15.6	16.4
Escambia Bay	7/18/91	-10.5	-30.7	-26.1	LOST	-20.2	2.2	1.9	11.1	15.6	LOST

B.D. = Below Detection

Table 4.13 Isotopic data collected from ITE sites during the 1991 Louisianian Province Demonstration. $\Delta^{13}C$ is the isotopic discrimination between suspended particulate matter and dissolved inorganic carbon.

sampled waters spanning the range of terrestrial and algal sources of organic matter (Fig. 4.1), consistent with the fact that these samples originated from both fresh and coastally-dominated waters. The most positive value (-17.3 ppt) was from Southern Laguna Madre where seagrasses, which are relatively enriched in ^{13}C, are known to contribute significant quantities of organic matter (Fry et al. 1987). At the opposite end, Escambia Bay SPM had anomalously light $\delta^{13}C$, -30.7 ppt, which is outside the range generally reported for terrestrially-derived organic matter in estuaries. The source of this enriched material is discussed below.

When the stations are ordered from most positive to negative $\delta^{13}C$ of SPM as in Figure 4.1, the corresponding and expected transition from terrestrial or sewage-derived nitrogen ($\delta^{15}N$ of -2 to +4) to coastal nitrogen ($\delta^{15}N$ of +8 to +12) was not observed for SPM (Fig. 4.2). Lavaca Bay, Arroyo Colorado, Galveston Bay, and the Houston Ship Channel all had significantly ^{15}N-enriched values, resulting from either degradation (Altabet and McCarthy 1986) or from uptake of isotopically enriched inorganic nitrogen (Mariotti et al. 1984; Cifuentes et al. 1988). Considering the $\delta^{13}C$ ratios, the C:N ratios of SPM (Fig. 4.3) were generally in the range reported for algae (7-10, Holligan et al. 1984) rather than bacteria (3-5, Lee and Furhman 1987) or vascular plant material (>50, Hedges and Mann 1979). Some values were highly enriched in nitrogen (C:N <5). These values could result from algae growing in nitrogen-enriched conditions, or from extensive bacterial colonization of particles. The combination of low C:N and negative $\delta^{13}C$ in SPM, therefore, is more likely the result of algae growing on isotopically light CO_2 (Fogel et al. 1992). The use of stable isotopes as a developmental indicator will be initiated in 1993.

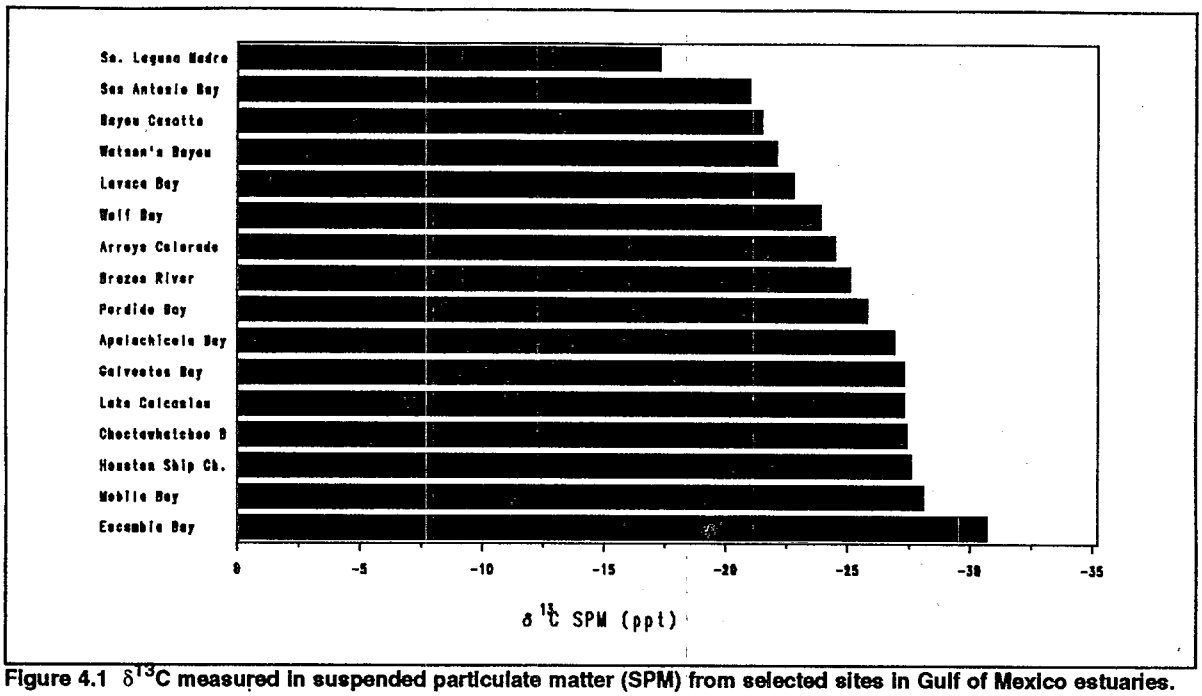

Figure 4.1 $\delta^{13}C$ **measured in suspended particulate matter (SPM) from selected sites in Gulf of Mexico estuaries.**

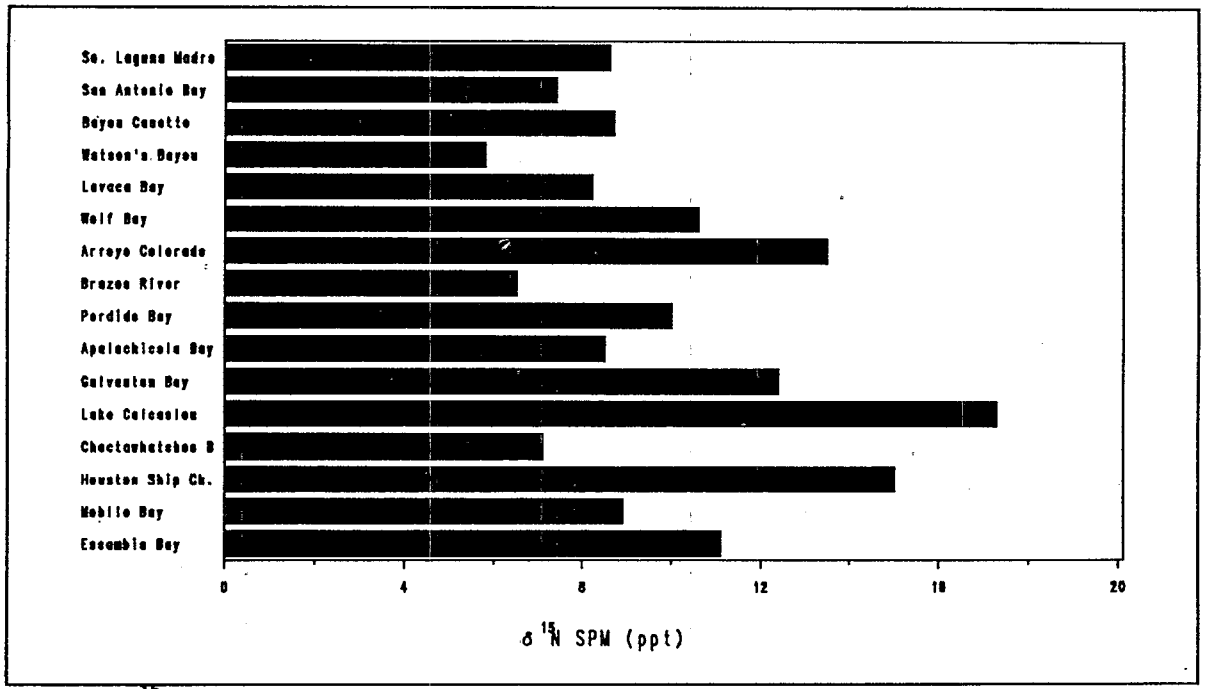

Figure 4.2 $\delta^{15}N$ **measured in suspended particulate matter (SPM) from selected sites in Gulf of Mexico estuarine systems.**

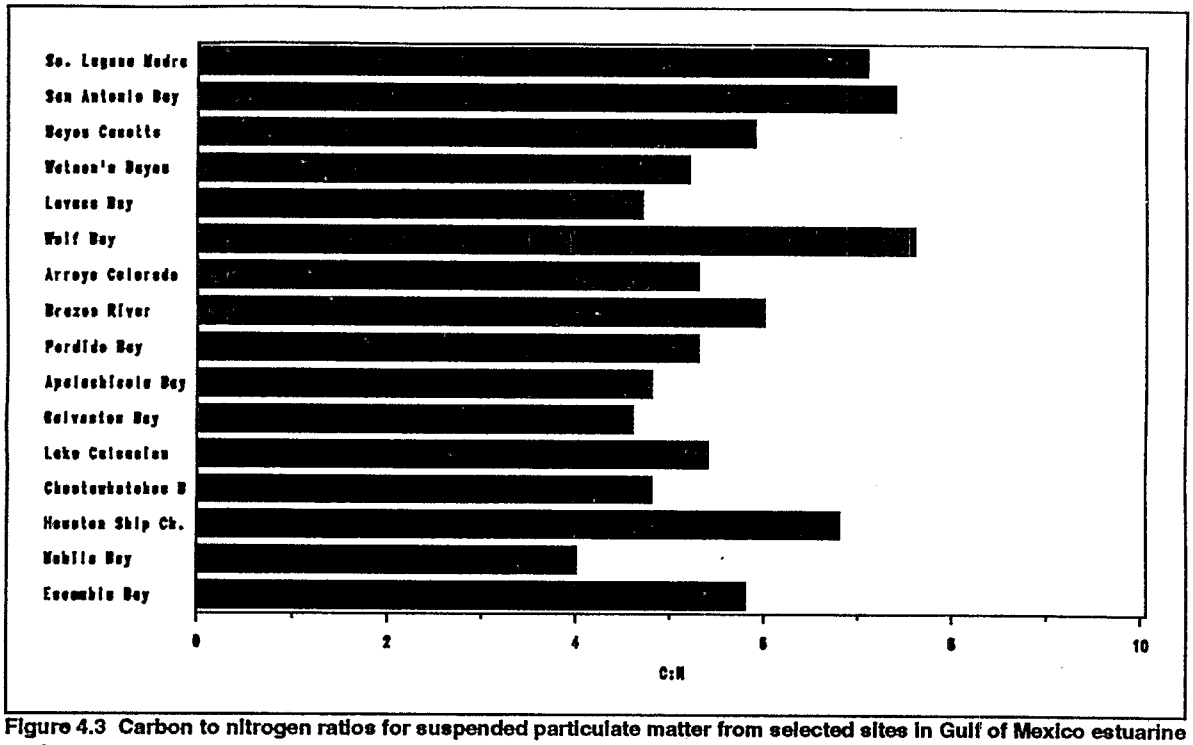

Figure 4.3 Carbon to nitrogen ratios for suspended particulate matter from selected sites in Gulf of Mexico estuarine systems.

SECTION 5

STATISTICAL ASSOCIATIONS BETWEEN RESPONSE AND EXPOSURE INDICATORS

One of the objectives of the EMAP-E Louisianian Province is to ascertain statistical associations for response indicators (e.g., benthic index, water clarity, fish tissue contaminant levels). These associations could relate, statistically, response variables with exposure indicators (e.g., sediment contaminants, sediment toxicity, hypoxia) or stressor indicators (e.g., human population, discharge volumes in watersheds, pesticide application rates). One of the reasons for collecting exposure indicators during EMAP sampling is to allow the determination of statistical associations. The existence of these associations does not imply causation but rather denotes a statistical correlation. The results of these associations can be used by researchers to hone research hypotheses that can be tested to determine causation. However, this research is not a part of the monitoring element of EMAP at present.

Several potential associations were investigated relating to response indicators used in the 1991 Louisianian Province Demonstration. These included examinations of the statistical relationships between the benthic index and various exposure indicators, between fish contaminant concentrations and sediment contaminant concentrations, and between sediment toxicity and sediment contaminants. Many other associations could be investigated but the purpose of this section is to demonstrate how the EMAP monitoring data can be used to evaluate associations. No statistical relationships were investigated between indicators and stressors, whether natural (e.g., climate) or anthropogenic (e.g., total watershed loadings) because the stressor data will not be collated from existing data bases until future years in EMAP.

5.1 ASSOCIATIONS WITH THE BENTHIC INDEX

The methodology used to create the benthic index was described in Section 2. The benthic index accounts for 90% of the variability observed in the 1991 subset of sites exhibiting environmental extremes and thus, the benthic index is assumed to be representative of an integrative indicator of ecological condition. While further testing through Years 2-4 in the Louisianian Province will be necessary to confirm this assumption, it is worthwhile to evaluate the potential associations between the benthic index and exposure indicators. The results of this analysis can be used to begin to assess the causes of degraded benthic communities in Gulf of Mexico estuaries through long-term structured research.

The associations between the benthic index and exposure indicators were determined using three statistical tools: (1) univariate correlations between the benthic index value at a site and corresponding exposure indicators at that site, (2) multivariate regressions between index values and exposure indicator values, and (3) multivariate regressions between categorized index values (i.e., < 4.1 represents poor conditions and > 6.5 represents good conditions) and exposure values. All of these assessments were completed for the Louisianian Province as a whole and for each of the three estuarine classes (i.e., large estuaries, large tidal rivers, and small estuaries). Other spatial divisions (Gulf States and individual estuaries) could be evaluated after several additional years of monitoring data are collected (i.e., a single year's information provides too small a sample size).

Univariate analysis comparing the benthic index to each exposure indicator showed that few exposure indicators accounted for significant portions of the variability in the observed benthic index (Table 5.1). While 44 individual sediment contaminants were significantly related to the benthic index value, only 9 contaminants accounted for > 10% of the variability in the index (2 alkanes, 2 pesticides, 4 PAHs, and 1 PCB). Of these sediment contaminants, only three accounted for > 20% of the variation: two alkanes (C10 and C13) and one pesticide (4,4' DDD). Univariate analysis of non-sediment contaminant indicators revealed only 3 indicators accounting for > 5% of

Sediment Contaminant Indicator	R^2	Significance
Alkanes		
C10	0.216	***
C12	0.059	*
C13	0.235	***
C14	0.069	**
C22	0.099	*
C23	0.047	*
C24	0.049	*
C25	0.043	*
C26	0.073	**
C34	0.042	*
Heavy Metals		
Cadmium	0.064	*
Chromium	0.066	*
Copper	0.064	*
Iron	0.081	**
Lead	0.089	**
Manganese	0.072	**
Mercury	0.076	**
Nickel	0.092	**
Selenium	0.043	*
Tin	0.094	**
Zinc	0.095	**
Anthropogenic Metals		
Mercury	0.051	*
Nickel	0.041	*
PCB Congeners		
Congener #52	0.145	***
Congener #66	0.043	*
Congeners #110/#77	0.044	*
Pesticides		
alpha-Chlordane	0.072	**
2,4'-DDD	0.097	**
4,4'-DDD	0.247	***
4,4'-DDE	0.099	**
4,4'-DDT	0.143	***
Dieldrin	0.093	**
trans-Nonachlor	0.066	*

Table 5.1 Significant univariate relationships between the benthic index and sediment contaminant indicators. (* = p < .05, ** = p < .01, and *** = p < .001).

Sediment Contaminant Indicator	R^2	Significance
PAHs		
Benzo(a)pyrene	0.043	*
Benzo(k)fluoranthene	0.046	*
Benzo(g,h,i)perylene	0.052	*
Biphenyl	0.088	**
C1-Naphthalene	0.147	***
C2-Naphthalene	0.091	**
2,6 Dimethylnaphthalene	0.127	***
Ideno(1,2,3,c,d)pyrene	0.050	*
1-methylnaphthalene	0.158	***
2-methylnaphthalene	0.134	**
Naphthalene	0.110	**

Table 5.1(Cont.) Significant univariate relationships between the benthic index and sediment contaminant indicators. (* = p < .05, ** = p < .01, and *** = p < .001).

Exposure Indicator	R^2	Significance
Instantaneous Bottom Dissolved Oxygen	0.001	
Ampelisca Bioassay	0.012	*
Mysid Bioassay	0.012	
Light Transmittance (PAR) at 1 meter	0.032	
RPD Depth	0.047	*
Light Transmittance (PAR) at bottom	0.007	
Bottom Salinity	0.073	**
Bottom pH	0.042	
Percent Silt-Clay	0.010	
Total Organic Carbon	0.030	
Acid Volatile Sulfides	0.001	
Bottom Temperature	0.034	

Table 5.2 Univariate relationships between the benthic index and exposure indicators other than sediment contaminants. (* = p < .05, ** = p < .01, and *** = p < .001).

observed variability and no indicators accounted for > 10% of variation (Table 5.2).

Univariate statistics can be used to examine if there are any significant differences in how benthic communities in the three estuarine classes relate to exposure variables. Benthic index values in all three classes, based on univariate correlations, showed associations with selected alkanes and heavy metals (Table 5.3) while large estuaries and large tidal rivers had some relationships with sediment and water quality attributes. Benthic communities in small estuaries were the only benthic assemblages to show any association with sediment pesticide concentrations. These analyses suggest that while some differences exist among the three estuarine classes with regard to poor benthic assemblages as determined by the benthic index, benthic communities in all three estuarine classes are associated with similar environmental exposure indicators.

We developed a stepwise multivariate regression model for the province-wide benthic index. While a successful model was created, it only accounted for 49% of the observed variation in the distribution of benthic index values (Fig. 5.1). The six variables that characterize this model are all sediment contaminants suggesting that the only significant associations between the benthic index and exposure variables are sediment contaminants (2 heavy metals, 1 pesticide, 1 alkane, 1 PAH, and 1 PCB) with the degraded form of DDT (4,4'-DDD) accounting for 25% of the variability. As was shown in the univariate analyses, some differences between the three

Exposure Variable	R^2		
	Large	Tidal	Small
Alkanes	(n=48)	(n=10)	(n=42)
Total	0.103*	0.132	0.001
C10	0.054	0.444*	0.123*
C16	0.000	0.529*	0.060
C18	0.133*	0.144	0.038
C19	0.211***	0.105	0.025
C20	0.154**	0.000	0.000
C21	0.102*	0.000	0.003
C22	0.195**	0.029	0.083
C23	0.191**	0.005	0.096
C24	0.144**	0.010	0.093
C25	0.196**	0.004	0.109*
C26	0.253***	0.000	0.152*
C27	0.095*	0.339	0.023
C28	0.010	0.485*	0.009
C30	0.232***	0.000	0.052
C31	0.219***	0.001	0.020
C32	0.284***	0.001	0.028
C33	0.209**	0.012	0.016
C34	0.249***	0.046	0.049
Heavy Metals			
Arsenic	0.066	0.044	0.288***
Cadmium	0.127*	0.039	0.037
Chromium	0.076	0.023	0.187**
Copper	0.172**	0.030	0.121*
Iron	0.167**	0.035	0.216**
Lead	0.195**	0.024	0.169*
Manganese	0.135*	0.021	0.028
Mercury	0.039	0.422*	0.021
Nickel	0.191**	0.037	0.227**
Selenium	0.196**	0.027	0.057
Tin	0.108*	0.078	0.210**
Zinc	0.122*	0.021	0.198**
PCB Congeners			
Congener #28	0.148**	0.029	0.023
Congener #126	0.086*	0.017	0.024
Pesticides			
4,4'-DDE	0.022	0.014	0.158*

Table 5.3 Univariate relationships between benthic index values and exposure indicators for large estuaries (Large), large tidal rivers (Tidal), and small estuaries (Small) (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).

Exposure Variable	R^2		
	Large	Tidal	Small
PAHs			
Fluorene	0.119	0.007	0.054
Naphthalene	0.010	0.017	0.143*
Phenanthrene	0.109*	0.030	0.043
Water Quality			
Bottom pH	0.041	0.534*	0.040
Sediment Quality			
Total Organic Carbon	0.276***	0.031	0.060
Mean RPD Depth	0.011	0.585***	0.010

Table 5.3 (Cont.) Univariate relationships between benthic index values and exposure indicators for large estuaries (Large), large tidal rivers (Tidal), and small estuaries (Small) (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).

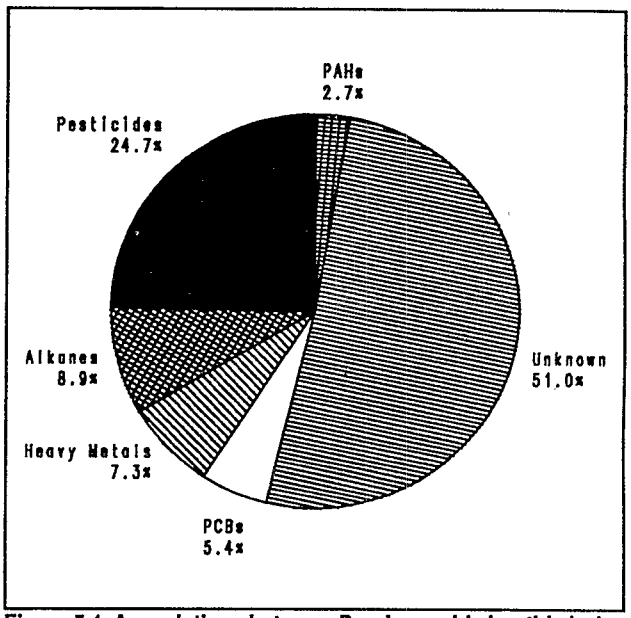

Figure 5.1 Associations between Province-wide benthic index and exposure indicators. Percentage associated with portion of pie portrays R^2 of indicator.

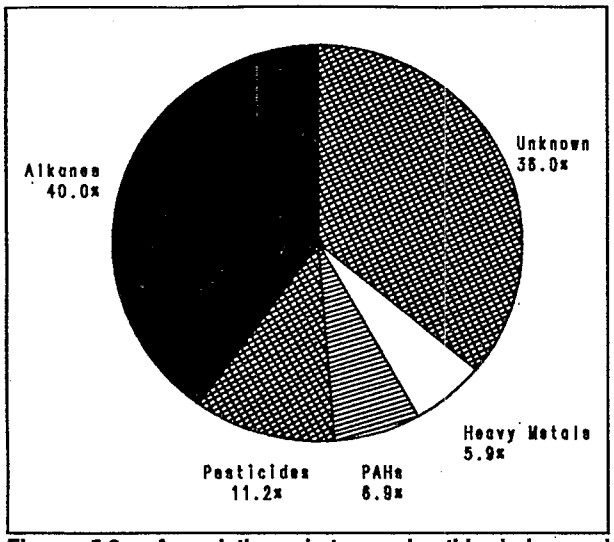

Figure 5.2 Associations between benthic index and exposure indicators for large estuaries. Percentages associated with portion of pie portrays R^2 of indicator.

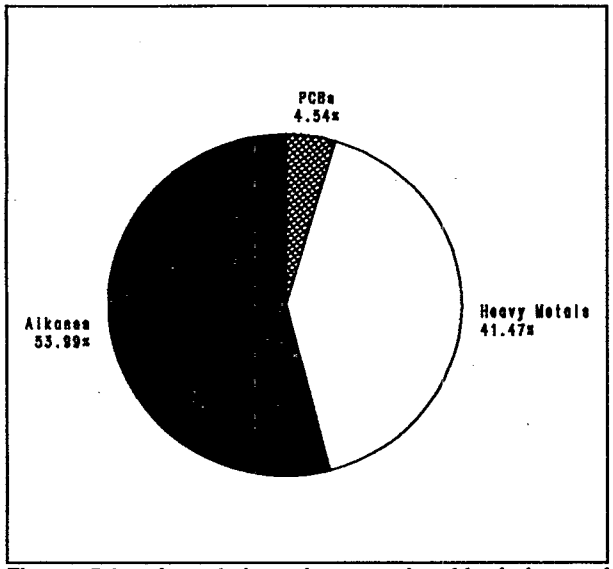

Figure 5.3 Associations between benthic index and exposure indicators for large tidal rivers. Percentages associated with portion of pie portrays R^2 of indicator.

estuarine classes may exist; therefore, stepwise multivariate regressions were developed for each estuarine class.

The multivariate regression for the large estuary class increased the overall accounted variability to 64% (Fig. 5.2) with sediment contaminants again comprising all of the explained variability (2 alkanes, 2 pesticides, 1 PAH, and 1 heavy metal). The concentration of alkanes in sediments contributed to 40% of the observed variability in the benthic index seen in large estuaries. Ninety-eight percent of the variability in benthic index values in large tidal rivers in the Louisianian Province (i.e., Mississippi River) was associated with sediment contaminants (1 alkanes, 1 PCB, and 1 heavy metal). Alkanes and heavy metals accounted for 54% and 41% of the variability in large tidal river benthic index values (Fig 5.3). Similarly, in small estuaries, the benthic index is associated with sediment contaminants that account for 66% of observed variability (Fig 5.4). Unlike large estuaries and tidal rivers, heavy metals in sediments accounted for 40% of the variability in the benthic index with the remaining 26% of the explained variability being associated with a PAH, a PCB, and a pesticide.

Because the benthic index represents an evolving response indicator, we tested the hypothesis that the index, based on a single year, only accurately discriminates between poor benthic community structure and good benthic community structure. Under this hypothesis, variability within these two classes has not been developed to the point to accurately represent the ordering among the estuaries, although the underlying structure exists and will take

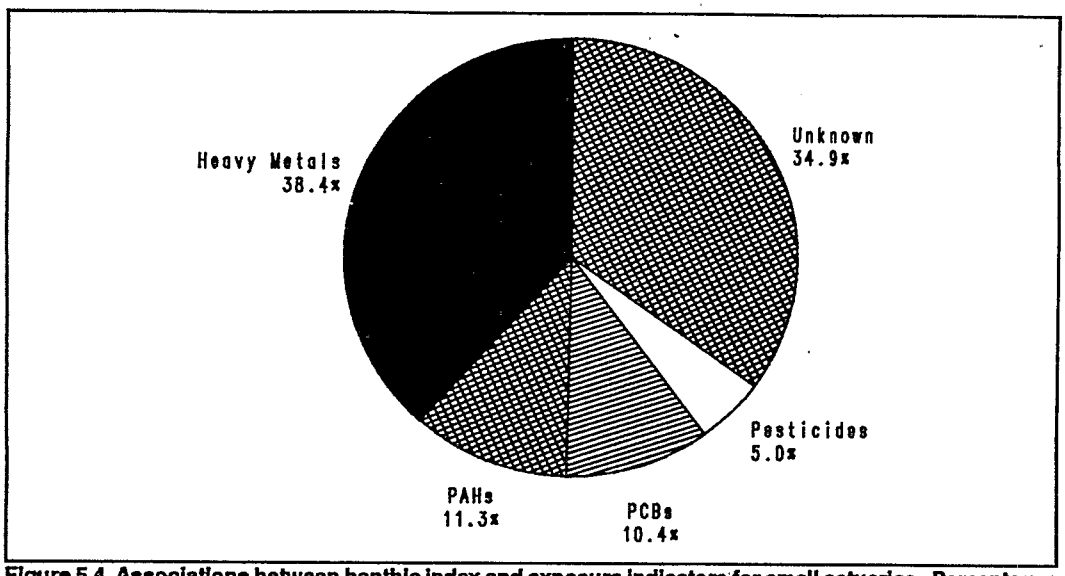

Figure 5.4 Associations between benthic index and exposure indicators for small estuaries. Percentages associated with portion of pie portrays R^2 of indicator.

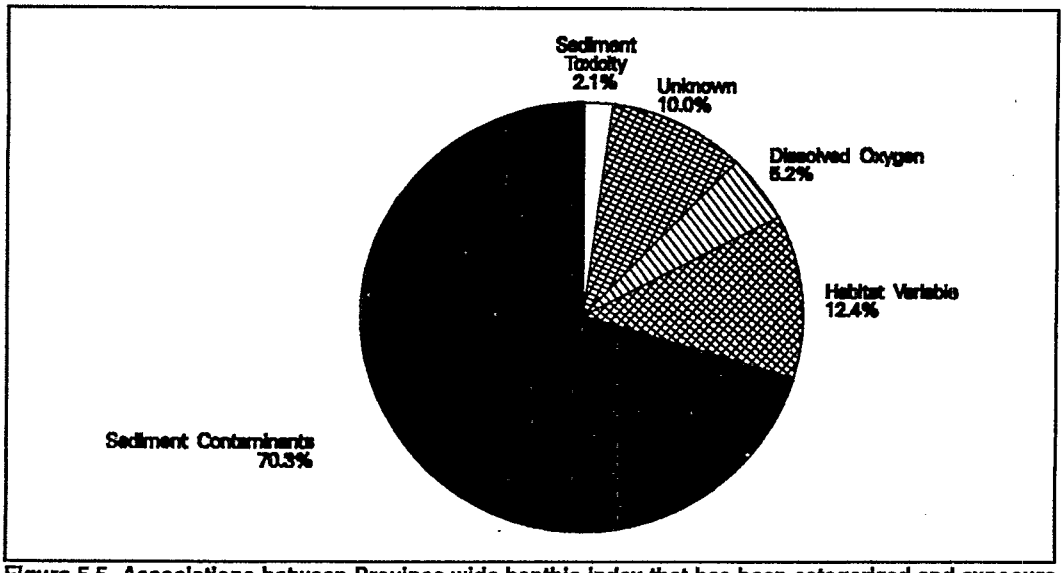

Figure 5.5 Associations between Province-wide benthic index that has been categorized and exposure indicators. Percentages associated with portion of pie portrays R^2 of indicator.

several years to ascertain. In this analysis, we assigned two categories to the benthic index: poor < 4.1 and good > 6.1. Using the categorical regression, 90% of the variability in these benthic index classes were associated with measured exposure indicators (Fig. 5.5). Sediment contaminant concentrations and sediment toxicity accounted for 72% of the variability between good and poor benthic index values with heavy metals contributing the greatest portion of this association (42%). Alkanes contributed 17% with other sediment contaminants (i.e., PCBs, PAHs, and pesticides contributing 11%. The degree of stratification, minimum dissolved oxygen concentration, and the percent of time dissolved oxygen concentrations are less than 2 ppm was associated with 5% of the differences between the index categories. Other water quality habitat

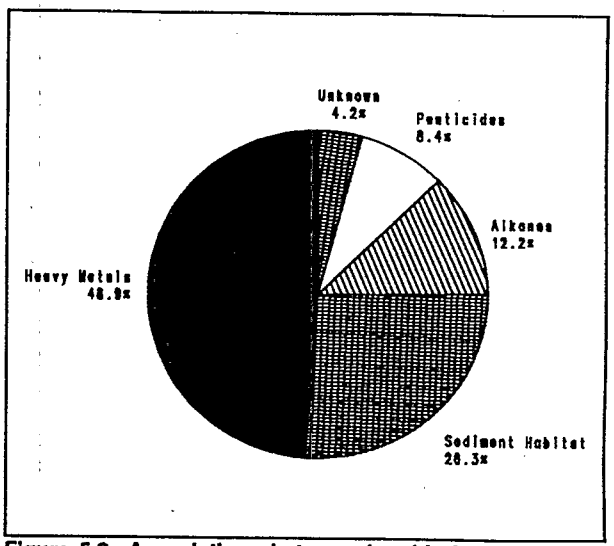

Figure 5.6 Associations between benthic index that has been categorized and exposure indicators for large estuaries. Percentages associated with portion of pie portrays r^2 of indicator.

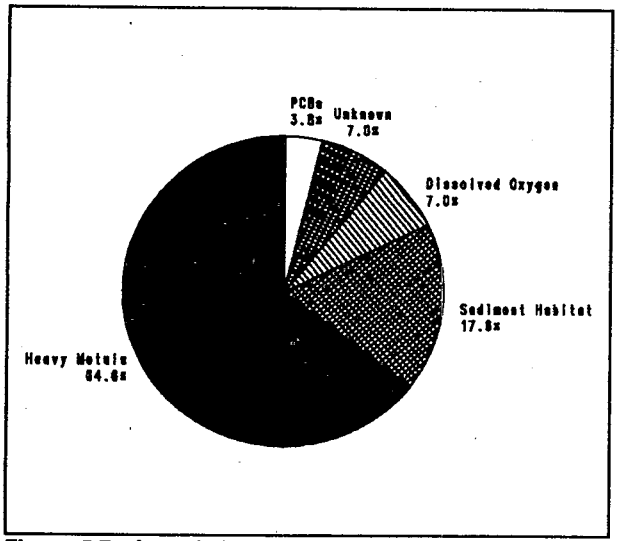

Figure 5.7 Associations between benthic index that has been categorized and exposure indicators for small estuaries. Percentages associated with portion of pie portrays r^2 of indicator.

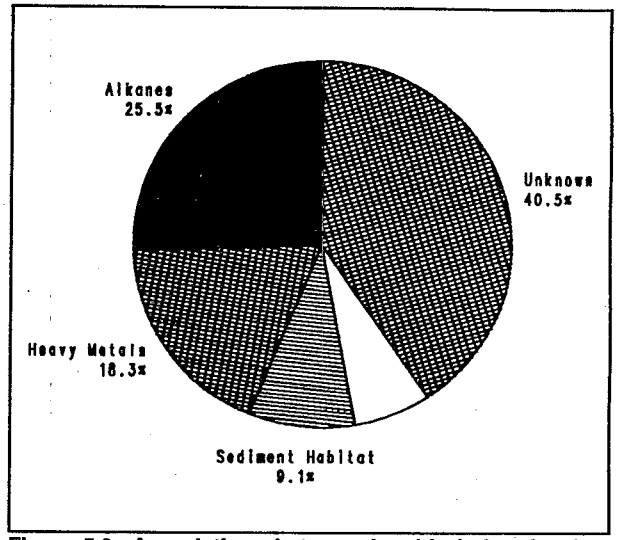

Figure 5.8 Associations between benthic index that has been categorized and exposure indicators for large tidal rivers. Percentages associated with portion of pie portrays r^2 of indicator.

Exposure Variable	r	
	Ampelisca	Mysids
Heavy Metals		
Aluminum		-0.36***
Antimony		-0.25*
Arsenic		-0.26**
Cadmium		-0.34***
Chromium		-0.27**
Copper		-0.38***
Iron		-0.40***
Lead		-0.35***
Manganese	-0.26**	-0.64***
Nickel		-0.40***
Selenium		-0.37***
Tin		-0.38***
Zinc		-0.48***
PCBs		
Congener #44	-0.22*	
Congener #110	-0.26**	
Congener #126	-0.21*	-0.33***
Congener #206	-0.21*	
PAHs		
C1-Chrysene	-0.22*	
C2-Chrysene	-0.22*	
C3-Chrysene	-0.21*	
C4-Chrysene	-0.25*	
C1-Fluoranthene	-0.20*	
C4-Phenanthrene	-0.20*	
Dibezo(a,h)anthracene	-0.21*	
Perylene		-0.46***
Pesticides		
Aldrin	-0.26**	
alpha-BHC		-0.22*
alpha-Chlordane	-0.35***	-0.51***
cis-Nonachlor	-0.28**	-0.33***
4,4'-DDD	-0.21*	-0.21*
4,4'-DDT	-0.38***	-0.33***
Dieldrin	-0.26**	-0.23*
gamma-Chlordane	-0.38***	-0.32**
Mirex	-0.40***	-0.50***
Oxychlordane	-0.37***	-0.48***
Composite Totals		
Total Alkanes		-0.23*
Alkanes > 7000 ppb[1]	-0.22*	-0.44***
Total Chlordanes	-0.33***	-0.27**
Number of Metals exceeding Criteria Value		-0.47***

Table 5.4 Significant univariate relationships between survival of Ampelisca and mysid in bioassays and sediment contaminant concentrations using Pearson correlation coefficient (r). (* = p < 0.05, ** = p < 0.01, *** = p < 0.001).

indicators (i.e., bottom pH, salinity, temperature and light transmittance) accounted for 12% of the variation. When the categorical regressions were developed by estuarine class, heavy metals in sediments were associated with major portions of the variation in index categories for large estuaries (49%) and small estuaries (65%) (Fig. 5.6 and 5.7, respectively). Heavy metals were less importantly associated with the index in large tidal rivers (18%) but alkanes were the major association with the index in the Mississippi River (26%). However, because the Mississippi River contributes < 1% of the area of the province, heavy metals were the predominate association between the benthic index and sediment contaminants.

Regardless of the statistical analysis used, variation in the benthic index is associated with sediment contaminants, clearly, showing that ecological integrity, as measured by the benthic index, is related to the degree of sediment contamination. While dissolved oxygen condition plays a significant role, its association with the benthic index accounts for a small portion of the observed variability. Although the index was adjusted for differences due to salinity, several other habitat variables apparently contribute to a small portion of the variability observed in the index.

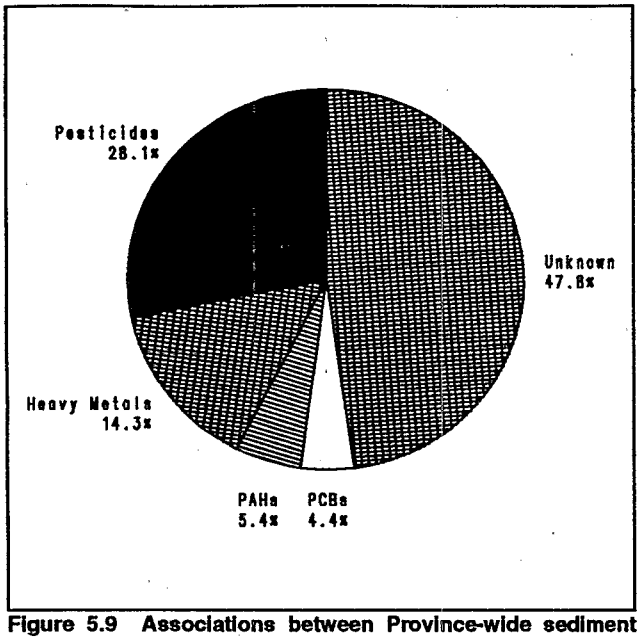

Figure 5.9 Associations between Province-wide sediment toxicity for (a) amphipods and (b) mysides and sediment contaminants. Percentage associated with portion of pie portrays R^2 of indicator.

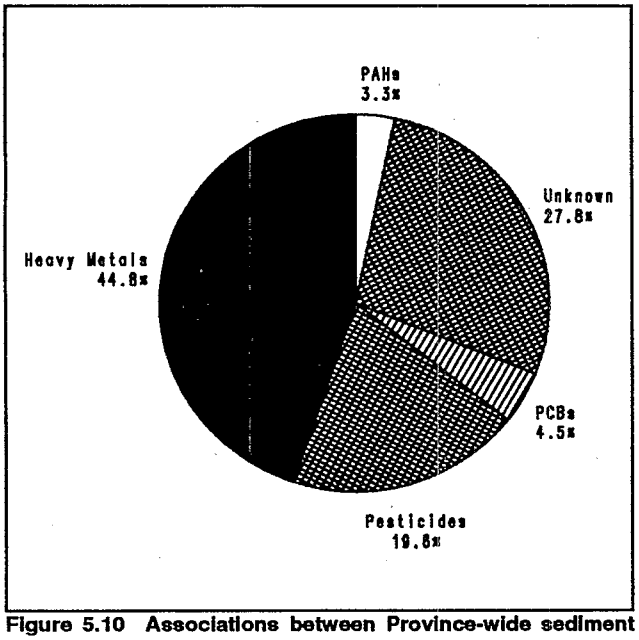

Figure 5.10 Associations between Province-wide sediment toxicity for (a) amphipods and (b) mysids and sediment contaminants. Percentage associated with portion of pie portrays R^2 of indicator.

5.2 ASSOCIATIONS WITH SEDIMENT TOXICITY

The associations between the sediment toxicity and sediment contaminant concentrations were determined using two statistical tools: (1) univariate correlations between the bioassay survival rate at a site and corresponding sediment contaminant concentrations at that site, and (2) multivariate regressions between index values and exposure indicator values. These assessments were completed for the Louisianian Province. Other spatial divisions (estuarine classes, Gulf States, and individual estuaries) could be evaluated after several additional years of monitoring data are collected (i.e., a single year's information provides too small a sample size).

Table 5.4 shows the results of the univariate analysis for metals, PCBs, PAHs, pesticides, composite measures of contaminants (e.g., total alkanes, total PCBs). Ten-day bioassays with ***Ampelisca abdita*** (amphipod) were most strongly associated with pesticides although the sediment concentration of any individual pesticide only accounted for no more than 16% of the variability seen in sediment toxicity. While the explained variance was small for any individual sediment contaminant, all significant correlations were negative showing the increased concentrations of particular contaminants were associated with decreased survivorship of amphipod in 10-day bioassays. Using multivariate regressions confirmed the univariate correlation results with 28.1% of the

variation in amphipod sediment toxicity being associated with pesticides (Fig. 5.9). Of the remaining 72% of the variation in sediment toxicity, 14% was associated with heavy metal concentrations and only 10% was associated with PAHs and PCBs combined.

Table 5.4 shows the results of the univariate analysis for sediment contaminants and 3-day mysid bioassays. *Mysidopsis* (mysid) bioassays were most strongly associated with heavy metals and pesticides. Several heavy metals and pesticides were associated with greater than 20% of the observed variance in the 3-day mysid tests (e.g., manganese, zinc, BHC, mirex, and oxychlordane). Several composite contaminant values were strongly associated with mysid bioassays. The number of heavy metals at a site exceeding critical values was associated with 22% of the variation in these bioassays while sites with total alkanes > 7000 ppb were strongly associated with mysid mortality (19%). About 72% of the variation in mysid survivorship was associated with sediment contaminants with higher concentrations of contaminants being associated with lower survival rates (Fig. 5.10). Heavy metals and pesticides were associated with 45% and 20% of the variation in mysid survival rates, respectively.

5.3 ASSOCIATIONS BETWEEN SEDIMENT CONTAMINANTS WITH ACID VOLATILE SULFIDES AND TOTAL ORGANIC CARBON

An analysis was performed to assess the extent to which acid volatile sulfides (AVS) and total organic carbon (TOC) were associated with contaminant concentrations in sediment using Pearson correlations. Five of the 12 heavy metals (raw concentrations) were significantly correlated with AVS (Table 5.5). Nine of the 12 heavy metals were significantly associated with concentrations of sediment organic carbon. Most of the R^2 values for individual metals were rather low (i.e., < 20%), the highest correlations were between AVS and mercury (0.44) and cadmium (0.43) and TOC and cadmium (0.57), lead (0.49), selenium (0.45), and mercury (0.43). Unlike raw concentrations, aluminum-adjusted metal concentrations were more strongly associated with TOC than AVS. Aluminum-adjusted lead (0.61), cadmium (0.58), selenium (0.44), mercury (0.43), and chromium (0.43) were strongly associated with TOC while only aluminum-adjusted lead was strongly related to AVS (0.47).

Many organic contaminants were significantly associated with AVS and TOC levels in sediments. Nineteen of 28 alkanes were significantly associated with TOC concentrations while 10 alkanes were associated with AVS. Correlations between alkanes and TOC were 50-100% stronger than those between AVS and alkanes (Table 5.5). Fifty-eight percent of PAHs were significantly associated with

TOC and 34% of PAHs were associated with AVS. Unlike other organic contaminants, chlorinated pesticides were more strongly associated with AVS than with TOC (Table 5.5).

These significant relationships suggest that AVS or TOC could be used as a possible covariate. In addition, the relationships between heavy metal or organic contaminant concentrations and AVS or TOC should be investigated to determine if this relationship is related to bioavailability.

5.4 ASSOCIATIONS WITH DISSOLVED OXYGEN

The associations among dissolved oxygen (DO) and habitat indicators were investigated using univariate analysis. Two dissolved oxygen parameters (instantaneous bottom DO concentration and the minimum DO concentration) were correlated with six habitat indicators (AVS, TOC, mean RPD depth, temperature, degree of stratification, and surface area of estuary). Province-wide associations were found between instantaneous bottom DO and degree of stratification and bottom temperature that accounted for 28% of the variation in DO concentrations. Minimum DO concentrations were associated with degree of stratification and AVS concentrations accounting for 17% of observed variation.

Examination of the three estuarine classes showed that instantaneous bottom DO concentrations were most strongly associated with habitat indicators in large tidal rivers. Ninety-four percent of the observed variability in instantaneous bottom DO was associated with degree of stratification, bottom temperature, and AVS concentrations. Only 31% of observed variability in bottom instantaneous DO in large estuaries was associated with a single habitat indicator (degree of stratification). In small estuaries, 27% of variation in dissolved oxygen was associated with degree of stratification and bottom temperature. Significantly smaller portions of overall variability in minimum DO were associated with habitat variables in large and small estuaries (11% and 22%, respectively).

Evaluation of these results suggest that a portion of the hypoxic conditions observed during the 1991 Louisianian Province Demonstration could be related to physical factors (stratification, temperature). Large portions of the observed variability in both instantaneous and minimum DO concentrations remained unexplained. Only DO concentrations in the Mississippi River were predominately associated with physical factors. If dissolved oxygen represents an endpoint for estuarine eutrophication, much of the unexplained variability in DO concentrations in large and small estuaries could be due to eutrophic conditions (e.g., nutrient concentrations, chlorophyll concentrations). As was shown in Section 4, stable isotope analysis of carbon and nitrogen suggested strongly eutrophic conditions (phytoplankton sources of carbon) at hypoxic sites. Additional indicators would have to be evaluated to assess the potential for eutrophication in Louisianian Province estuaries.

Metals	AVS r	TOC
Arsenic	0.27**	0.28**
Cadmium	0.43***	0.57***
Chromium	0.30**	0.40***
Copper		0.33***
Lead	0.39***	0.49***
Mercury	0.44***	0.43***
Al-Adjusted Metals		
Arsenic	0.24*	0.26**
Cadmium	0.43***	0.58***
Chromium		0.43***
Copper		0.39***
Iron		0.33***
Lead	0.47***	0.61***
Mercury	0.43***	0.43***
Selenium	0.32***	0.44***
Zinc	0.22*	
Alkanes		
C10	0.28**	0.47***
C15	0.24*	0.24*
C17	0.35***	0.49***
C19		0.22*
C20		0.31**
C21	0.26**	0.47***
C22	0.25*	0.53***
C23	0.27**	0.55***
C24	0.28**	0.58***
C25	0.26**	0.58***
C26	0.26**	0.62***
C27		0.33***
C28		0.21*
C30	0.27**	0.47***
C31		0.30**
C32		0.33***
C33		0.24*
C34		0.32**
Total		0.38***

Table 5.5 Significant Pearson correlations of sediment metal concentrations with acid volatile sulfides and total organic carbon. (* = p < .05, ** = p < .01, and *** = p < .001).

PAHs	AVS r	TOC
Acenaphthylene	0.35***	0.36***
Acenaphthene	0.27**	0.29**
Anthracene	0.31**	0.36***
Benzo(a)anthracene	0.52***	0.48***
Benzo(a)pyrene	0.48***	0.45***
Benzo(b)fluoranthene	0.61***	0.51***
Benzo(e)pyrene	0.54***	0.48***
Benzo(k)fluoranthene	0.58***	0.60***
Benzo(g,h,i)perylene	0.27**	0.29**
C1-Fluoranthene	0.26*	0.26*
C1-Naphthalene		0.35*
C2-Naphthalene		0.35*
C3-Naphthalene		0.25*
C4-Naphthalene		0.24*
Chrysene	0.55***	0.51***
2,6-Dimethylnaphthalene		0.32***
Fluorene		0.25**
Fluoranthene	0.55***	0.53***
Ideno (1,2,3,c,d)pyrene	0.40***	0.41***
1-Methylnaphthalene		0.37***
2-Methylnaphthalene		0.32***
1-Methylphenanthrene	0.38***	
Naphthalene		0.41***
Phenanthrene		0.27**
Pyrene	0.46***	0.47***
Total PAHs		0.30**
Pesticides		
2,4'-DDD	0.37***	0.36***
2,4'-DDE	0.30**	
4,4'-DDE	0.60***	0.45***
2,4'-DDT	0.35***	0.32***
delta-BHC		0.20*
cis-Nonachlor		0.28**
Total DDT	0.38***	0.30**
PCBs		
Total PCBs	0.50***	0.45***

Table 5.5(Cont.) Significant Pearson correlations of sediment metal concentrations with acid volatile sulfides and total organic carbon. (* = p < .05, ** = p < .01, and *** = p < .001).

SECTION 6

EVALUATION OF SAMPLING DESIGN ATTRIBUTES

The sampling design used in the 1991 Louisianian Province Demonstration is described in detail in Summers et al. (1993b). Only a brief description appears in this section in order to facilitate discussion of issues associated with the design. These design issues involve:

- Comparison of index sampling and random sampling in small estuaries;
- Evaluation of the grid scale density (Is the present grid density sufficient to characterize estuarine characteristics?);
- Examination of the degree of spatial autocorrelation within the Louisianian Province dataset;
- Evaluation of the degree of replication necessary to develop province- and class-wide descriptions.

6.1 COMPARISON OF INDEX SAMPLING AND RANDOM SAMPLING

During the planning of the statistical design for the 1991 Louisianian Province Demonstration, the method for locating the sites within selected small estuaries and within the segments of the large tidal rivers were actively discussed. Essentially two alternatives existed: (1) random location of sites within the bounds of the estuaries or segments or (2) the location of index sites believed to be representative of the sampling space (i.e., small estuary or river segment). Basically, random locations are probability-based with the assumption of representativeness when sampling for individual estuaries or they can be used as individual points in the total space occupied by the population of small estuaries or river segments. Index sites are probability-based only if enough knowledge exists to select sites that are representative of the sampling space being sampled. Generally, index sampling requires fewer total samples than random sampling (Overton, personal communication) because of the spatial variability of the resource. Given this fact, index sampling would have been selected for sampling small estuaries. However, it was difficult to develop criteria for the selection of index sites (e.g., and even more difficult to locate sites that fit these criteria). As a result, both index (i.e., located as well as possible) and random samples were located in each small estuary and large tidal river segment sampled.

All indicators were collected at both site types. The resulting datasets were tested to determine if the paired random and index sites are more alike than two randomly selected sites. If the sites are similar then there is no advantage to index sampling. If the sites are statistically different, then index sampling could be advantageous. Four methods were used to

examine the question which should in ideal cases all provide the same results. In marginal situations, differences between the four different tests should be helpful in discerning which test results are correct. The four methods were:

- Comparison of population-level cumulative distribution functions (CDFs) based on random sites and based on index sites (similar CDFs would show no differences between population distributions based on random sites or index sites);

- ANOVA to determine significant differences between random and index sites based on paired sites as a factor (station effect) (if a station effect can be shown then the pairs vary less than two randomly selected sites);

- Correlation analysis using Pearson's **r** as a first order approximation to any relationship (if the slope is not different from 1.0 and the intercept is not different from 0, then the random and index sites are not different); and

- Correlation analysis using Spearman's **r** to detect any consistent relationship based on ordered data.

The types of indicators were discussed separately below.

6.1.1 BENTHIC RESPONSE INDICATORS

Benthic variables showed no differences in the population-level distributions of number of species, biodiversity, abundance, abundance of large bivalves, and proportional contributions of amphipods, decapods, bivalves, and polychaetes in large river segments. Two of the CDFs corresponding to major variables comprising the benthic index biodiversity and proportion of total abundance as bivalves, are shown in Figure 6.1 and 6.2. Thus, at a population level (class) for benthic variables, there are no differences in index and random sampling. However, ANOVA which shows paired consistencies rather than total distribution consistencies showed only 3 of the 8 benthic variables were similar at index and random sites in river segments (Table 6.1). Similar results were seen using correlational analyses where only percent decapods and percent polychaetes showed no differences between index and random sites. In large tidal river segments, higher mean number of benthic species, higher mean benthic abundance, and higher mean benthic diversity occurred at index sites (Table 6.1). Lower mean abundance and mean proportion of bivalves were seen at index sites. While CDFs of population distributions of these variables are not different between index and random sites in rivers (i.e, class-wide estimates are not significantly different), the sites do not occur at the same locations along the distribution function. Thus, for large tidal rivers, random sites are not representative of the segments they are supposed to represent and multiple sites would be required to represent individual segments of the rivers.

Benthic variables showed no differences in the population-level distributions of number of species, biodiversity, abundance, abundance of large bivalves, and

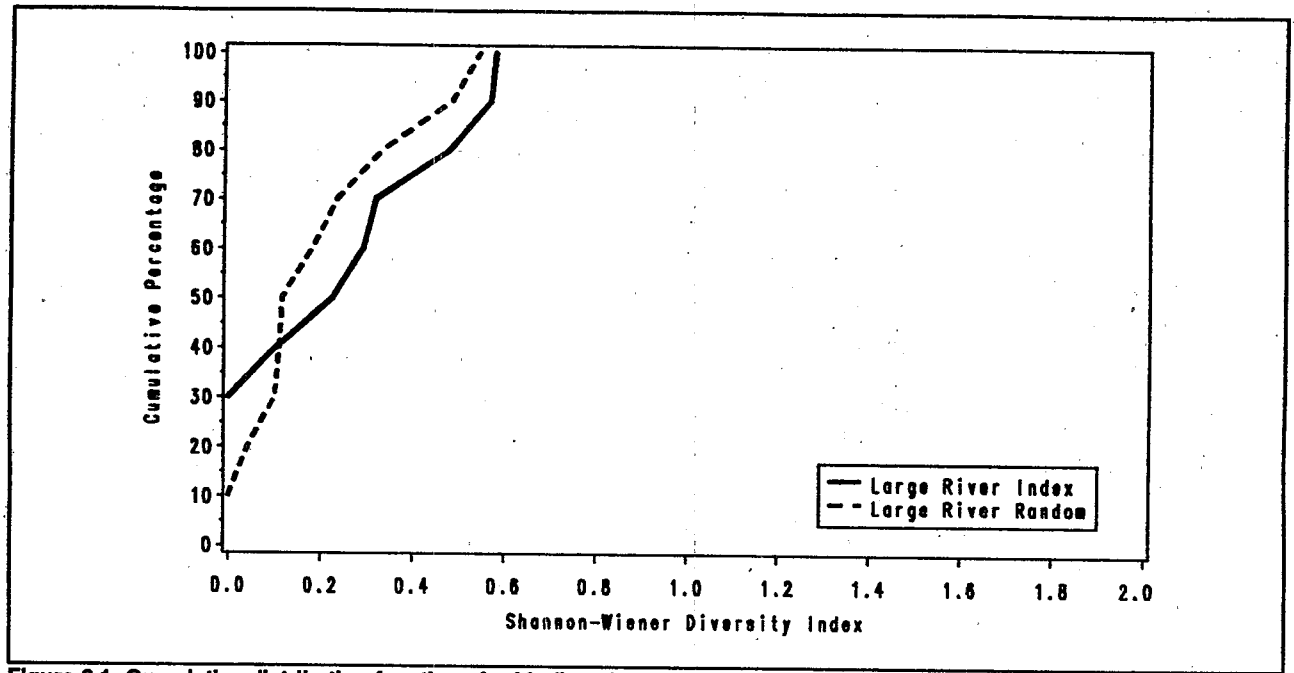

Figure 6.1 Cumulative distribution functions for biodiversity as bivalves for random and index sites in large tidal rivers.

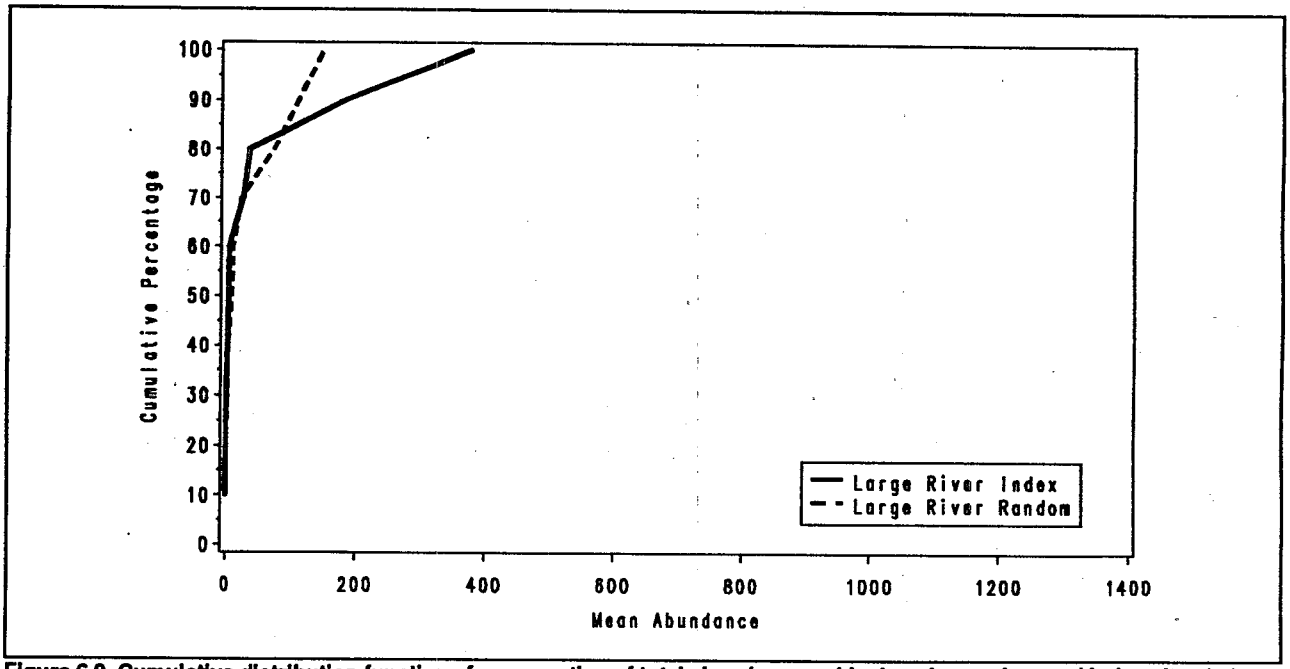

Figure 6.2 Cumulative distribution functions for proportion of total abundance as bivalves for random and index sites in large tidal rivers.

proportional contributions of amphipods,decapods, bivalves, and polychaetes in small estuaries/small tidal rivers. Two of the CDFs corresponding to major variables comprising the benthic index are shown in Figure 6.3: biodiversity and Figure 6.4: proportion of total abundance as bivalves. Thus, at a population level (class) for dissolved oxygen variables, there are no differences in index and random sampling. Similarly, the results of the ANOVA testing showed no significant differences for any benthic response variables except for abundance of large bivalves (Table 6.2). Pearson and Spearman correlations generally confirmed the lack of significant differences between random and index samples for benthic response variables (13 of 16 pairs were significantly correlated). The three correlative analyses (mean abundance, abundance of large bivalves, and proportion of bivalves showed relatively small differences [mean abundance (98.7 organisms versus 93.6 organisms), large bivalve abundance (6.1 versus 5.9), and percent bivalves (18.0% versus 15.1%)]. These small differences reflect the low variability seen in the small estuarine class whether using random or index sampling. Thus, random sampling is adequate to represent small estuaries at the class level or at reduced levels (e.g., small estuaries in individual states or individual estuaries).

Variable	Anova Pr > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Mean number of species	0.299	0.132	NS	0.134	NS	2.53	3.00
Mean abundance	0.691	-0.280	NS	-0.030	NS	38.67	63.60
Diversity	0.116	0.355	NS	0.411	NS	0.22	0.26
Large bivalve abundance	0.032					4.75	1.50
% Amphipods	0.521					3.27	0.00
% Decapods	0.007	0.997	.0001	0.745	.0133	0.11	0.30
% Bivalves	0.616	-0.048	NS	-0.102	NS	23.09	5.23
% Polychaete	0.000	0.872	.0010	0.908	.0003	31.89	27.77
Benthic Index	0.930	-0.508	NS	-0.444	NS	-1.29	-2.28

Table 6.1 Results of index and random sampling comparisons for benthic response variables in large tidal rivers using ANOVA ($p < 0.1$ = no significant difference) and Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

Variable	Anova Pr > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Mean number of species	0.000	0.714	.0001	0.658	.0001	11.11	14.89
Mean abundance	0.000	0.204	NS	0.520	.0003	98.67	93.56
Diversity	0.050	0.687	0001	0.633	.0001	0.63	0.75
Large bivalve	0.481	056	NS	0.334	.0375	6.13	5.86
% Amphipods	0.000	0.757	.0001	0.568	.0001	4.48	5.61
% Decapods	0.041	0.669	.0001	0.353	.0187	2.74	3.40
% Bivalves	0.000	0.240	NS	0.340	.0237	18.03	15.08
% Polychaetes	0.003	0.381	.0106	0.364	.0150	42.44	45.49

Table 6.2 Results of index and random sampling comparisons for benthic response variables in small estuaries/small tidal rivers using ANOVA (p < 0.1 = no significant difference), Pearson and Spearman correlations (p < 0.1 = no significant difference)

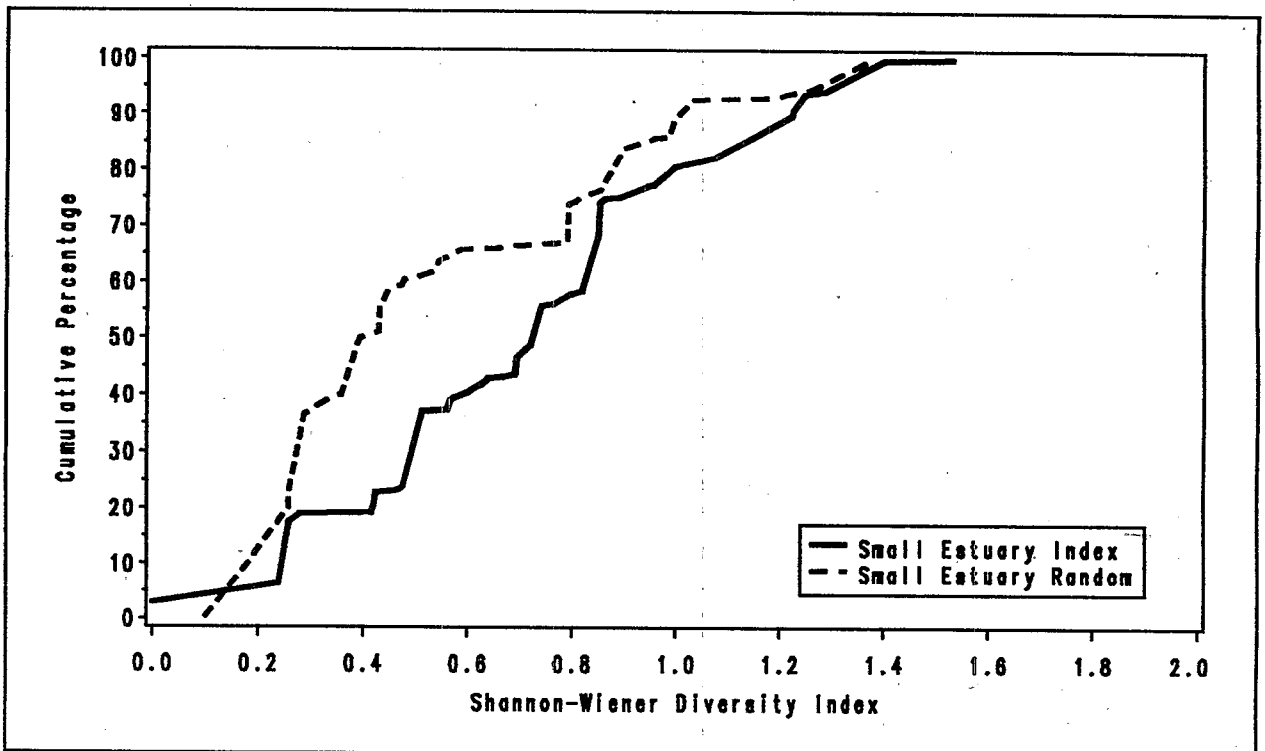

Figure 6.3 Cumulative distribution functions for biodiversity as bivalves for random and index sites in small estuaries/small tidal rivers.

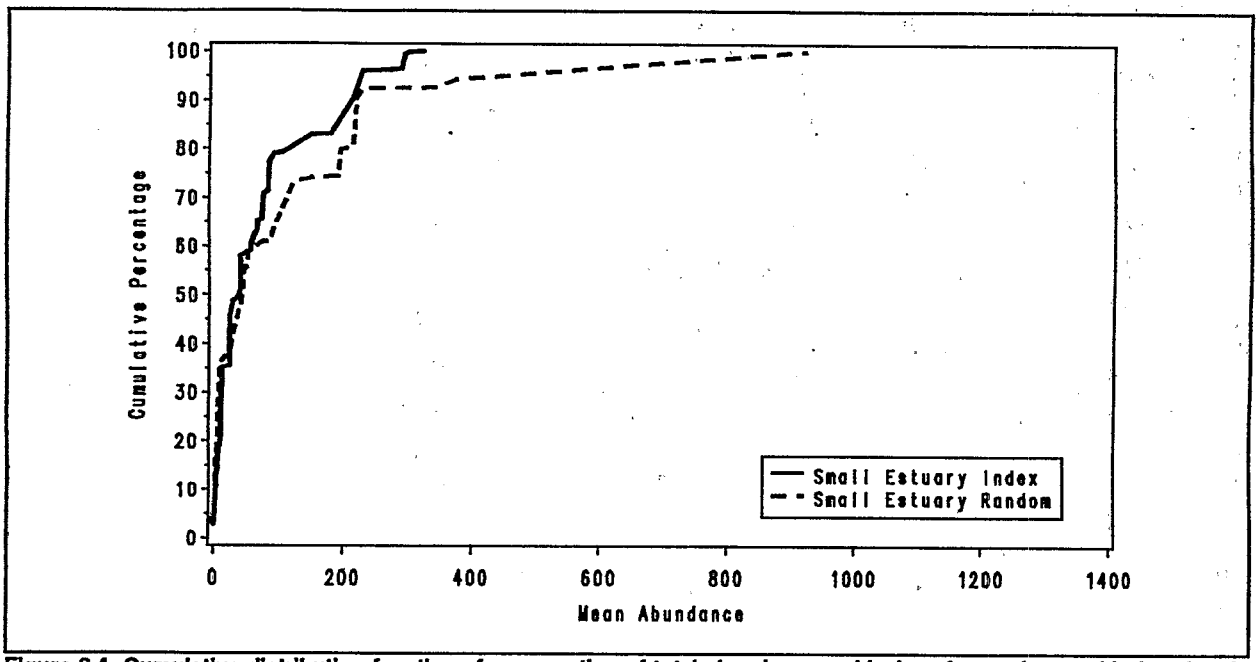

Figure 6.4 Cumulative distribution functions for proportion of total abundance as bivalves for random and index sites in small estuaries/small tidal rivers.

6.1.2 FINFISH RESPONSE INDICATORS

Finfish community response indicators showed no differences in the population-level distributions of number of species, abundance, and proportional contributions of marine catfish, puffers, sciaenids, clupeids, and bothids in large river segments. Two of the CDFs of number of finfish species and abundance/trawl are shown in Figure 6.5 and 6.6. Thus, at a population level (class) for finfish variables, there are no differences in index and random sampling. ANOVA and correlation analyses, however, showed significant differences between random and index sampling for all finfish indicators in large tidal rivers (Table 6.3). Major differences revealed, at random versus index sites, significantly higher proportions of catfish (70.2% versus 48%), higher proportions of bothids (flounders)(7.8% versus 0.9%), and lower proportions of clupeids (1.3% versus 11.4%). Like benthic response indicators, fish indicators would be better to represent individual segments but class-level population distributions based on random sites are no different than index sites.

Finfish community response indicators showed no differences in the population-level distributions of number of species, abundance, and proportional contributions of marine catfish, puffers, sciaenids, clupeids, and bothids in small estuaries/small tidal rivers. Two of the CDFs of number of finfish species and abundance/trawl are shown in Figures 6.7 and 6.8. ANOVA and correlation analyses

generally showed no differences between random and index sites in small estuary with the exception of puffers (Table 6.4). Due to the low proportional abundance of puffers (0.6% and 0.4% in random and index sites, respectively), the statistical difference does not represent any ecological difference. Random sampling for finfish indicators is adequate to represent small estuaries at the class level or at reduced levels (e.g., small estuaries in individual states or individual estuaries).

6.1.3 CONTINUOUS DISSOLVED OXYGEN

Continuous dissolved oxygen parameters (i.e., minimum concentration, percent of time with DO < 2 ppm, and percent of time with DO < 5 ppm) were collected at random and index sites only in the small estuary class. The above dissolved oxygen variables showed no differences in the population-level distributions in small estuaries/small tidal rivers. The CDFs corresponding to these variables are shown

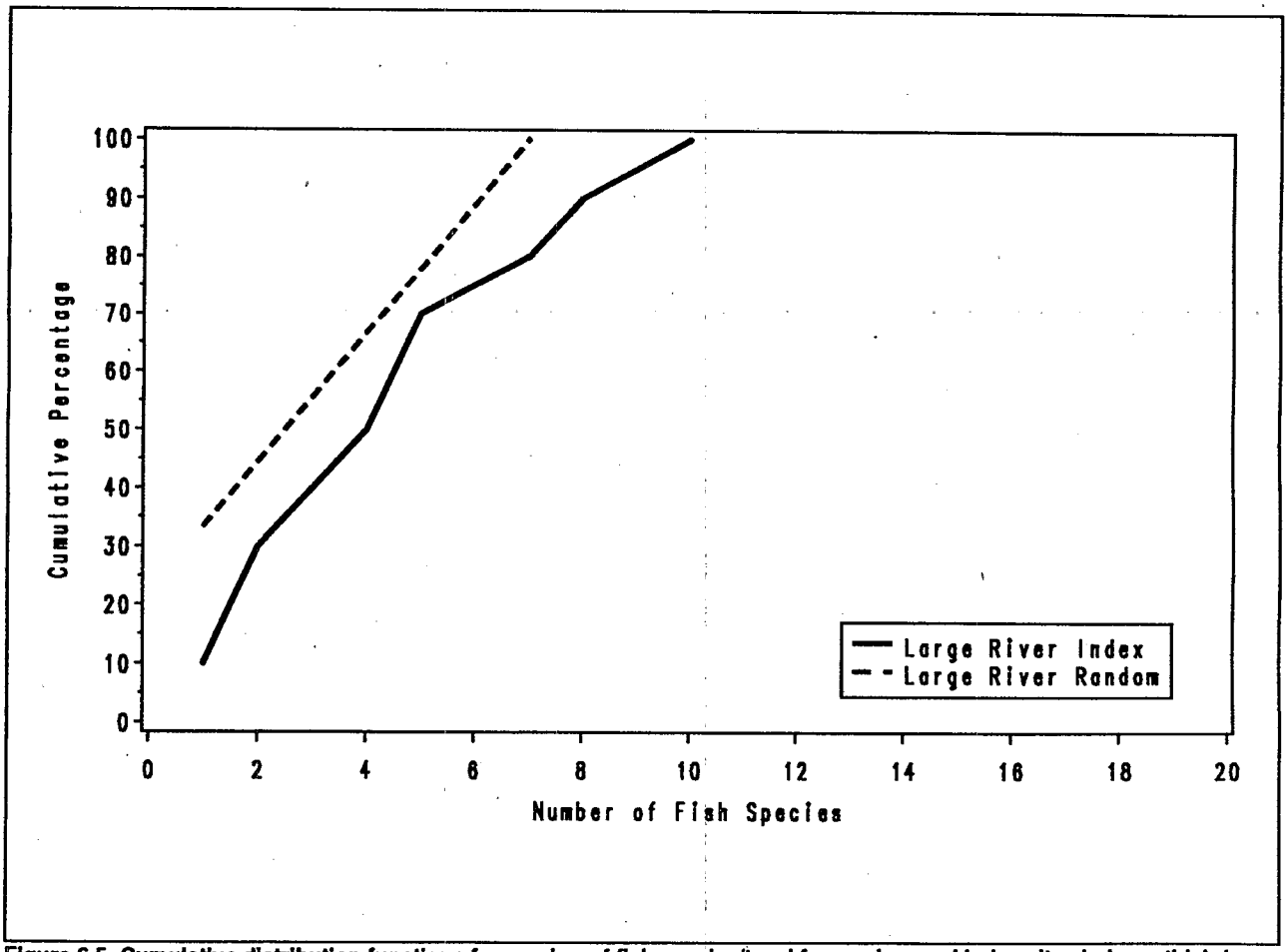

Figure 6.5 Cumulative distribution functions for number of fish species/trawl for random and index sites in large tidal rivers.

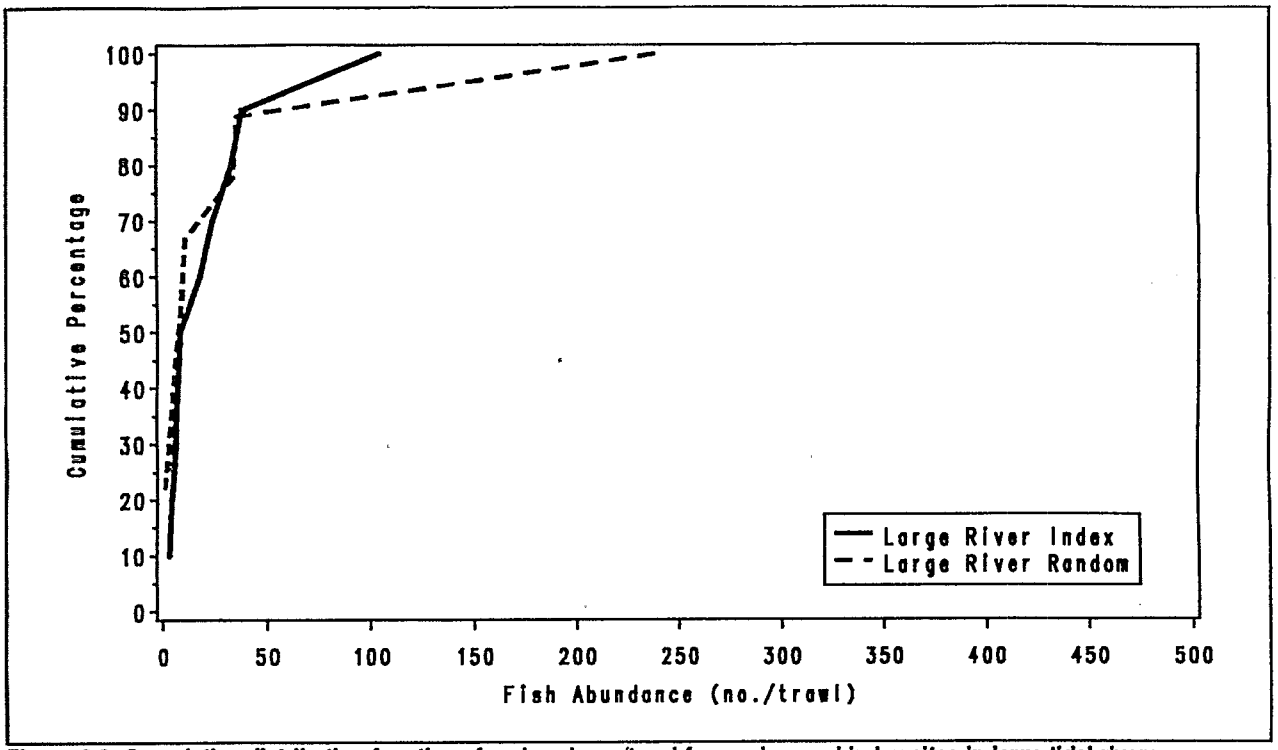

Figure 6.6 Cumulative distribution functions for abundance/trawl for random and index sites in large tidal rivers.

Variable	Anova Pr > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
# Fish species	0.043	0.587	.09	0.558	NS	3.44	4.70
Fish abundance	0.562	-0.053	NS	0.555	NS	38.00	24.60
Fish index	0.045	0.625	.07	0.607	.08	1.88	2.82
% Catfish	0.234	0.462	NS	0.492	NS	70.20	48.03
% Puffers						0.00	0.00
% Scianids	0.174	0.370	NS	0.204	NS	12.49	16.67
% Clupeids	0.593	-0.136	NS	0.219	NS	1.28	11.35
% Bothids	0.649	-0.178	NS	-0.244	NS	7.80	0.82

Table 6.3 Results of index and random sampling comparisons for finfish response variables in large tidal rivers using ANOVA ($p < 0.1$ = no significant difference) and Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

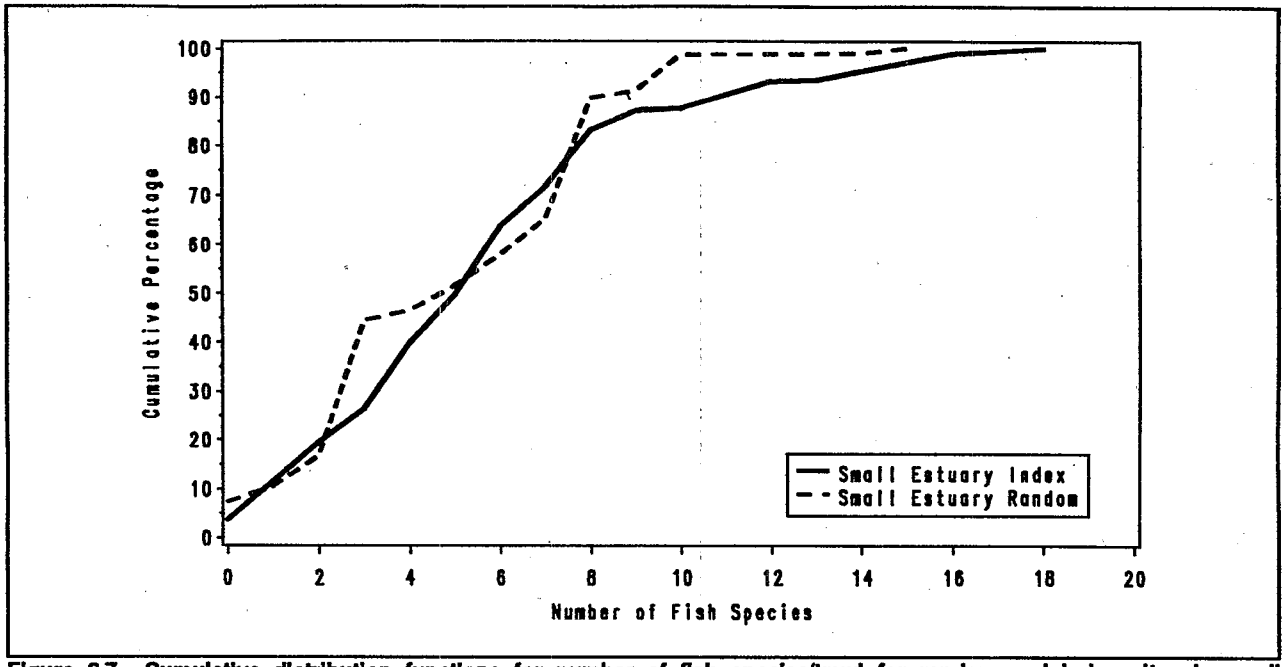

Figure 6.7 Cumulative distribution functions for number of fish species/trawl for random and index sites in small estuaries/small tidal rivers.

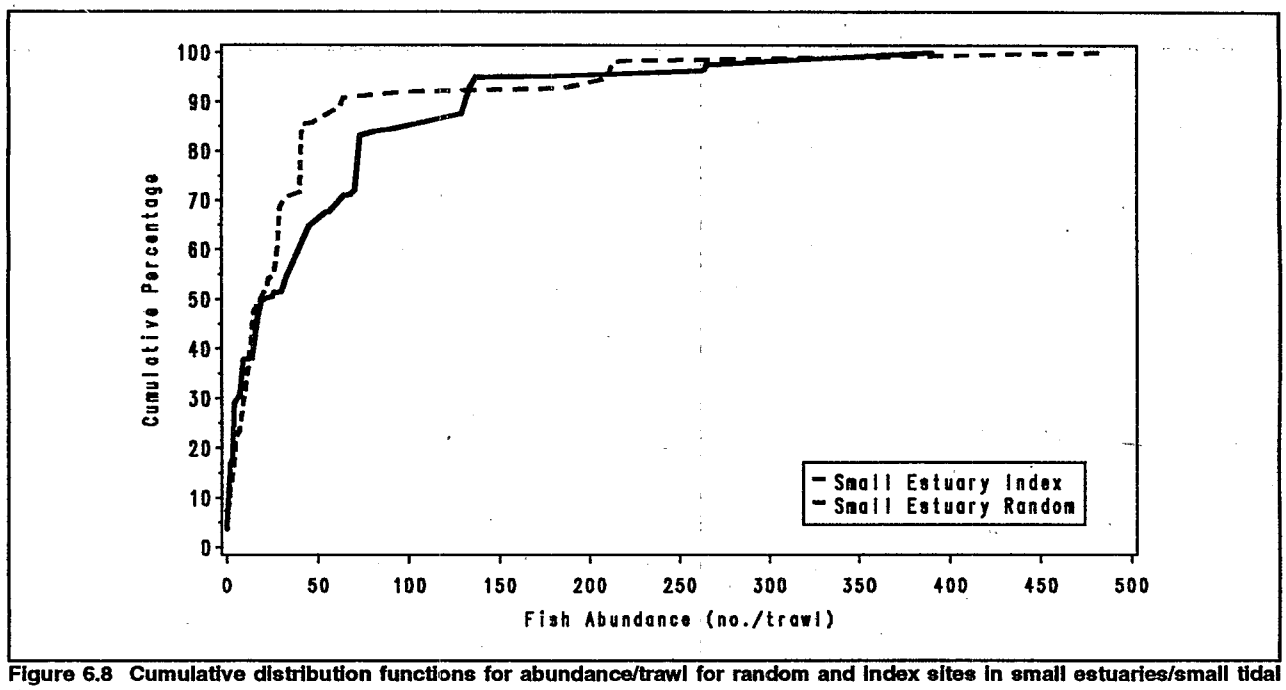

Figure 6.8 Cumulative distribution functions for abundance/trawl for random and index sites in small estuaries/small tidal rivers.

Variable	Anova Pr > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
# Fish spp	0.019	0.357	.0257	0.318	.0484	6.08	7.38
Fish abundance	0.001	0.442	.0034	0.360	0192	73.21	70.19
% Catfish	0.008	0.390	.0142	0.234	NS	17.29	14.96
% Puffers	0.487	-0.106	NS	-0.143	NS	0.56	0.35
% Scianids	0.006	0.310	.0551	0.343	.0325	23.36	23.47
% Clupeids	0.009	0.194	NS	0.448	.0043	10.92	8.05
% Bothids	0.063	0.251	NS	0.519	.0007	0.93	1.48

Table 6.4 Results of index and random sampling comparisons for finfish response variables in small estuaries/small tidal rivers using ANOVA ($p < 0.1$ = no significant difference) and Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

in Figures 6.9-6.11. Thus, at a population level (class) for benthic variables, there are no differences in index and random sampling. ANOVA and correlational analyses confirmed this similarity between random and index sites for continuous dissolved oxygen indicators (Table 6.5). Random sampling for dissolved oxygen indicators is adequate to represent small estuaries at the population level or at reduced spatial scales (e.g., state small estuaries).

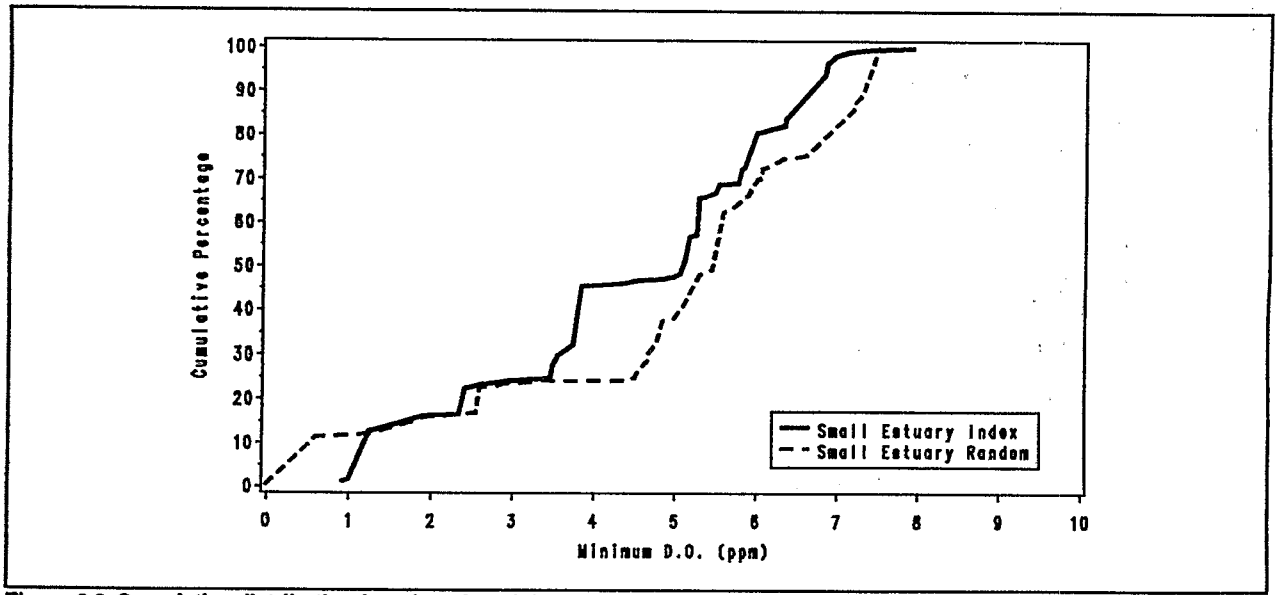

Figure 6.9 Cumulative distribution functions for minimum dissolved oxygen concentration for random and index sites in small estuaries/small tidal rivers.

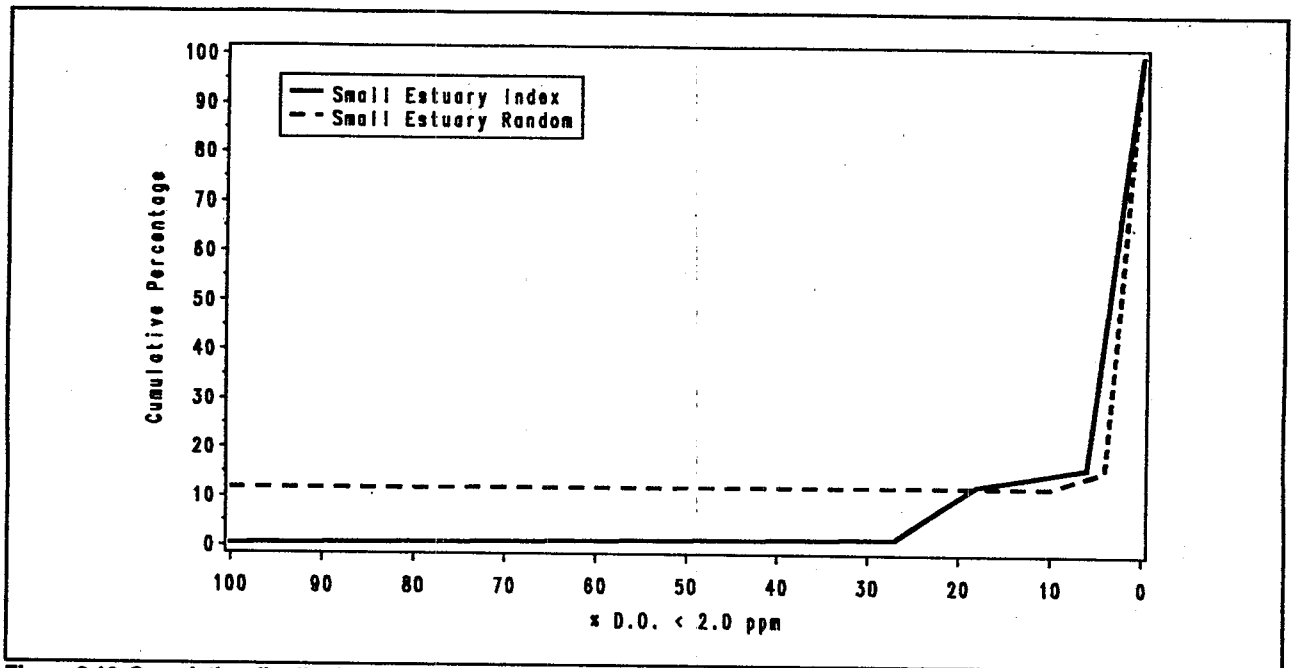

Figure 6.10 Cumulative distribution functions for percent of time at concentrations < 2 ppm for random and index sites in small estuaries/small tidal rivers.

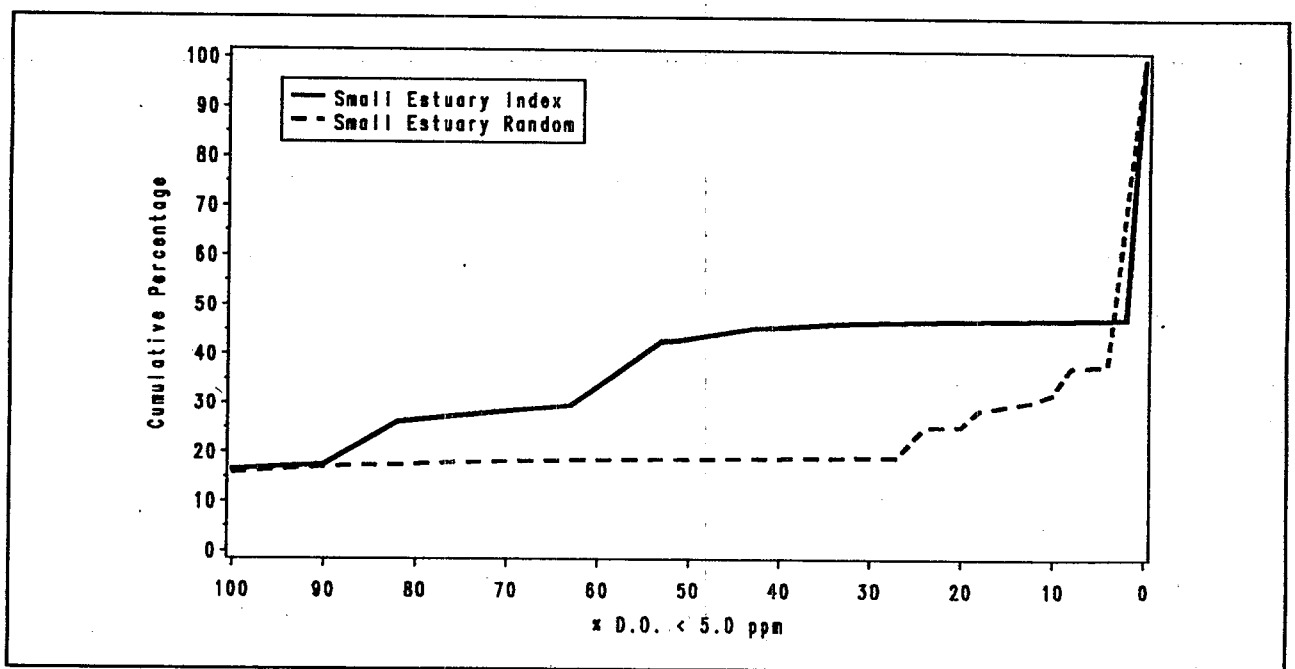

Figure 6.11 Cumulative distribution functions for percent of time at concentrations < 5 ppm for random and index sites in small estuaries/small tidal rivers.

Variable	Anova Pr > F	Pearson r	Pearson p	Spearman r	Spearman p	Random	Mean Index
Minimum DO	0.000	0.541	.0002	0.464	.0015	4.81	4.77
% Time DO < 2	0.001	0.626	.0001	0.628	.0001	7.14	3.43
% Time DO < 5	0.000	0.437	.0030	0.358	.0170	26.50	27.36

Table 6.5 Results of index and random sampling comparisons for dissolved oxygen response variables in small estuaries/small tidal rivers using ANOVA and Pearson and Spearman correlations (p < 0.1 = no significant difference).

6.1.4 HUMAN USE INDICATORS

Human use response indicators showed no differences in the population-level distributions of presence of water clarity, marine debris and contaminants in edible fish fillets in large river segments.

Cumulative distribution functions for water clarity, total PCB and mercury concentrations in catfish are shown in Figures 6.12-14. Thus, at a population level (class) for human use variables, there are no differences in index and random sampling. ANOVA and correlation analyses show differences between

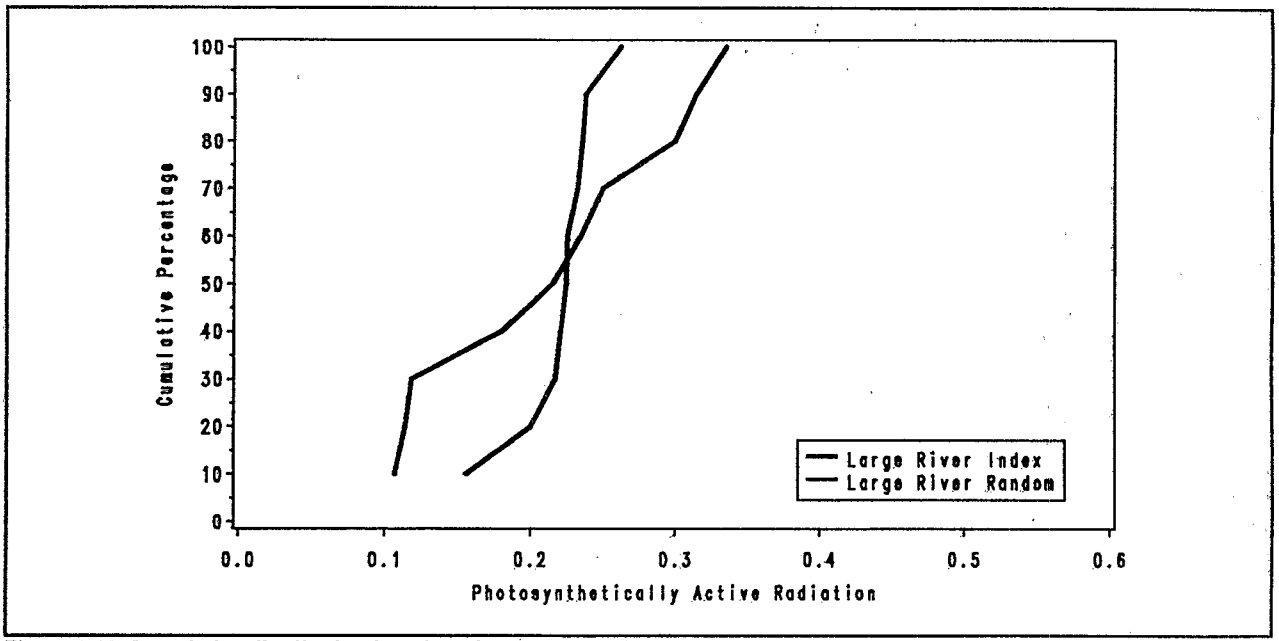

Figure 6.12 Cumulative distribution functions for percent surface light transmittance at 1 m for random and index sites in large tidal rivers.

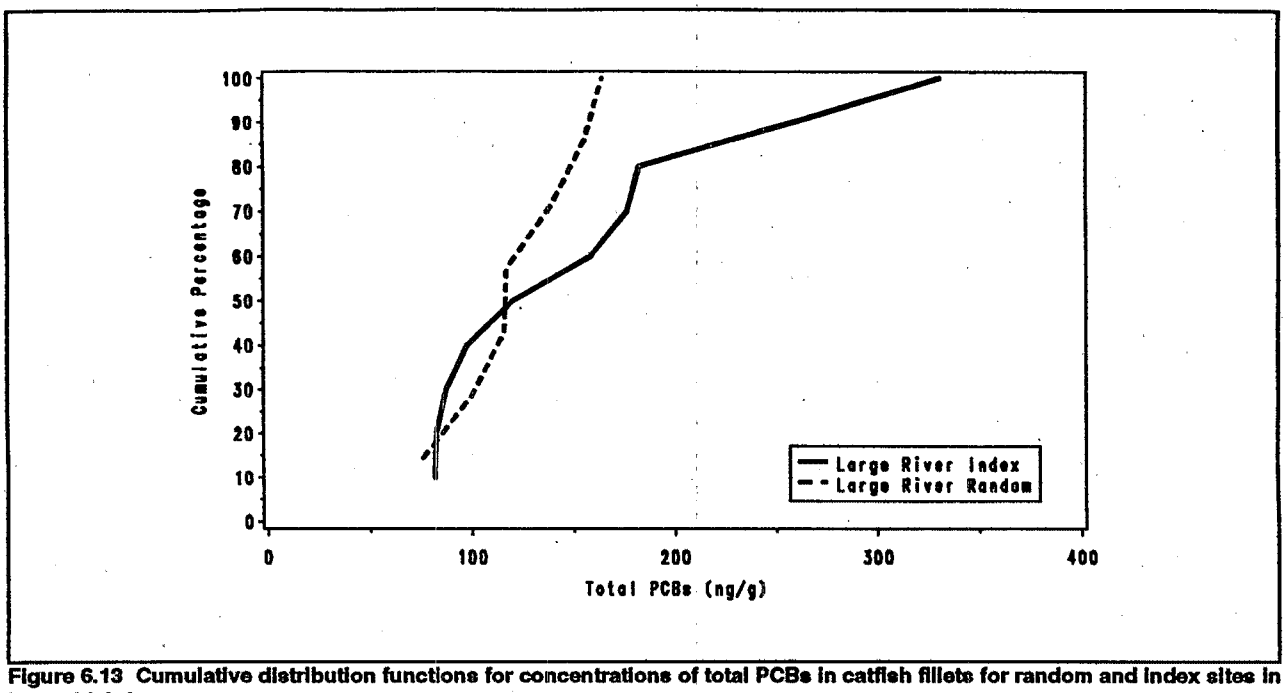

Figure 6.13 Cumulative distribution functions for concentrations of total PCBs in catfish fillets for random and index sites in large tidal rivers.

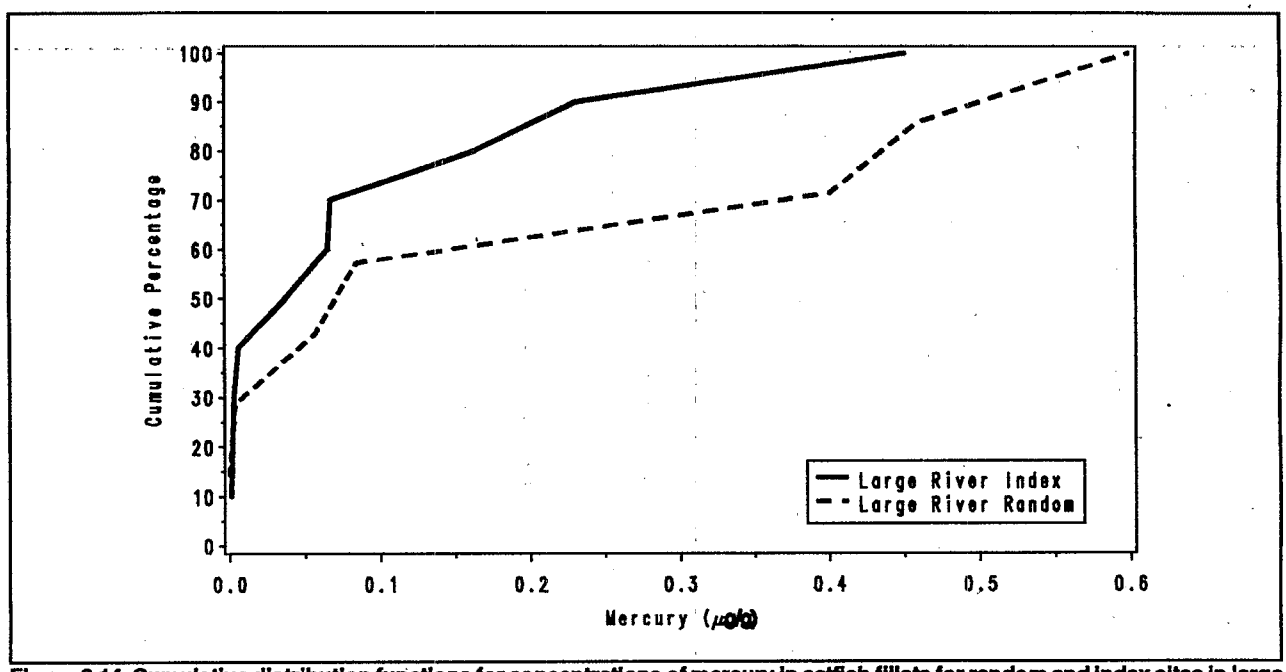

Figure 6.14 Cumulative distribution functions for concentrations of mercury in catfish fillets for random and index sites in large tidal rivers.

random and index sites for tissue contaminant concentrations and presence of marine debris but no significant differences for water clarity (Table 6.6). Therefore, a single random sample for human use indicators may not be adequate to represent large tidal rivers at reduced spatial scales (e.g., segments).

Human use response indicators showed no differences in the population-level distributions of presence of water clarity, marine debris and contaminants in edible fish fillets in small estuaries/small tidal rivers. Cumulative distribution functions for water clarity, total PCB and mercury concentrations in catfish are shown in Figures 6.15 through 6.17. Thus, at a population level (class) for human use variables, there are no differences in index and random sampling. ANOVA and correlation analyses show no differences between random and index sites for human use indicators (Table 6.7). Therefore, random sampling for human use indicators is adequate to represent small estuaries/small tidal rivers at the population level or at reduced spatial scales (e.g., states).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PAR	0.420	.02	NS	.05	NS	.21	.22
Aldrin	0.718	0.700	.08	0.291	NS	2.14	2.01
Chlordane	0.578	0.764	.05	0.618	NS	8.97	8.51
2,4'-DDD	0.638	-0.073	NS	0.144	NS	75.73	80.44
4,4'-DDD	0.795	-0.183	NS	0.218	NS	11.17	17.85
2,4'-DDE	0.920	-0.383	NS	-0.127	NS	9.11	5.03
4,4'-DDE	0.928	-0.313	NS	-0.315	NS	1.97	6.34
2,4'-DDT	0.842	0.498	NS	0.432	NS	33.01	17.00
4,4'-DDT	0.004	-0.563	NS	-0.468	NS	22.98	32.17
Dieldrin	0.689	-0.346	NS	0.036	NS	49.46	16.12
Endosulfan	0.514	0.399	NS	0.221	NS	2.36	0.86
Endrin	0.787	-0.202	NS	0.164	NS	5.94	3.22
Gamma BHC						0.00	0.00
HCB	0.733	0.461	NS	0.530	NS	6.30	3.34
Hepta Epox	0.901	-0.461	NS	-0.360	NS	8.26	4.90
Heptachlor	0.934					0.00	2.27
Mirex	0.259	-0.270	NS	-0.162	NS	26.41	9.43
Tot. PEST	0.732	0.091	NS	0.468	NS	267.81	216.83
Total PCB	0.950	-0.375	NS	-0.306	NS	133.07	157.70
Toxaphene	0.847	-0.192	NS	-0.254	NS	558.33	791.67
Transnona	0.913	0.634	NS	0.559	NS	6.95	8.98

Table 6.6 Results of index and random sampling comparisons for human use response variables in large tidal rivers using ANOVA ($p < 0.1$ = no significant difference) and Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PCB 8	0.850					0.00	0.61
PCB 18	0.850					0.00	0.80
PCB 28	0.866	0.306	NS	0.273	NS	1.14	1.14
PCB 44	0.981	-0.380	NS	-0.394	NS	1.73	2.54
PCB 52	0.990	-0.630	NS	-0.491	NS	5.48	3.96
PCB 66	0.971	0.093	NS	-0.164	NS	5.60	5.72
PCB 77	0.919	-0.263	NS	0.432	NS	10.63	11.70
PCB 99	0.873	-0.272	NS	-0.288	NS	12.87	12.71
PCB 101	0.919	-0.143	NS	-0.198	NS	9.67	9.23
PCB 105	0.886	-0.920	.01	-0.691	.09	7.14	13.75
PCB 118	0.829	0.172	NS	0.631	NS	13.54	11.94
PCB 126	0.922	-0.335	NS	-0.270	NS	7.44	10.74
PCB 128	0.978	0.247	NS	-0.108	NS	2.13	2.89
PCB 138	0.000	0.510	NS	0.455	NS	0.67	5.28
PCB 153	0.531	0.361	NS	0.468	NS	24.60	28.89
PCB 170	0.596	0.028	NS	0.054	NS	6.47	10.11
PCB 180	0.826	0.119	NS	0.000	NS	14.14	20.58
PCB 187	0.393	0.482	NS	0.512	NS	4.88	5.20
PCB 195	0.285	0.611	.06	0.444	NS	3.19	2.03
PCB 206	0.659	0.442	NS	0.430	NS	4.38	3.83
PCB 209	0.702	0.244	NS	0.178	NS	6.76	9.96
Silver	0.317	0.028	NS	-0.100	NS	0.24	0.30
Aluminum	0.728	-0.721	NS	-0.900	.03	8.92	41.08
Arsenic	0.391	0.328	NS	0.600	NS	4.84	2.09
Cadmium	0.897	-0.456	NS	-0.456	NS	0.08	0.04
Chromium	0.901	-0.620	NS	-0.574	NS	0.11	0.18
Copper	0.672	-0.107	NS	0.200	NS	12.78	6.60
Iron	0.363	-0.485	NS	-0.100	NS	44.88	38.52
Mercury	0.155	-0.345	NS	-0.100	NS	0.22	0.10
Nickel	0.616	-0.299	NS	0.083	NS	0.34	0.21
Lead						0.00	0.00
Selenium	0.311	0.748	NS	0.632	NS	0.48	0.33
Tin	0.545	-0.134	NS	0.100	NS	2.51	0.45
Zinc	0.268	0.435	NS	0.500	NS	53.65	34.05

Table 6.6 (Cont.) Results of index and random sampling comparisons for human use response variables in large tidal rivers using ANOVA ($p < 0.1$ = no significant difference) and Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

6.1.5 HABITAT INDICATORS

Habitat indicators showed no differences in the population-level distributions of temperature, salinity, pH, instantaneous bottom dissolved oxygen concentration, degree of stratification, RPD depth, acid volatile sulfide concentration, percent total organic carbon and percent silt-clay presence in large river segments. Cumulative distribution functions for bottom pH, stratification, and bottom salinity are shown in Figures 6.18-6.20. Thus, at a population level (class) for habitat variables, there are no differences in index and random sampling. ANOVA and correlation analyses showed significant differences for bottom dissolved oxygen, degree of stratification, bottom pH, bottom salinity, mean RPD depth, acid volatile sulfides, total organic carbon, and percent silt-clay (Table 6.8). However, the mean differences are rather small with 0.2 ppm for dissolved oxygen differences, 0.8 ppt for stratification differences, <0.1 pH units, 1.2 ppt salinity, 0.4 ppb AVS, 0.1% TOC, and < 1% silt-clay. While the small variability observed in the random and index sites results in significant differences, these differences do not appear to be ecologically meaningful. Only the differences observed for mean RPD depth seems a "real" statistical difference with index sites having RPD depths about 19 mm deeper than those at random sites. However, the difference between 58 mm at random sites and 76 mm at index sites does not appear to be ecologically significant. Random sampling is adequate to represent habitat variables in large tidal rivers.

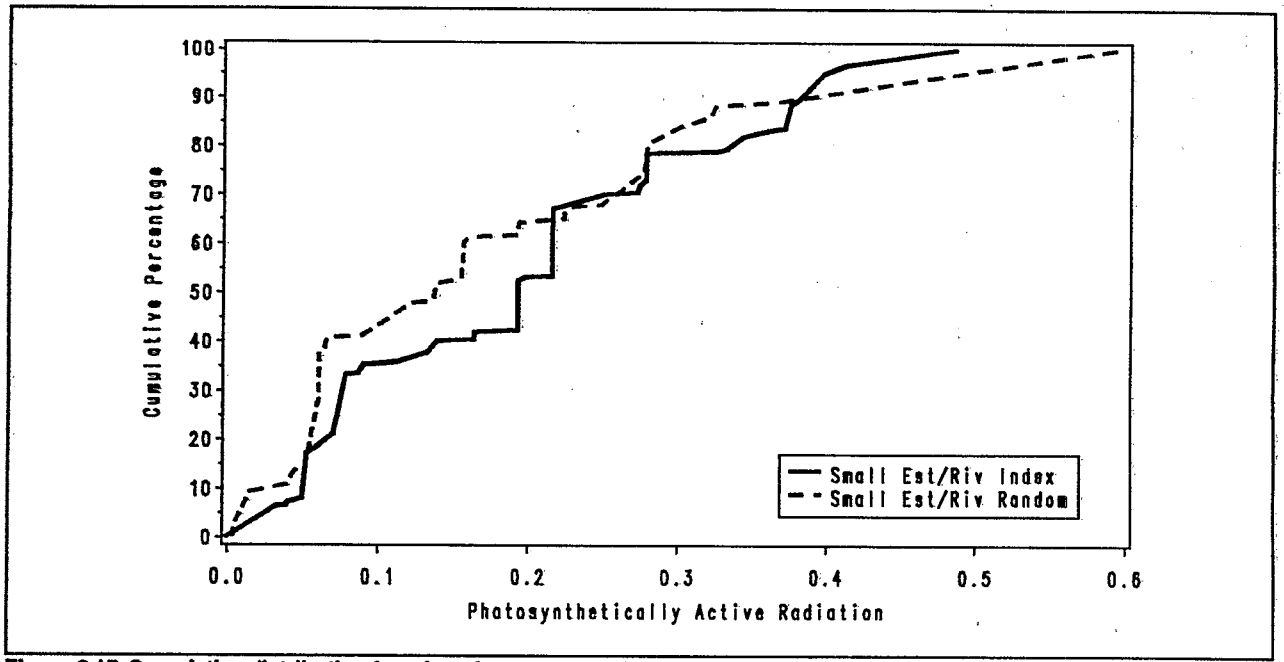

Figure 6.15 Cumulative distribution functions for percent surface light transmittance at 1 m for random and index sites in small estuaries/small tidal rivers.

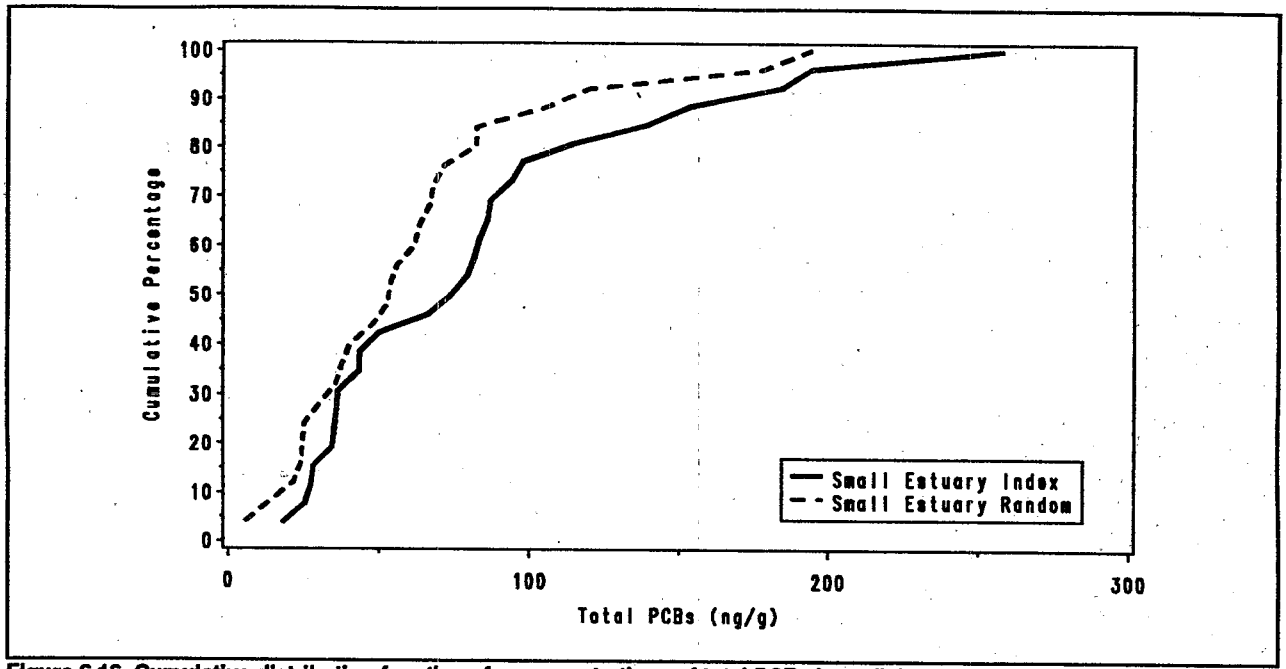

Figure 6.16 Cumulative distribution functions for concentrations of total PCBs in catfish fillets for random and index sites in small estuaries/small tidal rivers.

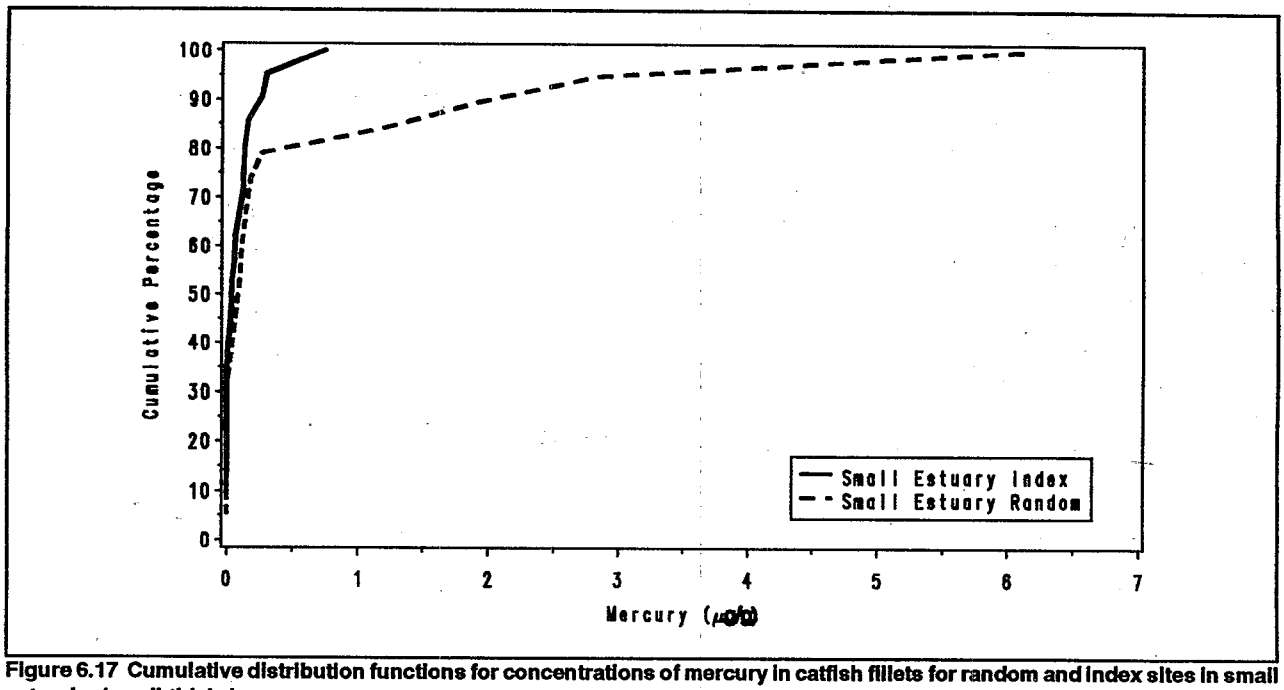

Figure 6.17 Cumulative distribution functions for concentrations of mercury in catfish fillets for random and index sites in small estuaries/small tidal rivers.

Habitat indicators showed no differences in the population-level distributions of temperature, salinity, pH, instantaneous bottom dissolved oxygen concentration, degree of stratification, RPD depth, acid volatile sulfide concentration, percent total organic carbon and percent silt-clay presence in small estuaries. Cumulative distribution functions for bottom pH, stratification, and bottom salinity are shown in Figure 6.21-23. Thus, at a population level (class) for habitat variables, there are no differences in index and random sampling. ANOVA and correlation analyses showed significant differences for only bottom pH and total organic carbon (ANOVA test only). Correlation analyses were showed index and random sites to be similar for TOC and the mean differences between random and index sites were 0.1 pH units and < 0.1% TOC. These differences in small estuarine environments do not represent ecological differences; therefore, random sampling is adequate for representing habitat variables in small estuaries at the population level and at reduced scales.

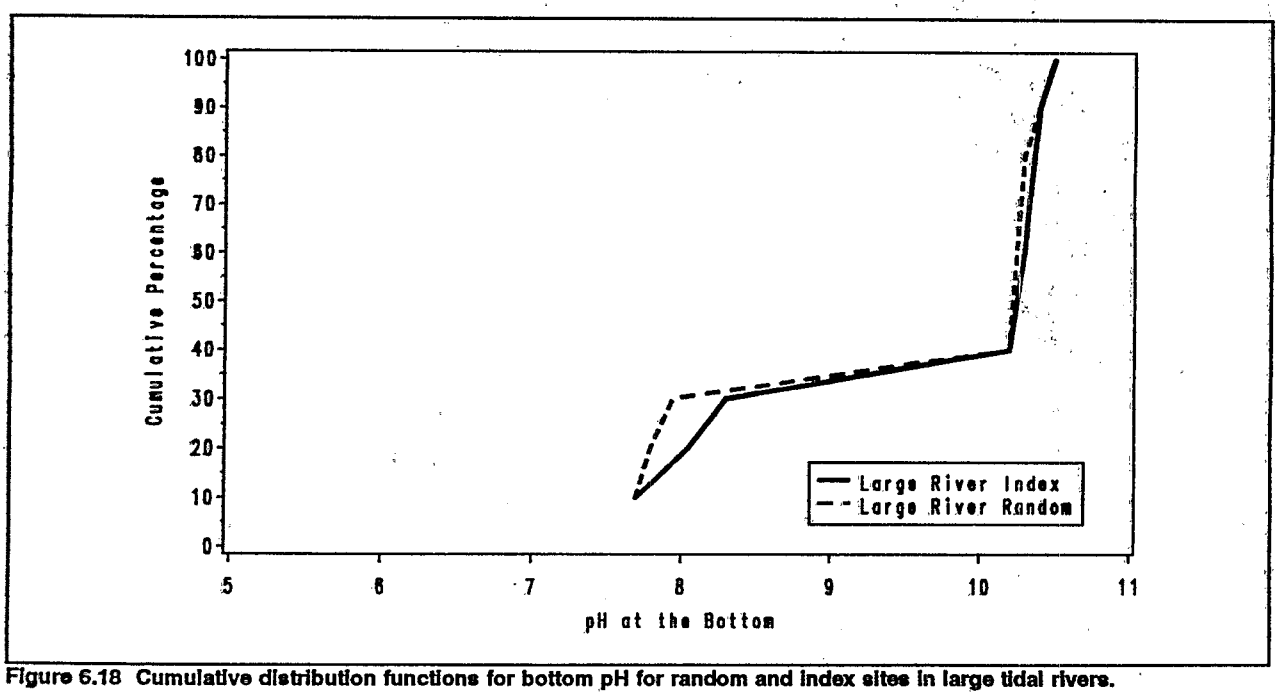

Figure 6.18 Cumulative distribution functions for bottom pH for random and index sites in large tidal rivers.

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PAR	.001	.64	.001	.75	.001	.16	.20
CROAKER							
Aldrin	0.030	-0.138	NS	-0.228	NS	1.13	0.64
Chlordane	0.001	-0.188	NS	-0.127	NS	1.65	3.53
2,4'-DDD	0.032	0.585	.017	0.310	NS	8.97	11.59
4,4'-DDD	0.780	-0.172	NS	-0.136	NS	0.66	1.41
2,4'-DDE	0.108	-0.166	NS	-0.287	NS	1.39	1.64
4,4'-DDE	0.954	-0.246	NS	-0.272	NS	0.60	0.69
2,4'-DDT	0.001	-0.316	NS	-0.231	NS	1.42	2.00
4,4'-DDT	0.000	0.302	NS	0.639	.007	15.08	44.42
Dieldrin	0.000	0.161	NS	0.179	NS	3.45	2.02
Endosulfan	0.101					0.00	1.03
Endrin	0.135	0.073	NS	0.310	NS	1.99	1.29
Gamma BHC	0.000					0.21	0.00
HCB	0.000	-0.064	NS	-0.091	NS	20.89	0.49
Hepta Epox	0.000	0.160	NS	0.244	NS	1.96	5.68
Heptachlor	0.254	0.120	NS	0.424	NS	0.60	1.73
Mirex	0.492	-0.110	NS	0.164	NS	9.47	12.57
Tot. PEST	0.000	0.296	NS	0.336	NS	50.26	91.38
Total PCB	0.192	0.026	NS	0.519	.04	38.36	37.16
Toxaphene	0.000	-0.072	NS	-0.081	NS	1700.00	100.00
Transnona	0.301	-0.032	NS	0.011	NS	1.27	1.62
PCB 8	0.883	-0.067	NS	-0.067	NS	1.69	0.21
PCB 18	0.112					2.22	0.00
PCB 28	0.030	0.366	NS	0.443	.09	0.65	0.72
PCB 44	0.057	0.293	NS	0.318	NS	1.02	0.88
PCB 52	0.001	0.224	NS	0.015	NS	1.40	4.54
PCB 66	0.282	-0.110	NS	0.196	NS	1.71	2.13
PCB 77	0.141	-0.215	NS	-0.294	NS	1.37	1.13
PCB 99	0.002	-0.111	NS	0.098	NS	1.92	2.31
PCB 101	0.365	-0.237	NS	-0.114	NS	1.75	2.94
PCB 105	0.556	-0.183	NS	-0.316	NS	2.11	7.33
PCB 118	0.309	-0.249	NS	0.138	NS	2.28	5.70
PCB 126	0.092	0.651	.006	0.443	.09	1.71	1.85
PCB 128	0.323	-0.294	NS	-0.214	NS	0.83	1.03
PCB 138	0.114	-0.067	NS	0.023	NS	0.36	0.63
PCB 153	0.266	0.378	NS	0.438	.09	5.74	3.05
PCB 170	0.000	0.518	.040	0.322	NS	1.06	1.67
PCB 180	0.570	-0.012	NS	0.099	NS	2.16	3.72
PCB 187	0.000	0.489	.050	0.619	.01	2.01	0.90
PCB 195	0.064	-0.276	NS	-0.336	NS	1.71	1.12
PCB 206	0.013	-0.026	NS	0.164	NS	3.53	0.88
PCB 209	0.520	0.170	NS	0.191	NS	5.21	2.32
Silver	0.035	0.466	NS	0.423	NS	0.26	0.16
Aluminum	0.660	0.257	NS	0.189	NS	6.74	4.94

Table 6.7 Results of index and random sampling comparisons for human use response variables in small estuaries/small tidal rivers using ANOVA ($p < 0.1$ = no significant difference), Pearson and Spearman correlations ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Arsenic	0.024	0.233	NS	0.210	NS	1.92	1.70
Cadmium	0.423	-0.167	NS	-0.167	NS	0.01	0.01
Chromium	0.439	-0.175	NS	-0.010	NS	0.14	0.22
Copper	0.047	-0.040	NS	-0.094	NS	1.05	1.11
Iron	0.685	-0.211	NS	-0.088	NS	28.30	24.44
Mercury	0.047	0.320	NS	0.292	NS	0.20	0.03
Nickel	0.003	0.676	.03	0.722	.02	0.35	0.23
Lead	0.000	0.262	NS	0.100	NS	0.18	0.50
Selenium	0.091	0.294	NS	0.266	NS	0.60	0.38
Tin	0.007	0.192	NS	0.363	NS	0.72	0.59
Zinc	0.219	0.130	NS	0.319	NS	27.96	28.32
SHRIMP							
Aldrin	0.849					0.18	1.16
Chlordane	0.850	0.086	NS	0.312	NS	1.10	0.72
2,4'-DDD	0.052	0.164	NS	0.141	NS	3.66	2.12
4,4'-DDD	0.057	0.738	.02	0.586	.09	0.99	1.09
2,4'-DDE	0.993	-0.189	NS	-0.189	NS	0.46	1.02
4,4'-DDE	0.345	0.661	.05	0.661	.05	0.68	0.90
2,4'-DDT	0.768					0.08	0.32
4,4'-DDT	0.603	0.593	.09	0.339	NS	24.04	59.78
Dieldrin	0.759	0.127	NS	0.344	NS	0.76	0.69
Endosulfan						0.00	0.00
Endrin						0.00	0.00
Gamma BHC						0.00	0.00
HCB	0.067	0.259	NS	0.435	NS	2.13	1.95
Hepta Epox	0.024	0.819	<.01	0.802	<.01	3.12	2.67
Heptachlor						0.00	0.00
Mirex	0.500	0.629	.06	0.068	NS	19.24	18.65
Tot. PEST	0.381	0.485	NS	0.734	.02	57.31	92.35
Total PCB	0.465	0.287	NS	0.295	NS	16.60	34.11
Toxaphene						0.00	0.00
Transnona	0.041	0.468	NS	0.713	.03	0.84	1.23
PCB 8						0.00	0.00
PCB 18						0.00	0.00
PCB 28	0.987	-0.189	NS	-0.189	NS	0.17	0.65
PCB 44	0.057	0.388	NS	0.317	NS	1.02	1.12
PCB 52	0.001	0.131	NS	0.388	NS	1.40	3.64
PCB 66	0.282	0.448	NS	0.855	<.01	1.71	2.70
PCB 77	0.141	0.614	.03	0.783	<.01	1.37	1.44
PCB 99	0.068	0.495	NS	0.700	.03	1.22	1.15
PCB 101	0.208	0.597	.09	0.851	<.01	0.75	0.92
PCB 105	0.625	0.499	NS	0.614	.08	1.18	1.71
PCB 118	0.720	-0.227	NS	0.135	NS	1.62	1.52
PCB 126	0.522	-0.115	NS	0.169	NS	0.71	1.02
PCB 128	0.559	0.291	NS	0.423	NS	0.32	0.32

Table 6.7(Cont) Results of index and random sampling comparisons for human use response variables in small estuaries/small tidal rivers using ANOVA (p < 0.1 = no significant difference), Pearson and Spearman correlations (p < 0.1 = no significant differ

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Random	Mean Index
PCB 138						0.00	0.00
PCB 153	0.612	0.150	NS	0.581	NS	1.85	1.81
PCB 170	0.421	0.574	NS	0.071	NS	1.15	2.45
PCB 180	0.093	0.565	NS	0.371	NS	0.76	0.78
PCB 187	0.991	-0.250	NS	-0.246	NS	0.20	0.20
PCB 195	0.813	0.829	<.01	0.594	.09	0.88	5.10
PCB 206	0.534	0.784	<.01	0.284	NS	1.61	1.71
PCB 209	0.845	-0.083	NS	-0.034	NS	3.05	13.89
Silver	0.644	-0.235	NS	-0.181	NS	0.15	0.15
Aluminum	0.951	0.493	NS	0.051	NS	107.58	73.62
Arsenic	0.258	-0.307	NS	-0.154	NS	3.28	2.78
Cadmium	0.279	0.598	NS	0.583	NS	0.10	0.04
Chromium	0.983	0.585	NS	0.676	NS	3.37	0.18
Copper	0.808	-0.032	NS	0.051	NS	17.98	10.36
Iron	0.710	0.074	NS	0.103	NS	73.93	63.23
Mercury	0.772	0.598	NS	0.564	NS	0.21	0.22
Nickel	0.985	0.401	NS	0.631	NS	4.87	0.48
Lead	0.709	0.410	NS	0.663	NS	0.01	0.08
Selenium	0.492	0.321	NS	0.359	NS	0.25	0.16
Tin	0.700	-0.023	NS	-0.051	NS	1.30	0.84
Zinc	0.899	0.372	NS	0.410	NS	48.21	41.66
CATFISH							
Aldrin	0.241	0.105	NS	-0.057	NS	1.93	0.96
Chlordane	0.104	0.360	NS	0.149	NS	3.45	4.27
2,4'-DDD	0.551	0.140	NS	0.239	NS	32.67	85.22
4,4'-DDD	0.251	0.187	NS	0.338	NS	2.27	4.37
2,4'-DDE	0.034	0.568	<.01	0.154	NS	2.38	3.24
4,4'-DDE	0.932	-0.081	NS	0.014	NS	1.04	0.91
2,4'-DDT	0.009	0.650	<.01	0.289	NS	3.63	4.07
4,4'-DDT	0.240	0.004	NS	0.050	NS	24.45	26.66
Dieldrin	0.018	0.558	.01	0.572	<.01	1.31	1.65
Endosulfan	0.381					0.81	0.00
Endrin	0.689	0.003	NS	0.111	NS	1.28	0.87
Gamma BHC	0.000					0.30	0.00
HCB	0.006	0.487	.03	0.837	<.01	1.71	1.31
Hepta Epox	0.427	0.180	NS	0.258	NS	1.54	2.80
Heptachlor	0.002					0.72	0.00
Mirex	0.091	-0.003	NS	0.244	NS	14.75	12.87
Tot. PEST	0.591	0.048	NS	0.303	NS	96.81	154.00
Total PCB	0.046	0.364	NS	0.527	<.01	59.86	83.66
Toxaphene	0.491					0.00	103.45
Transnona	0.102	0.403	.07	0.359	NS	3.32	4.73

Table 6.7 (Cont) Results of index and random sampling comparisons for human use response variables in small estuaries/small tidal rivers using ANOVA (p < 0.1 = no significant difference), Pearson and Spearman correlations (p < 0.1 = no significant differe

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PCB 8	0.000					0.59	0.00
PCB 18	0.539					0.00	0.21
PCB 28	0.955	-0.206	NS	-0.268	NS	0.86	0.58
PCB 44	0.992	-0.137	NS	-0.165	NS	1.39	0.76
PCB 52	0.717	-0.192	NS	-0.065	NS	2.33	1.81
PCB 66	0.105	0.209	NS	0.393	.09	1.83	2.24
PCB 77	0.000	0.266	NS	0.367	NS	2.24	4.81
PCB 99	0.002	0.466	.03	0.617	<.01	5.03	6.83
PCB 101	0.021	0.312	NS	0.309	NS	2.12	2.64
PCB 105	0.426	0.336	NS	0.356	NS	4.97	8.14
PCB 118	0.011	0.489	.03	0.523	.01	4.80	5.75
PCB 126	0.129	0.434	.05	0.586	<.01	3.03	5.31
PCB 128	0.272	0.086	NS	-0.212	NS	0.83	1.45
PCB 138	0.204	0.616	<.01	0.661	<.01	0.15	0.88
PCB 153	0.618	0.142	NS	0.169	NS	12.39	20.18
PCB 170	0.243	0.242	NS	0.322	NS	2.67	3.95
PCB 180	0.095	0.394	.09	0.408	.07	4.95	7.43
PCB 187	0.295	0.096	NS	0.304	NS	2.53	2.96
PCB 195	0.101	0.316	NS	0.199	NS	1.77	2.71
PCB 206	0.948	-0.106	NS	0.128	NS	3.03	5.07
PCB 209	0.501	0.086	NS	0.014	NS	7.26	7.30
Silver	0.970	-0.065	NS	0.220	NS	0.28	0.05
Aluminum	0.928	0.208	NS	-0.147	NS	35.70	47.67
Arsenic	0.000	-0.116	NS	-0.321	NS	2.67	7.19
Cadmium	0.144	0.392	NS	0.524	.09	0.03	0.04
Chromium	0.000	0.211	NS	0.173	NS	0.19	0.46
Copper	0.580	0.244	NS	0.400	NS	1.41	4.14
Iron	0.800	0.254	NS	0.200	NS	47.65	48.69
Mercury	0.803	-0.243	NS	0.207	NS	0.90	0.10
Nickel	0.657	0.281	NS	0.254	NS	0.27	0.43
Lead	0.960	-0.100	NS	-0.100	NS	0.08	0.10
Selenium	0.550	-0.064	NS	0.338	NS	0.40	0.25
Tin	0.780	-0.054	NS	-0.032	NS	0.99	0.92
Zinc	0.829	-0.406	NS	-0.132	NS	66.93	106.83

Table 6.7 (Cont) Results of Index and random sampling comparisons for human use response variables in small estuaries/small tidal rivers using ANOVA (p < 0.1 = no significant difference), Pearson and Spearman correlations (p < 0.1 = no significant differe

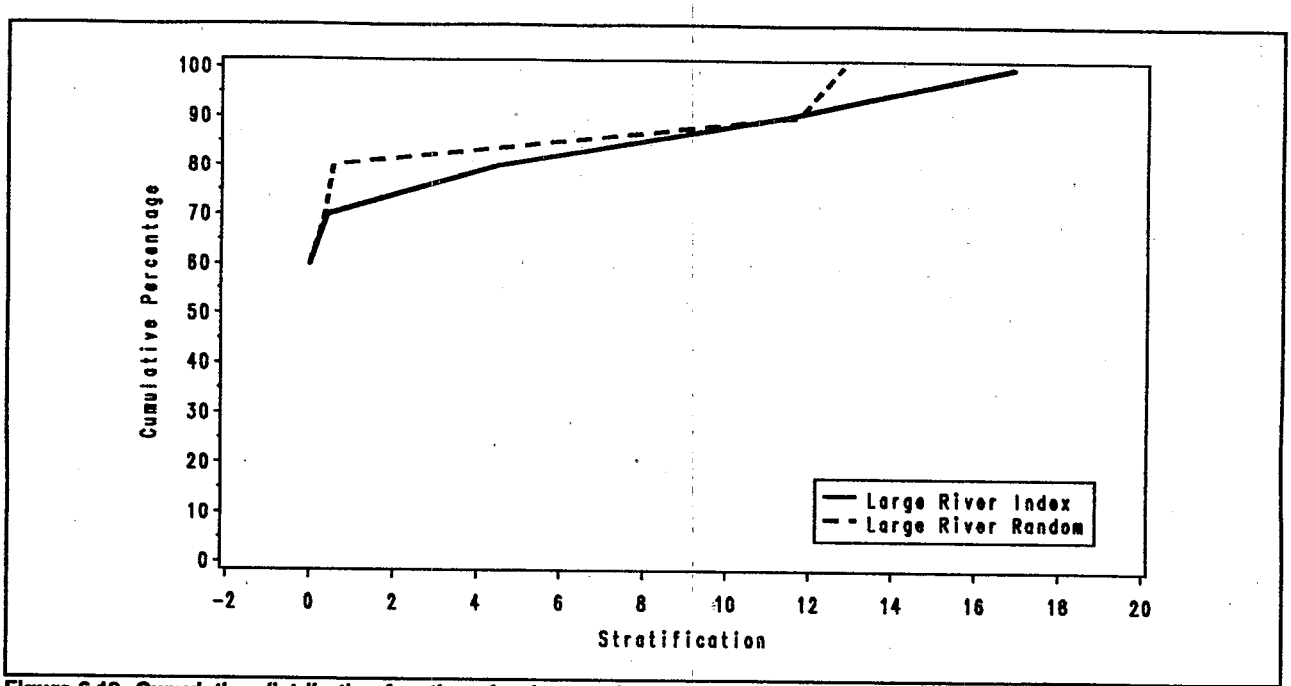

Figure 6.19 Cumulative distribution functions for degree of stratification for random and index sites in large tidal rivers.

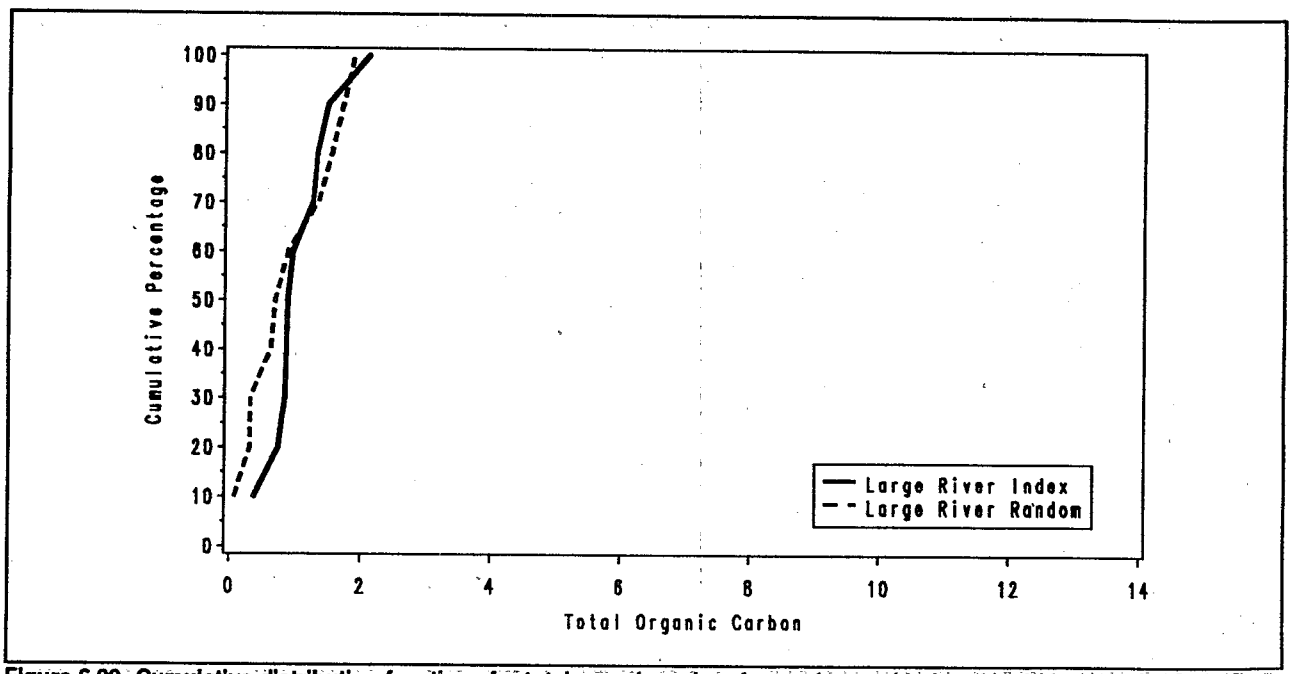

Figure 6.20 Cumulative distribution functions for total organic carbon for random and index sites in large tidal rivers.

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
STRATIFICATION	.07	.42	NS	.94	<.01	2.54	3.32
BOTTOM SALINITY	.054	.48	NS	.94	<.01	2.76	4.07
BOTTOM pH	.36	.07	NS	.37	NS	9.57	9.65
BOTTOM TEMPERATURE	.014	.61	.06	.65	.04	30.43	30.42
SURFACE SALINITY	.049	.81	<.01	.77	.01	0.22	0.75

Table 6.8 Results of index and random sampling comparisons for habitat variables in large tidal rivers using ANOVA Pearson and Spearman correlations and random versus index means (p < 0.1 = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
STRATIFICATION	.02	.31	.051	.31	.05	1.79	2.77
BOTTOM SALINITY	<.01	.95	<.01	.92	<.01	15.23	17.27
BOTTOM pH	.14	16	NS	.49	<.01	8.05	7.91
BOTTOM TEMPERATURE	<.01	.60	<.01	.68	<.01	29.93	29.67
SURFACE SALINITY	<.01	.93	<.01	.90	<.01	12.82	14.56

Table 6.9 Results of index and random sampling comparisons for habitat variables in small estuaries/small tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means (p < 0.1 = no significant difference).

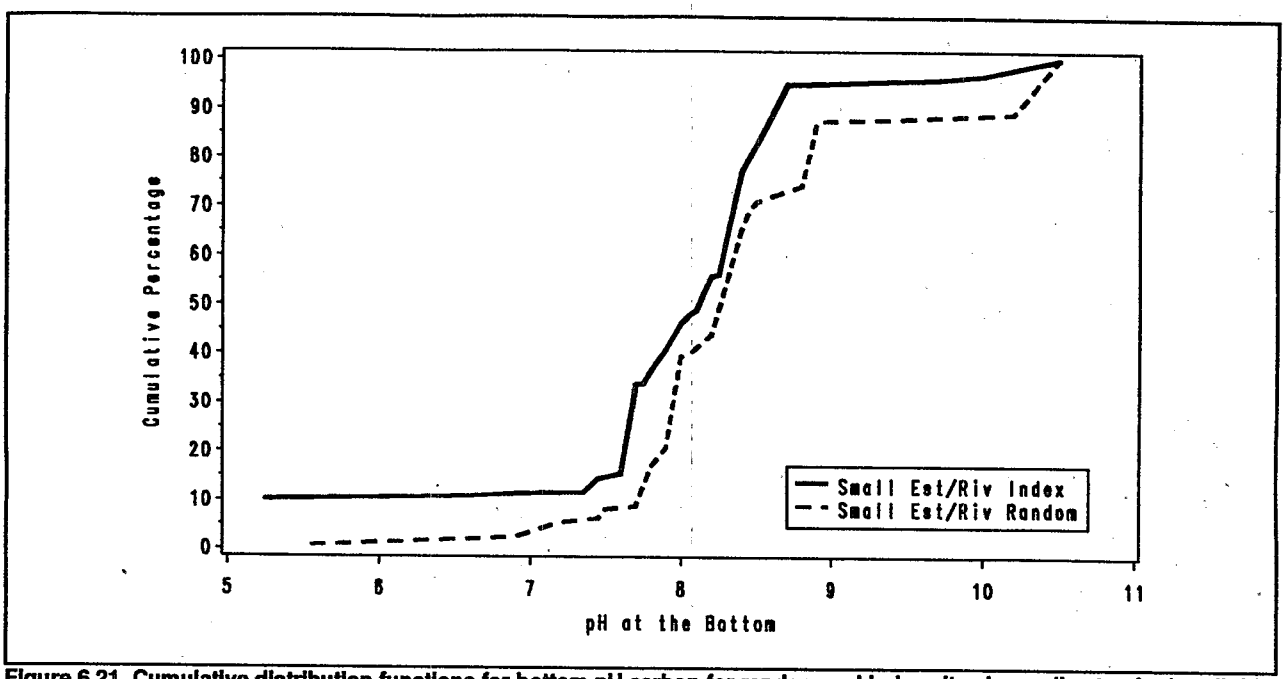

Figure 6.21 Cumulative distribution functions for bottom pH carbon for random and index sites in small estuaries/small tidal rivers.

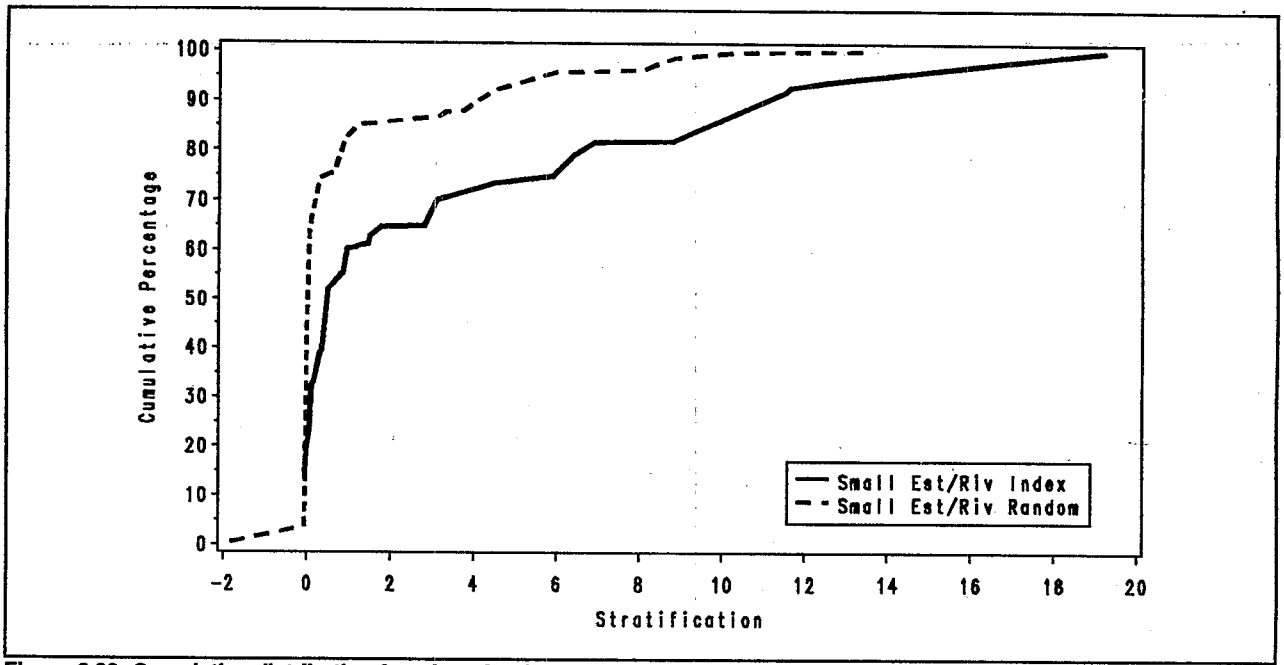

Figure 6.22 Cumulative distribution functions for degree of stratification for random and index sites in small estuaries/small tidal rivers.

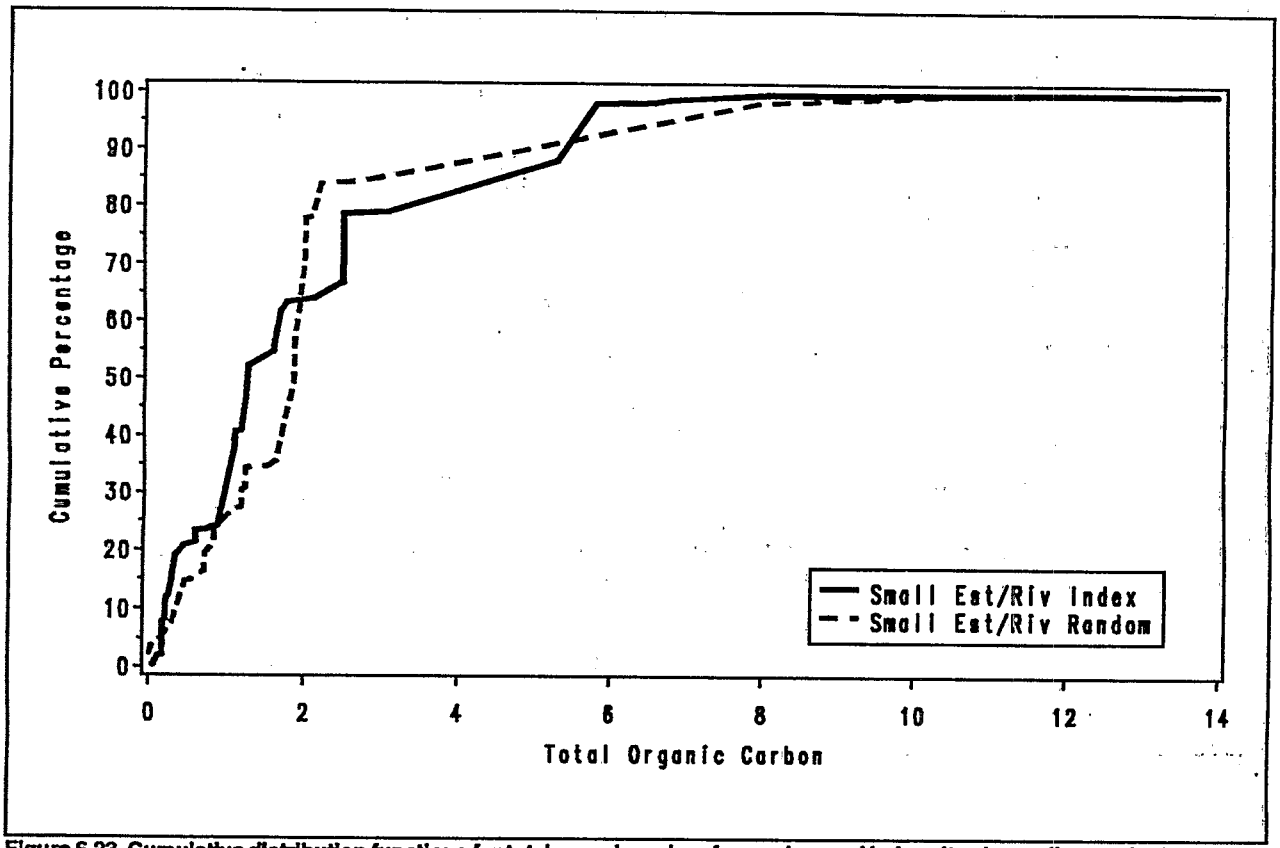

Figure 6.23 Cumulative distribution functions for total organic carbon for random and index sites in small estuaries/small tidal rivers.

6.1.6 SEDIMENT CONTAMINANTS

Sediment contaminant exposure indicators showed no differences in the population-level distributions of heavy metals, alkanes, PAHs, PCBs, and pesticides in large river segments. Cumulative distribution functions for mercury, total alkanes, total PAHs, total PCBs, and 4,4'-DDT are shown in Figures 6.24-6.28. Thus, at a population level (class) for sediment contaminant variables, there are no differences in index and random sampling. ANOVA and correlation analyses showed differences between random and index sites in large tidal rivers for all heavy metals (Table 6.10), all alkanes (Table 6.11), all PAHs (Table 6.12), all PCBs except PCB congener #206 and #209 (Table 6.13), and all pesticides with measurable concentrations (Table 6.14). Concentrations of heavy metals between random and index sites in large tidal rivers generally differed by < 2 ppm for all metals except chromium (6 ppm), manganese (37 ppm), and zinc (7 ppm). Alkane concentrations between random and index sites differed by 1-143 ppb while mean concentrations of PAHs between random and index sites in large tidal rivers differed by only 1-20 ppb. These differences represent statistical differences due to small variability and small sample size but they do not represent significant ecological variability. Differences in PCB and pesticides concentrations between random and index sites were < 1 ppb except for total DDT (< 2ppb).

Sediment contaminant exposure indicators showed no differences in the population-level distributions of heavy metals, alkanes, PAHs, PCBs, and pesticides in small estuaries/small tidal rivers. Cumulative distribution functions for mercury, total alkanes, total PAHs, total PCBs, and 4,4'-DDT are shown in Figures 6.29-6.33. Thus, at a population level (class) for sediment contaminant variables, there are no differences in index and random sampling. ANOVA and correlation analyses showed no differences between random and index sites for heavy metals (Table 6.15) except for mercury whose mean concentration difference was < 0.02 ppm showing low variabililty rather than significant ecological difference. Table 6.16 shows no differences for individual alkanes except for C11 for which the mean difference was about 1 ppb; again not ecologically significant. Unlike metals and alkanes in small estuaries, several significant differences in PAH, PCB, and pesticide concentrations existed between random and index sites (18 of 40 PAHs, 17 of 20 PCB congeners, and 21 of 25 pesticides) with random sites having the greater concentrations for PAHs (Table 6.17) and no consistent pattern of differences for PCBs (Table 6.18) and pesticides (Table 6.19). Although these differences are not large in terms of concentrations, significant variability exists within individual small estuaries for PAHs and PCBs. All mean concentrations are low so that the differences probably have little ecological significance. Because of the significant statistical variability in many PAH and PCB concentrations, multiple samples within an estuary would be required to characterize an individual estuary but random samples appear adequate to represent the population distribution of PAHs and PCBs in small estuaries.

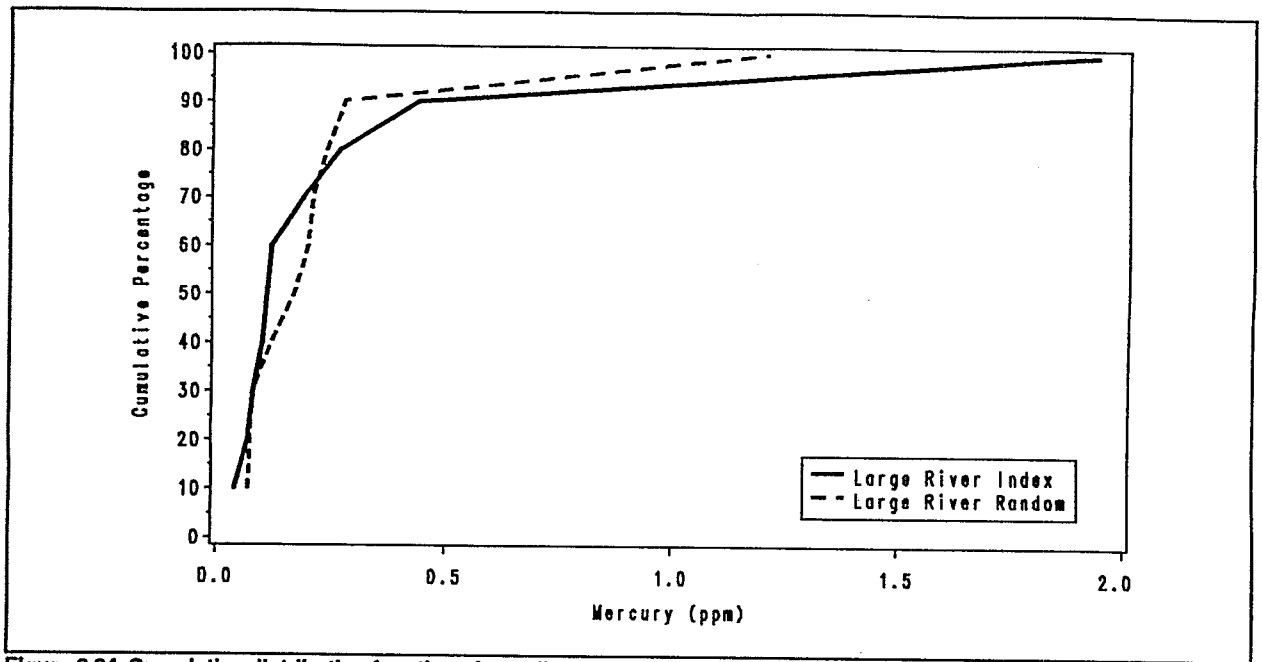

Figure 6.24 Cumulative distribution functions for sediment concentrations of (a) mercury for random and index sites in large tidal rivers.

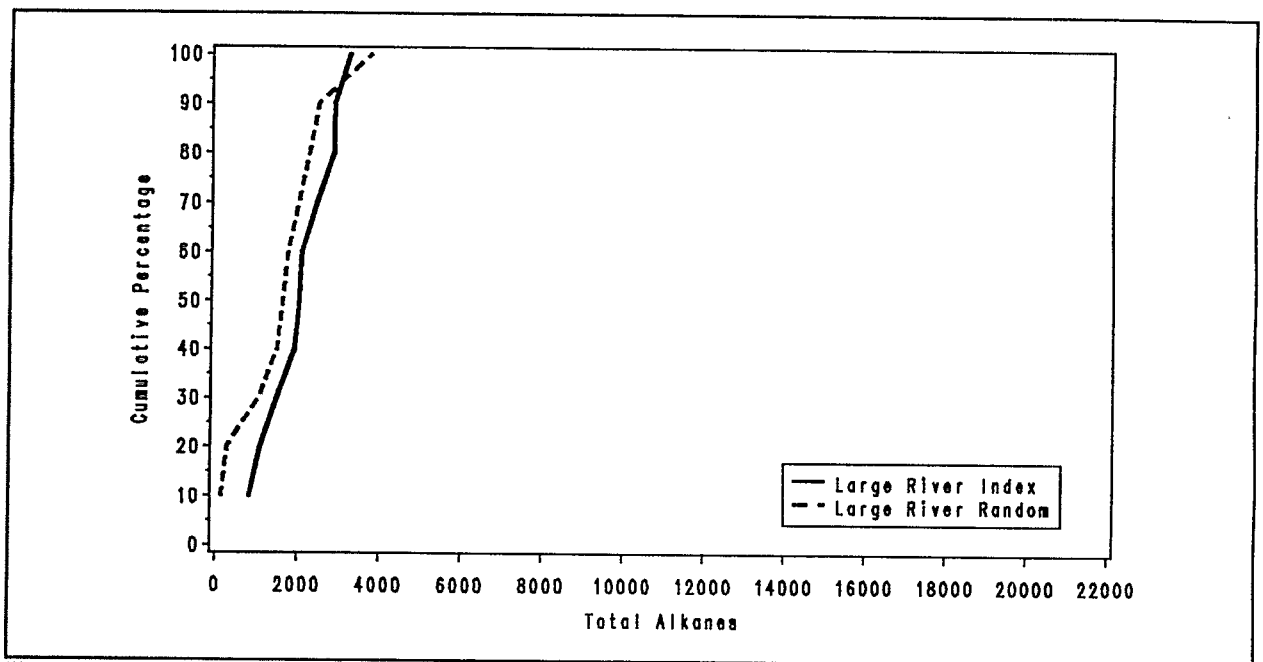

Figure 6.25 Cumulative distribution functions for sediment concentrations of total alkanes for random and index sites in large tidal rivers.

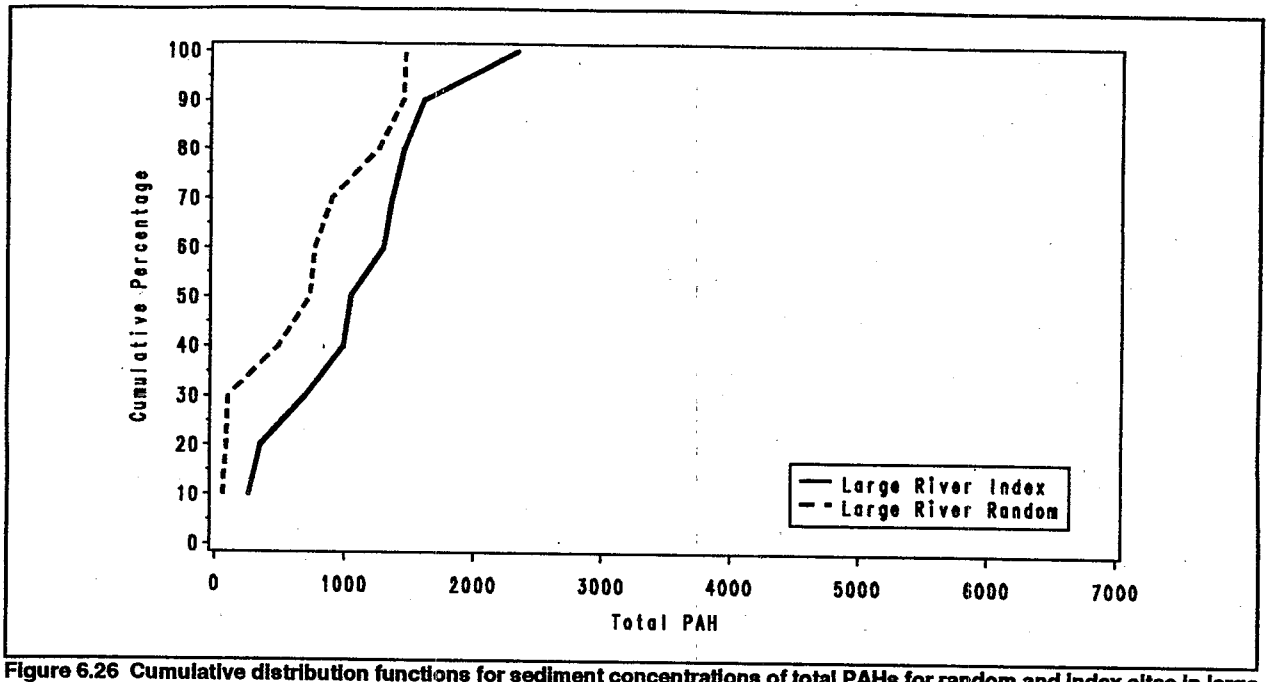

Figure 6.26 Cumulative distribution functions for sediment concentrations of total PAHs for random and index sites in large tidal rivers.

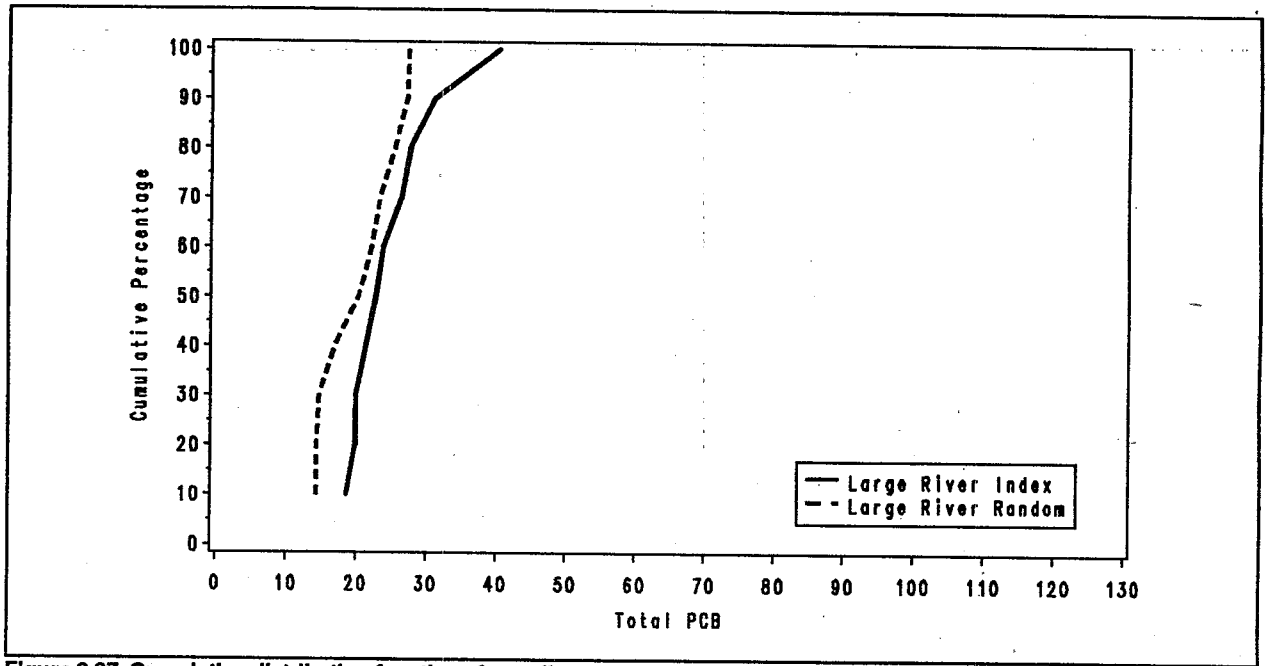

Figure 6.27 Cumulative distribution functions for sediment concentrations of total PCBs for random and index sites in large tidal rivers.

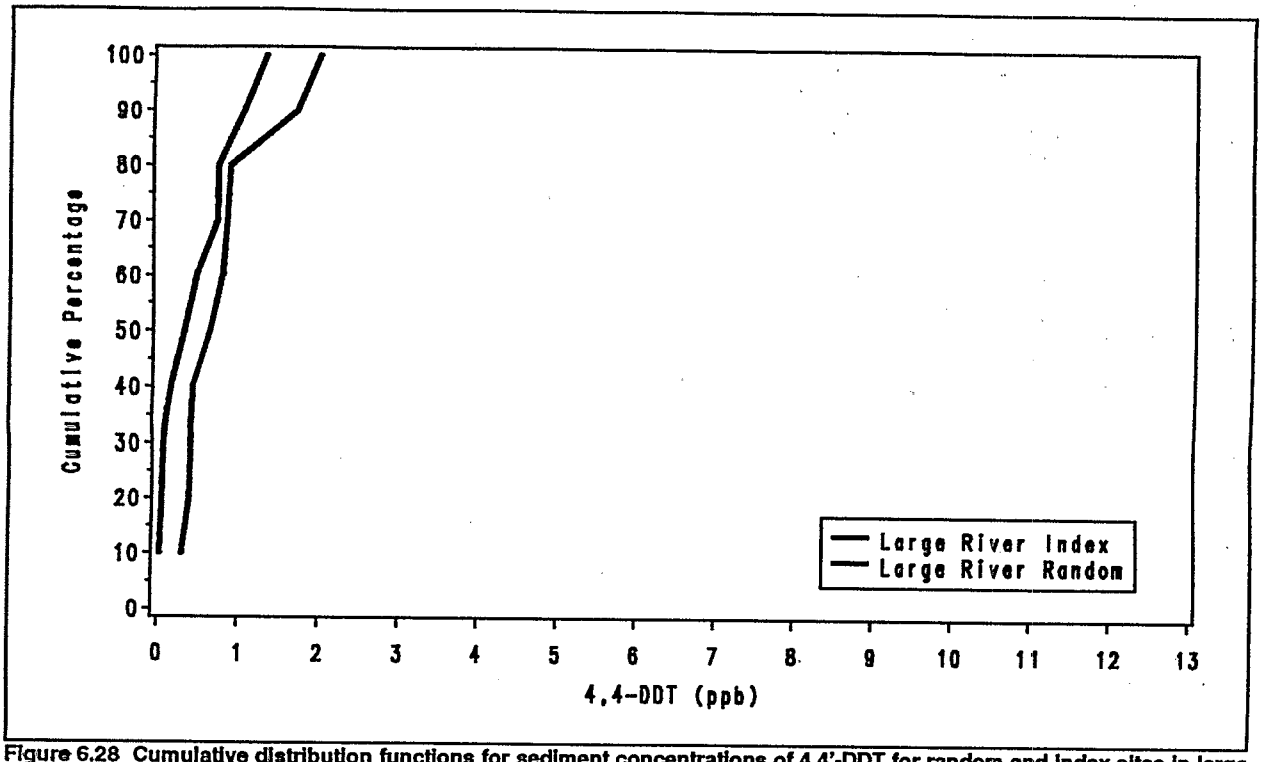

Figure 6.28 Cumulative distribution functions for sediment concentrations of 4,4'-DDT for random and index sites in large tidal rivers.

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Silver	0.77	-0.18	NS	-0.23	NS	0.17	0.24
Aluminum	0.37	0.08	NS	0.07	NS	5.10	5.31
Arsenic	0.14	0.37	NS	0.15	NS	6.75	7.73
Cadmium	0.06	0.49	NS	0.45	NS	0.31	0.35
Chromium	0.14	0.33	NS	0.45	NS	58.50	52.60
Copper	0.17	0.29	NS	0.19	NS	13.43	14.86
Iron	0.60	-0.15	NS	-0.40	NS	0.26	0.33
Manganese	0.10	0.39	NS	0.16	NS	771.10	733.70
Nickel	0.18	0.28	NS	0.16	NS	22.73	24.83
Lead	0.09	0.43	NS	0.35	NS	18.64	19.04
Antimony	0.33	0.12	NS	0.15	NS	0.91	0.95
Selenium	0.20	0.26	NS	0.23	NS	0.31	.380
Tin	0.03	0.57	NS	0.59	NS	1.87	2.11
Zinc	0.11	0.38	NS	0.47	NS	78.10	85.50

Table 6.10 Results of index and random sampling comparisons for sediment heavy metals concentrations in large tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
C10	0.91	-0.66	.04	-0.64	.04	20.08	15.17
C11	0.17	0.35	NS	0.44	NS	17.26	22.89
C12	0.16	0.35	NS	0.48	NS	20.78	25.27
C13	0.21	0.28	NS	0.32	NS	19.71	22.13
C14	0.16	0.33	NS	0.31	NS	24.18	29.45
C15	0.33	0.19	NS	0.42	NS	23.24	34.11
C16	0.36	0.06	NS	0.18	NS	19.81	21.61
C17	0.22	0.28	NS	0.22	NS	35.74	52.28
PrisTane	0.45	0.05	NS	0.19	NS	37.9	56.8
C18	0.48	-0.02	NS	-0.05	NS	17.6	22.5
Phytane	0.32	0.13	NS	0.16	NS	26.5	34.1
C19	0.42	0.04	NS	0.21	NS	22.8	28.9
C20	0.50	-0.07	NS	0.18	NS	36.6	35.4
C21	0.09	0.41	NS	0.19	NS	37.5	42.2
C22	0.052	0.47	NS	0.16	NS	34.7	37.7
C23	0.28	0.19	NS	0.07	NS	50.5	37.7
C24	0.29	0.17	NS	0.03	NS	39.8	47.7
C25	0.50	0.03	NS	0.08	NS	79.5	105.4
C26	0.46	0.05	NS	-0.04	NS	40.2	52.2
C27	0.96	-0.63	.05	-0.50	NS	165.3	177.4
C28	0.90	-0.67	.03	-0.39	NS	63.3	57.9
C29	0.95	-0.63	.05	-0.48	NS	365.2	387.0
C30	0.47	0.04	NS	0.13	NS	47.3	63.6
C31	0.63	-0.07	NS	-0.15	NS	289.4	432.1
C32	0.64	-0.08	NS	-0.22	NS	56.2	37.3
C33	0.77	-0.23	NS	-0.41	NS	149.8	88.4
C34	0.57	-0.07	NS	-0.18	NS	15.2	10.0
Alkanes	0.74	-0.24	NS	-0.08	NS	108	168

Table 6.11 Results of index and random sampling comparisons for sediment alkane concentrations in large tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	p	Spearman r	p	Mean Random	Index
Acenaphthene	0.38	0.20	NS	0.03	NS	2.45	4.24
Acenaphthylene	0.17	0.33	NS	-0.10	NS	2.03	2.81
Anthracene	0.53	0.04	NS	0.13	NS	5.12	11.68
Benzo(a)anthracene	0.47	0.10	NS	0.04	NS	17.05	30.37
Benzo(a)pyrene	0.46	0.10	NS	0.08	NS	19.21	31.60
Benzo(b)fluoranthene	0.37	0.16	NS	0.30	NS	22.81	36.25
Benzo(e)pyrene	0.37	0.15	NS	0.19	NS	17.232	26.16
Benzo(k)fluoranthene	0.23	0.36	NS	0.38	NS	14.15	24.23
Benzo(f,h,i)perylene	0.40	0.13	NS	0.08	NS	14.24	21.55
Biphenyl	0.37	0.17	NS	0.30	NS	3.39	4.79
C1-chrysene	0.43	0.14	NS	0.07	NS	22.96	38.66
C2-chrysene	0.36	0.19	NS	0.02	NS	21.89	34.58
C3-chrysene	0.44	0.16	NS	0.03	NS	9.43	16.43
C4-chrysene	0.26	0.28	NS	0.13	NS	8.87	13.88
C1-dibenzothio	0.53	0.04	NS	0.02	NS	4.84	7.55
C2-dibenzothio	0.51	0.06	NS	0.28	NS	12.35	19.75
C3-dibenzothio	0.37	0.21	NS	0.44	NS	16.16	25.65
C1-fluoranthpyrene	0.48	0.04	NS	0.13	NS	34.53	49.01
C1-fluorene	0.38	0.13	NS	0.26	NS	4.19	6.85
C2-fluorene	0.57	0.01	NS	-0.07	NS	9.07	13.94
C3-fluorene	0.54	0.02	NS	0.04	NS	13.90	21.08
C1-naphthalene	0.32	0.21	NS	0.33	NS	22.46	31.21
C2-naphthalene	0.37	0.12	NS	0.10	NS	23.64	31.74
C3-naphthalene	0.45	0.15	NS	0.02	NS	24.81	36.80
C4-naphthalene	0.50	0.11	NS	0.15	NS	18.68	27.68
C1-phenanthrene	0.56	-0.05	NS	0.13	NS	25.65	36.58
C2-phenanthrene	0.52	0.05	NS	0.26	NS	31.79	51.03
C3-phenanthrene	0.40	0.19	NS	0.28	NS	31.72	50.67
C4-phenanthrene	0.27	0.32	NS	0.58	NS	24.82	37.75
Chrysene	0.47	0.08	NS	0.24	NS	24.81	41.91
Dibenzo(a,h)anthracene	0.50	0.09	NS	0.18	NS	3.68	6.53
Dibenzothio	0.37	0.22	NS	0.01	NS	1.68	2.72
2,6-dimethylnaphthalene	0.44	0.08	NS	0.20	NS	8.78	12.26
Fluorene	0.26	0.32	NS	0.14	NS	3.64	6.39
Fluoranthene	0.27	0.31	NS	0.24	NS	30.36	51.13
(i)1,2,3-c,d-pyrene	0.48	0.08	NS	0.18	NS	12.89	13.87
2,3,5-trimethylnaphthalene	0.49	0.12	NS	0.01	NS	5.76	8.92
1-methylnaphthalene	0.29	0.23	NS	0.36	NS	9.39	12.97
2-methylnaphthalene	0.34	0.19	NS	0.24	NS	13.07	18.24
1-methylphenanthrene	0.48	0.12	NS	0.28	NS	4.58	8.36
TOT PAHS	.41	.16	NS	.20	NS	731.05	1139.00

Table 6.12 Results of index and random sampling comparisons for sediment PAH concentrations in large tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PCB 8	0.495					0.0	0.02
PCB 18	0.542					0.0	0.01
PCB 28	0.277	0.514	NS	0.536	NS	0.05	0.17
PCB 44	0.086	0.837	<.01	0.861	<.01	0.07	0.18
PCB 52	0.479	-0.001	NS	0.292	NS	0.50	0.71
PCB 66	0.216	0.326	NS	0.437	NS	0.14	0.25
PCB 101	0.235	0.394	NS	0.337	NS	0.18	0.33
PCB 105	0.716	0.178	NS	0.184	NS	0.01	0.15
PCB 110	0.602	-0.034	NS	-0.215	NS	0.81	1.43
PCB 118	0.418	0.091	NS	0.168	NS	0.14	0.22
PCB 126	0.641	-0.167	NS	-0.167	NS	0.01	0.00
PCB 128	0.299	0.133	NS	0.131	NS	0.11	0.13
PCB 138	0.476	-0.010	NS	0.012	NS	0.68	0.80
PCB 153	0.159	0.757	.01	0.685	.02	0.20	0.59
PCB 170	0.418	0.113	NS	0.000	NS	0.12	0.27
PCB 180	0.344	0.157	NS	0.107	NS	0.33	0.47
PCB 187	0.134	0.451	NS	0.179	NS	0.12	0.21
PCB 195	0.682	-0.199	NS	0.048	NS	0.02	0.01
PCB 206	0.039	0.594	.07	0.596	.06	0.04	0.03
PCB 209	0.006	0.792	<.01	0.750	.01	0.04	0.08
TOTAL PCBs	0.294	0.328	NS	0.280	NS	20.66	25.34

Table 6.13 Results of index and random sampling comparisons for sediment PCB concentrations in large tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Aldrin	0.095	0.382	NS	0.383	NS	0.01	0.02
Alpha BHC	0.148	0.325	NS	0.364	NS	0.05	0.03
Alpha Chlordane	0.620	-0.078	NS	-0.201	NS	0.11	0.17
Beta BHC	0.711	-0.242	NS	-0.248	NS	0.03	0.04
Cis-nonachlor	0.095	0.444	NS	0.727	.01	0.07	0.07
2,4'-DDD	0.578	0.024	NS	0.047	NS	0.07	0.12
4,4'-DDD	0.411	0.069	NS	0.042	NS	1.41	1.97
Delta BHC						0.00	0.00
2,4'-DDE	0.495					0.05	0.00
4,4'-DDE	0.454	0.027	NS	0.103	NS	0.90	1.18
2,4'-DDT	0.353	0.092	NS	0.042	NS	0.02	0.03
4,4'-DDT	0.548	0.016	NS	0.310	NS	0.48	0.83
Dieldrin	0.610	-0.104	NS	-0.018	NS	0.38	0.55
Endosulfan 1						0.00	0.00
Endosulfan 2						0.00	0.00
Endrin	0.495					0.00	0.02
Gamma BHC	0.344	0.125	NS	0.028	NS	0.01	0.02
Gamma Chlordane	0.507	0.016	NS	-0.144	NS	0.17	0.26
HCB	0.756	-0.274	NS	-0.249	NS	0.38	0.46
Heptachlor Epoxide	0.366	0.816	<.01	0.581	.07	0.00	0.07
Heptachlor						0.00	0.00
Mirex						0.00	0.00
Oxychlordane	0.517	-0.111	NS	-0.111	NS	0.00	0.00
Total BHC	0.400	0.029	NS	0.219	NS	0.09	0.10
Total Chlordane	0.272	0.278	NS	0.253	NS	0.47	0.73
Total DDT	0.411	0.089	NS	0.152	NS	2.96	4.15
Toxaphene						0.00	0.00
Transnonachlor	0.674	-0.174	NS	-0.236	NS	0.20	0.14

Table 6.14 Results of index and random sampling comparisons for sediment pesticide concentrations in large tidal rivers using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

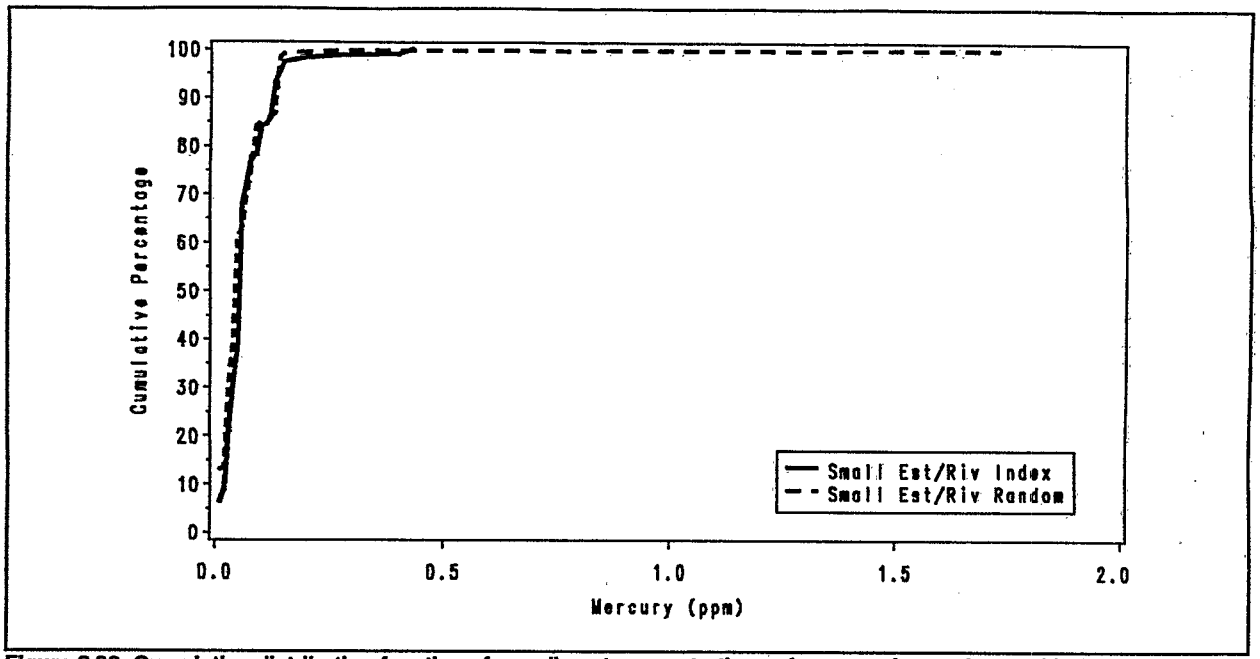

Figure 6.29 Cumulative distribution functions for sediment concentrations of mercury for random and index sites in small estuaries/small tidal rivers.

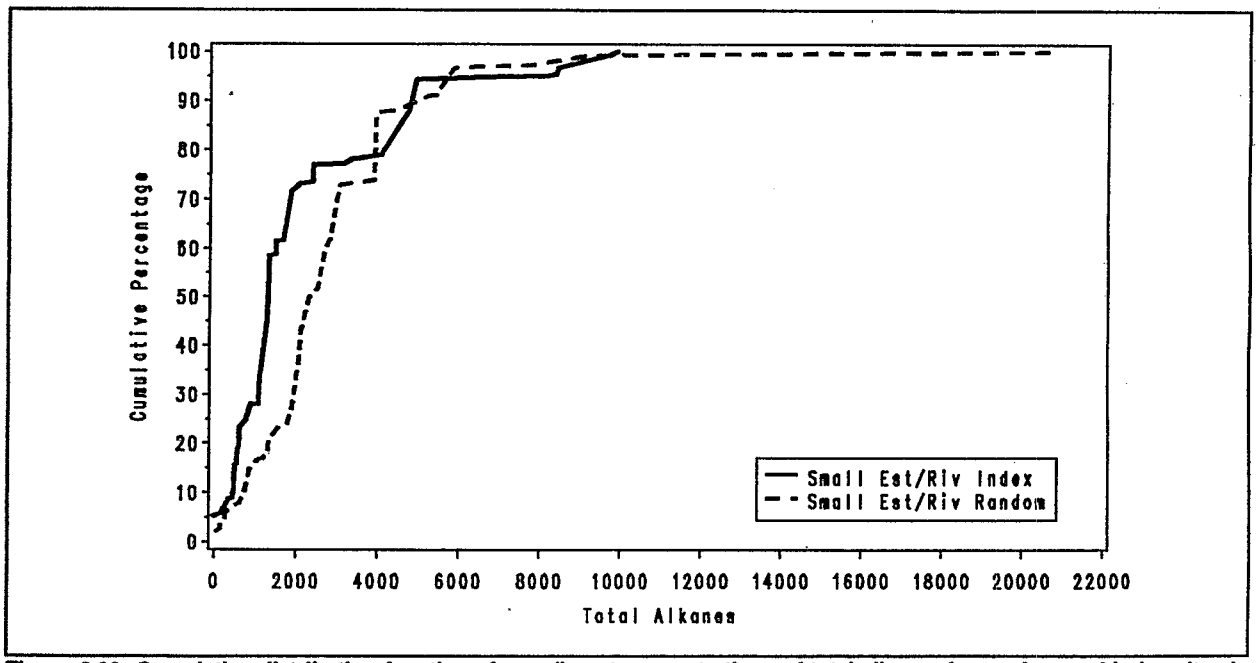

Figure 6.30 Cumulative distribution functions for sediment concentrations of total alkanes for random and index sites in small estuaries/small tidal rivers.

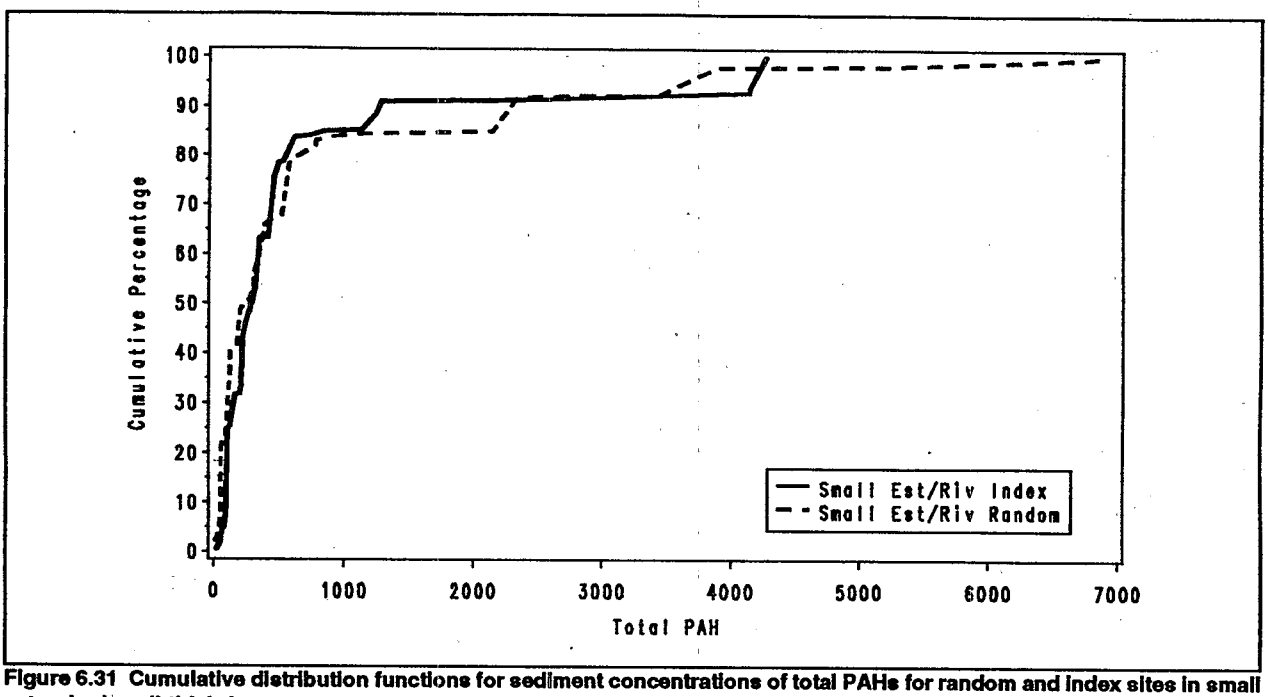

Figure 6.31 Cumulative distribution functions for sediment concentrations of total PAHs for random and index sites in small estuaries/small tidal rivers.

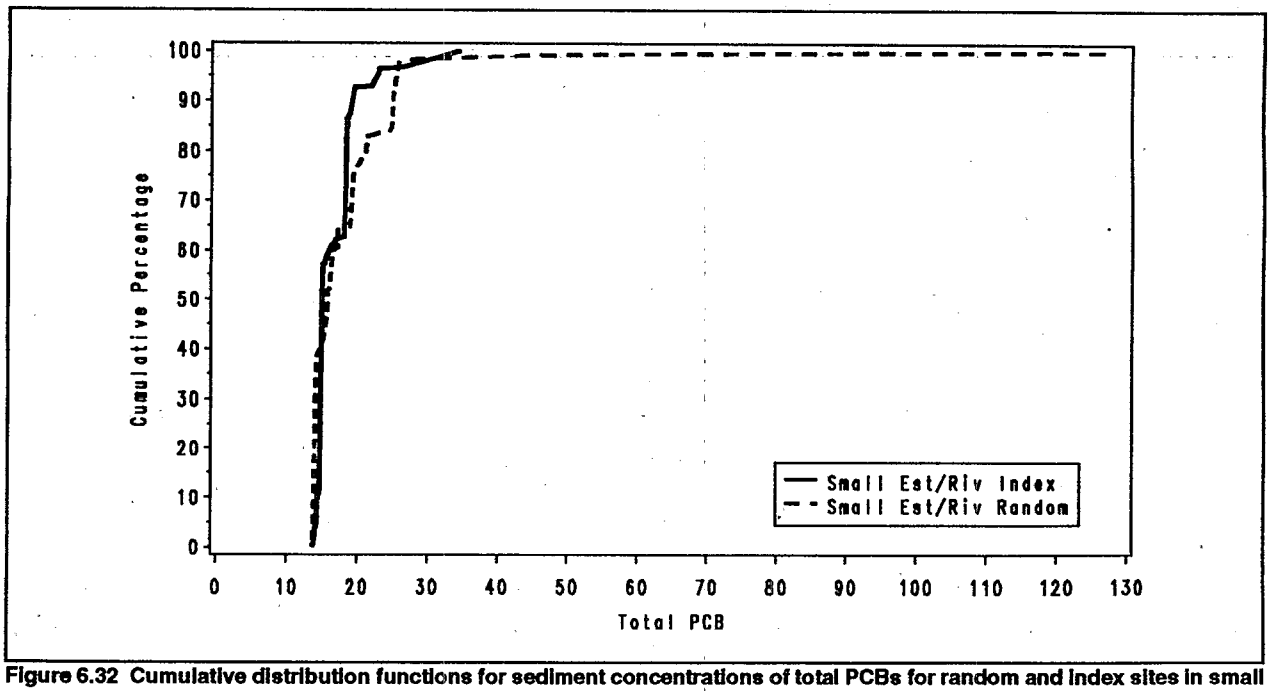

Figure 6.32 Cumulative distribution functions for sediment concentrations of total PCBs for random and index sites in small estuaries/small tidal rivers.

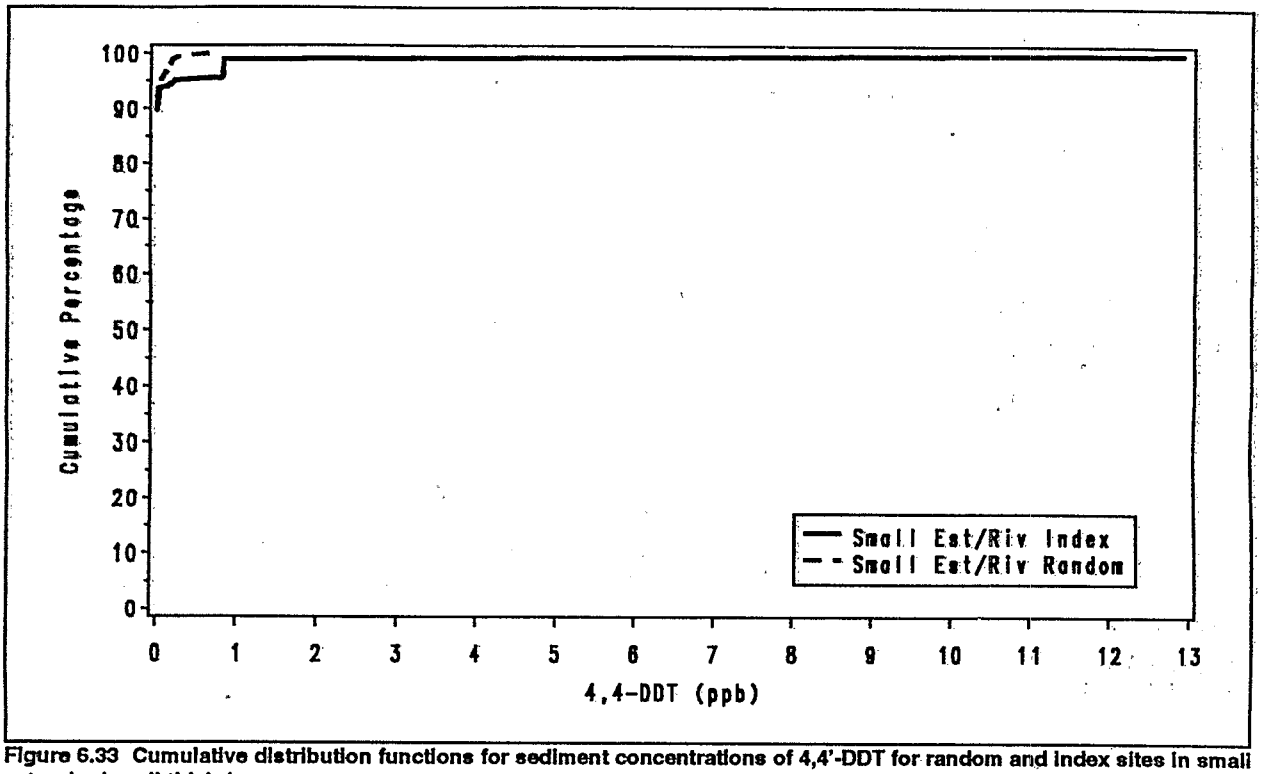

Figure 6.33 Cumulative distribution functions for sediment concentrations of 4,4'-DDT for random and index sites in small estuaries/small tidal rivers.

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Silver	.012	0.39	.01	0.55	<.01	.14	.12
Aluminum	.060	0.57	<.01	0.59	<.01	4.06	3.80
Arsenic	.001	0.32	.04	0.48	<.01	5.35	6.30
Cadmium	.080	0.28	NS	0.53	<.01	.19	.14
Chromium	.001	0.49	<.01	0.59	<.01	46.56	40.76
Copper	.001	0.64	<.01	0.61	<.01	10.42	9.13
Iron	.001	0.52	<.01	0.57	<.01	1.82	1.76
Hg	.660	-0.07	NS	0.31	.04	.11	.09
Manganese	.001	0.46	<.01	0.64	<.01	358.2	352.6
Nickel	.001	0.48	<.01	0.53	<.01	14.6	13.8
Lead	.005	0.36	.02	0.56	<.01	16.7	13.8
Antimony	.010	0.38	.01	0.51	<.01	.66	.54
Selenium	.030	0.31	.04	0.35	.02	.29	.29
Tin	.001	0.53	<.01	0.51	<.01	1.4	1.2
Zinc	.001	0.49	<.01	0.51	<.01	60.4	51.6

Table 6.15 Results of index and random sampling comparisons for sediment heavy metals concentrations in small estuaries using ANOVA, Pearson and Spearman correlations and random versus index means (p < 0.1 = no significant difference).

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
C10	.001	0.51	.001	0.36	.020	8.28	7.55
C11	.280	0.49	.001	0.61	.001	8.08	6.97
C12	.001	0.09	NS	0.49	.001	9.01	6.49
C13	.010	0.33	.030	0.60	.001	5.62	4.47
C14	.010	0.43	.004	0.67	.001	15.76	11.08
C15	.001	0.47	.001	0.54	.001	50.61	35.92
C16	.002	0.70	.001	0.81	.001	58.69	38.53
C17	.001	0.53	.001	0.65	.001	216.80	158.48
Pristane	.001	0.63	.001	0.67	.001	202.8	129.0
C18	.001	0.60	.001	0.71	.001	70.6	48.1
Phytane	.001	0.57	.001	0.79	.001	200.6	127.2
C19	.001	0.58	.001	0.75	.001	103.0	70.4
C20	.001	0.55	.001	0.71	.001	60.0	47.4
C21	.030	0.36	.020	0.46	.002	79.0	56.9
C22	.010	0.29	NS	0.51	.002	28.4	22.0
C23	.001	0.64	.001	0.54	.001	75.3	65.6
C24	.003	0.40	.010	0.63	.001	47.8	36.5
C25	.001	0.59	.001	0.51	.001	126.1	108.1
C26	.001	0.47	.001	0.52	.001	52.3	40.3
C27	.001	0.71	.001	0.53	.001	234.8	182.5
C28	.001	0.78	.001	0.53	.001	90.4	66.5
C29	.002	0.91	.001	0.55	.001	611.8	430.0
C30	.001	0.43	.003	0.47	.001	96.5	69.7
C31	.001	0.65	.001	0.54	.010	402.0	316.8
C32	.001	0.47	.001	0.47	.001	67.0	93.8
C33	.002	0.69	.001	0.53	.001	149.5	199.8
C34	.001	0.40	.010	0.44	.003	15.9	19.1
Alkanes	.001	0.67	.001	0.54	.001	233	317

Table 6.16 Results of index and random sampling comparisons for sediment alkane concentrations in small estuaries using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

6.2 EFFECTS OF GRID DENSITY OF PARAMETER ESTIMATION IN LARGE ESTUARIES

Although some historical sediment contaminants data (O'Connor 1990) was examined to estimate the sample sizes necessary to estimate contaminant concentrations with program objectives, is was unknown whether this sample size would be relevant to large geographic areas. As in the Virginian Province (Weisberg et al. 1992), the necessary sample size corresponded to a systematic grid with a density creating 280 km^2 sampling spaces for the Louisianian Province. In order the test the appropriateness of this spatial scale for the systematic grid, sampling was conducted at a grid scale four times denser in Mobile Bay, AL for all indicators. This supplemental sampling data can be used to address two questions:

- Would sampling at this increased scale improve the estimates of the sampled indicators in terms of accuracy or precision for the large estuary class or the Louisianian Province?

Variable	Anova Station P-Value	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Acenaphthene	.200	0.22	NS	0.59	.001	2.36	1.11
Acenaphthylene	.260	0.18	NS	0.46	.002	1.38	.63
Anthracene	.350	0.19	NS	0.47	.002	4.28	2.18
Benzo(a)anthracene	.400	0.10	NS	0.45	.002	12.62	5.34
Benzo(a)pyrene	.240	0.18	NS	0.50	.001	11.84	5.82
Benzo(b)fluoranthene	.350	0.06	NS	0.42	.005	19.73	8.44
Benzo(e)pyrene	.450	0.14	NS	0.49	.001	13.40	5.84
Benzo(k)fluoranthene	.100	0.26	NS	0.51	.001	9.79	5.23
Benzo(f,h,i)perylene	.120	0.29	.060	0.54	.001	9.99	5.31
Biphenyl	.250	0.57	.001	0.53	.001	1.97	1.36
C1-chrysene	.001	0.40	.010	0.52	.001	19.72	6.41
C2-chrysene	.250	0.55	.001	0.57	.001	23.00	6.05
C3-chrysene	.260	0.45	.002	0.64	.001	11.65	2.75
C4-chrysene	.001	0.67	.001	0.58	.001	7.02	2.43
C1-dibenzothio	.056	0.59	.001	0.72	.001	23.77	17.86
C2-dibenzothio	.001	0.58	.001	0.77	.001	47.65	32.34
C3-dibenzothio	.001	0.37	.014	0.72	.001	36.85	21.75
C1-fluoranthpyrene	.340	0.16	NS	0.61	.001	25.86	12.13
C1-fluorene	.001	0.55	.001	0.72	.001	21.56	14.09
C2-fluorene	.001	0.49	.001	0.80	.001	63.65	44.71
C3-fluorene	.001	0.49	.001	0.67	.001	77.79	49.85
C1-naphthalene	.060	0.28	.070	0.49	.001	7.54	4.71
C2-naphthalene	.002	0.59	.001	0.64	.001	11.90	7.61
C3-naphthalene	.001	0.51	.001	0.68	.001	74.20	41.07
C4-naphthalene	.004	0.45	.003	0.81	.001	145.63	86.56
C1-phenanthrene	.001	0.53	.001	0.70	.001	72.85	52.54
C2-phenanthrene	.013	0.51	.001	0.70	.001	82.79	59.09
C3-phenanthrene	.001	0.35	.020	0.71	.001	53.87	34.64
C4-phenanthrene	.290	0.17	NS	0.71	.001	36.71	17.18
Chrysene	.060	0.11	NS	0.48	.001	16.27	7.21
Dibenzo(a,h)anthracene	.370	0.50	.001	0.57	.001	2.93	1.33
Dibenzothio	.001	0.67	.001	.64	.001	6.59	4.40
2,6-dimethylnaphthalene	.003	0.50	.001	.58	.001	4.00	2.65
Fluorene	.430	0.56	.001	.61	.001	4.56	2.58
Fluoranthene	.001	0.09	NS	.51	.001	26.18	11.01
(i)1,2,3-c,d-pyrene	.080	0.29	.060	.53	.001	8.96	5.00
2,3,5-trimethylnaphthalene	.001	0.59	.001	.74	.001	22.19	13.05
1-methylnaphthalene	.070	0.29	.060	.49	.001	2.93	1.89
2-methylnaphthalene	.040	0.28	.070	.50	.001	4.61	2.82
1-methylphenanthrene	.001	0.61	.001	.73	.001	16.35	12.10
TOT PAHS	.004	.45	.003	.64	.001	1147.	700.4

Table 6.17 Results of index and random sampling comparisons for sediment PAH concentrations in small estuaries using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

- Would sampling at this increased scale improve the estimates of the sampled indicators in terms or accuracy or precision for Mobile Bay?

Variable	Anova Station PR > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
PCB 8	0.002	0.400	.0071	0.430	.0035	0.09	0.08
PCB 18	0.088	0.115	NS	0.267	.0795	0.02	0.02
PCB 28	0.167	0.460	.0017	0.496	.0006	0.15	0.03
PCB 44	0.643	-0.104	NS	-0.062	NS	0.04	0.02
PCB 52	0.015	0.322	.0328	0.432	.0034	0.24	0.15
PCB 66	0.517	-0.018	NS	0.241	NS	0.07	0.02
PCB 101	0.329	0.152	NS	0.554	.0001	0.32	0.08
PCB 105	0.401	0.134	NS	0.471	.0013	0.11	0.03
PCB 110	0.190	0.190	NS	0.356	.0177	0.48	0.19
PCB 118	0.381	0.141	NS	0.440	.0028	0.20	0.05
PCB 126	0.000	0.589	.0001	0.337	.0253	0.02	0.25
PCB 128	0.240	0.038	NS	0.271	.0750	0.11	0.02
PCB 138	0.446	0.321	.0337	0.444	.0025	0.75	0.31
PCB 153	0.412	0.121	NS	0.500	.0005	0.56	0.09
PCB 170	0.259	0.209	NS	0.426	.0086	0.51	0.22
PCB 180	0.389	0.150	NS	0.633	.0001	0.33	0.07
PCB 187	0.335	0.153	NS	0.570	.0001	0.25	0.04
PCB 195	0.400	0.158	NS	0.137	NS	0.05	0.01
PCB 206	0.152	0.697	.0001	0.180	NS	0.08	0.01
PCB 209	0.212	0.595	.0001	0.500	.0005	0.24	0.03

Table 6.18 Results of index and random sampling comparisons for sediment PCB concentrations in small estuaries using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

For the first question, sampling for the Mobile Bay increases the sample size for the large estuarine class from 56 to 69 (+23%). If the supplements are better characterizing the indicators within the large estuarine class, an increase of 23% in the sample size should result in a cumulative distribution function that is significantly different than the CDF based only on the large estuarine base samples. For the second question, the increase in sample size from 3 to 13 (+333%) with the supplemental samples should result in significantly different CDFs if the supplements better characterize the EMAP-E indicators.

Although all indicators were examined with regard to these questions, only selected indicators of each indicator type are discussed here; namely, benthic biodiversity (biological response), water clarity (human use response), minimum dissolved oxygen (water quality), total organic carbon (habitat), and total DDT (exposure). Comparison of province-wide CDFs for benthic biodiversity (Fig. 6.34), light transmittance to 1 m (Fig. 6.35), bottom minimum dissolved oxygen concentrations (Fig. 6.36), TOC (Fig. 6.37), and total sediment DDT concentration (Fig. 6.38) show that the addition of the supplemental sites do not significantly alter the province-wide distributions of these indicators. Unlike the province-wide distributions, the addition of the supplemental samples does significantly alter the indicator CDFs observed for Mobile Bay (Figures 6.39-43). Thus, the present spatial scale for the systematic grid

Variable	Anova Station PR > F	Pearson r	Pearson p	Spearman r	Spearman p	Mean Random	Mean Index
Aldrin	0.383	0.017	NS	0.262	.0854	0.00	0.00
Alpha BHC	0.008	0.186	NS	0.469	.0013	0.01	0.01
Alpha Chlordane	0.079	0.336	.0257	0.512	.0004	0.06	0.04
Beta BHC	0.575	-0.067	NS	-0.073	NS	0.01	0.00
Cis-nonachlor	0.042	0.393	.0084	0.497	.0006	0.04	0.01
2,4'-DDD	0.071	-0.026	NS	0.215	NS	0.03	0.01
4,4'-DDD	0.561	0.221	NS	0.588	.0001	0.17	0.19
Delta BHC	0.005	0.354	.0186	0.383	.0103	0.00	0.00
2,4'-DDE	0.174	0.202	NS	0.588	.0001	0.02	0.07
4,4'-DDE	0.472	0.237	NS	0.472	.0012	0.52	0.36
2,4'-DDT	0.058	-0.024	NS	0.220	NS	0.00	0.00
4,4'-DDT	0.428	0.045	NS	0.346	.0213	0.04	0.35
Dieldrin	0.396	0.028	NS	0.433	.0033	0.04	0.08
Endosulfan 1						0.00	0.00
Endosulfan 2						0.00	0.00
Endrin	0.293	0.312	.0391	0.476	.0011	0.00	0.03
Gamma BHC	0.037	0.290	NS	0.310	.0409	0.00	0.00
Gamma Chlordane	0.000	0.212	NS	0.221	NS	0.13	0.03
HCB	0.288	0.974	.0001	0.652	.0001	0.73	0.39
Heptachlor Epoxide	0.253	0.516	.0004	0.200	NS	0.11	0.01
Heptachlor	0.438					0.00	0.00
Mirex	0.468	-0.023	NS	-0.023	NS	0.00	0.00
Oxychlor	0.438					0.00	0.00
Total BHC	0.092	0.172	NS	0.381	.0108	0.03	0.03
Total Chlordane	0.221	0.247	NS	0.526	.0002	0.42	0.12
Total DDT	0.319	0.054	NS	0.419	.0046	0.79	0.99
Toxaphene						0.00	0.00
Transnonachlor	0.052	0.489	.0008	0.339	.0246	0.05	0.01

Table 6.19 Results of index and random sampling comparisons for sediment pesticide concentrations in small estuaries using ANOVA, Pearson and Spearman correlations and random versus index means ($p < 0.1$ = no significant difference).

corresponding to sampling spaces of 280 km^2 represents the indicator as well as the reduced 70 km^2 sampling spaces for province-wide estimates (as well as for large estuary distributions which comprise 75% of the province). However, if the objective is to characterize a specific estuary, the 280 km^2 is inadequate to characterize estuaries the size of Mobile Bay (about 600 km^2).

6.3 DEGREE OF SPATIAL AUTOCORRELATION FOR SITES SELECTED BASED ON THE GRID

During the 1991 Louisianian Province Demonstration, multiple stations were often sampled within large estuaries (i.e., > 250 km^2)(e.g., Apalachee Bay, Galveston Bay, Lake Pontchartrain). Because the initial sampling spaces (280 km^2 hexagons) were organized based on the random placement of a systematic sampling grid, some spatial relationship among proximally located stations might exist. The CDF for the distances among proximal stations is shown in Figure 6.44 and illustrates that distances range from 6 km to 39 km.

Because sampling points in the Louisianian Province were not equidistant, a spatial autocorrelation analysis was completed to determine if an interdependence of parameter values existed due to proximity. The analysis uses autocorrelation statistics that are basically descriptive in nature and can be used in tests of hypotheses. The spatial autocorrelation statistics are functions of both the data values and a weighing function which assigns values to pairs of sites to represent their geographic arrangement. The choice of weighing function determines the hypothesis tested and for one analysis presented here, examination of several tests simultaneously is used to ascertain the maximum distance of influence for selected parameters. The statistic employed for this spatial analysis is Moran's I (Odland 1988).

Most pertinent indicators were examined using a weighing function equal to the

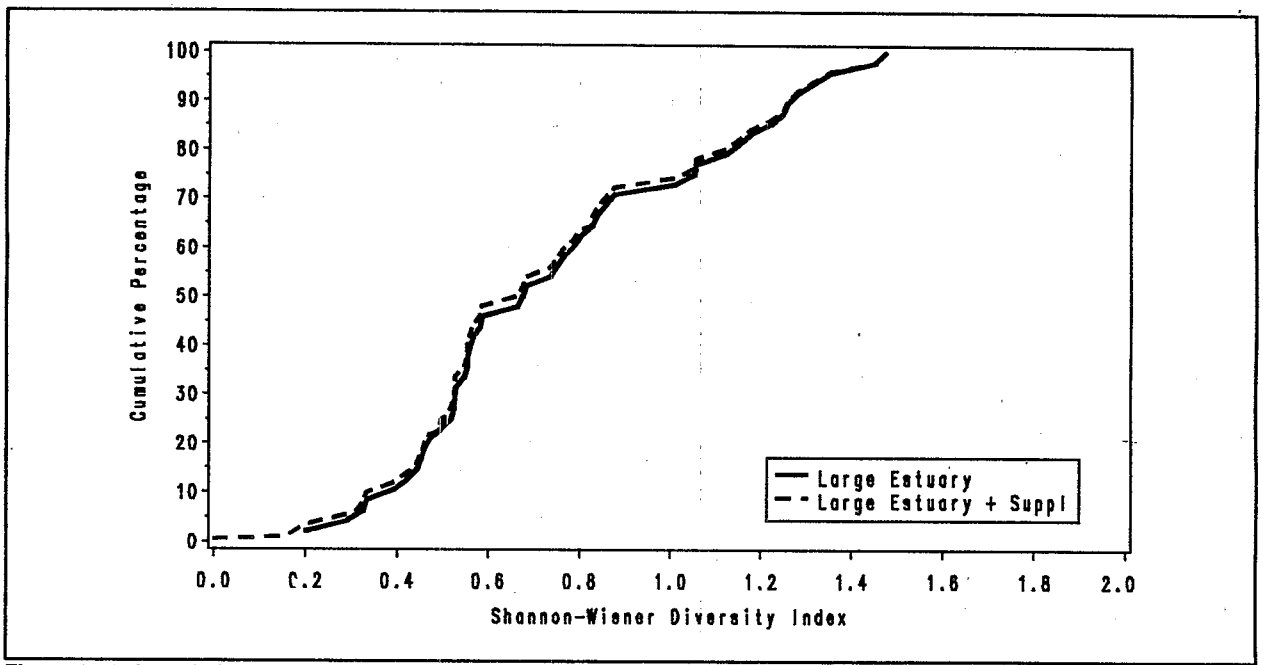

Figure 6.34 Cumulative distribution functions for benthic biodiversity incorporating supplemental sampling for Louisianian Province.

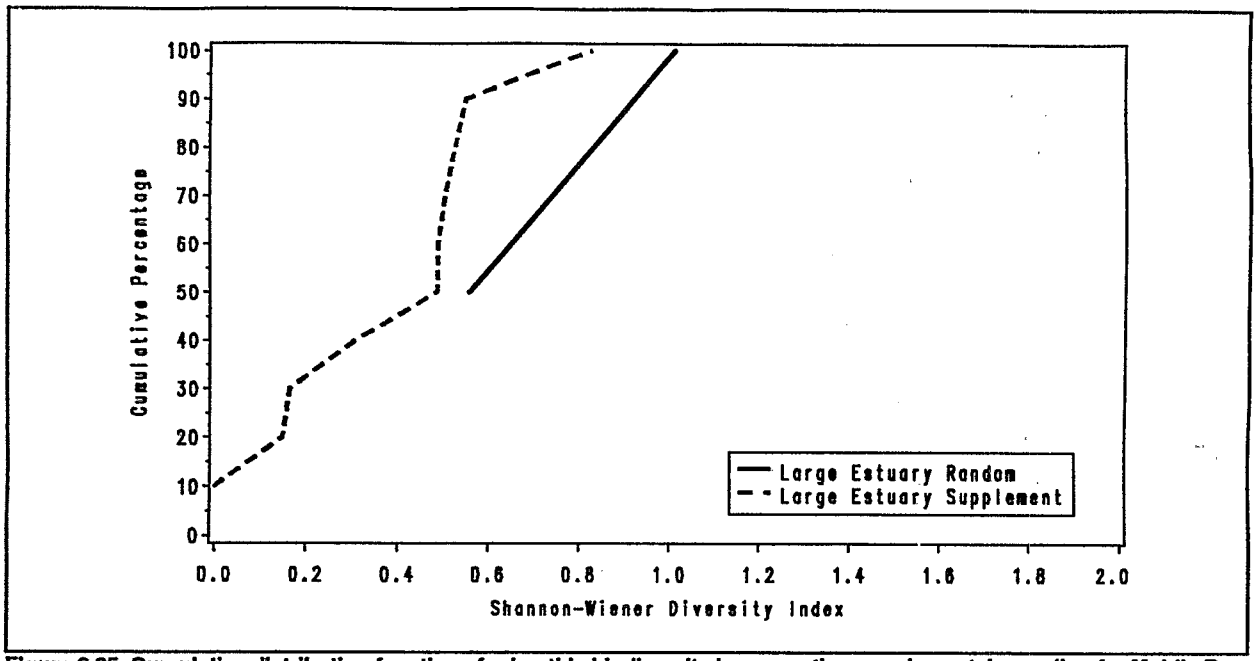

Figure 6.35 Cumulative distribution functions for benthic biodiversity incorporating supplemental sampling for Mobile Bay, AL.

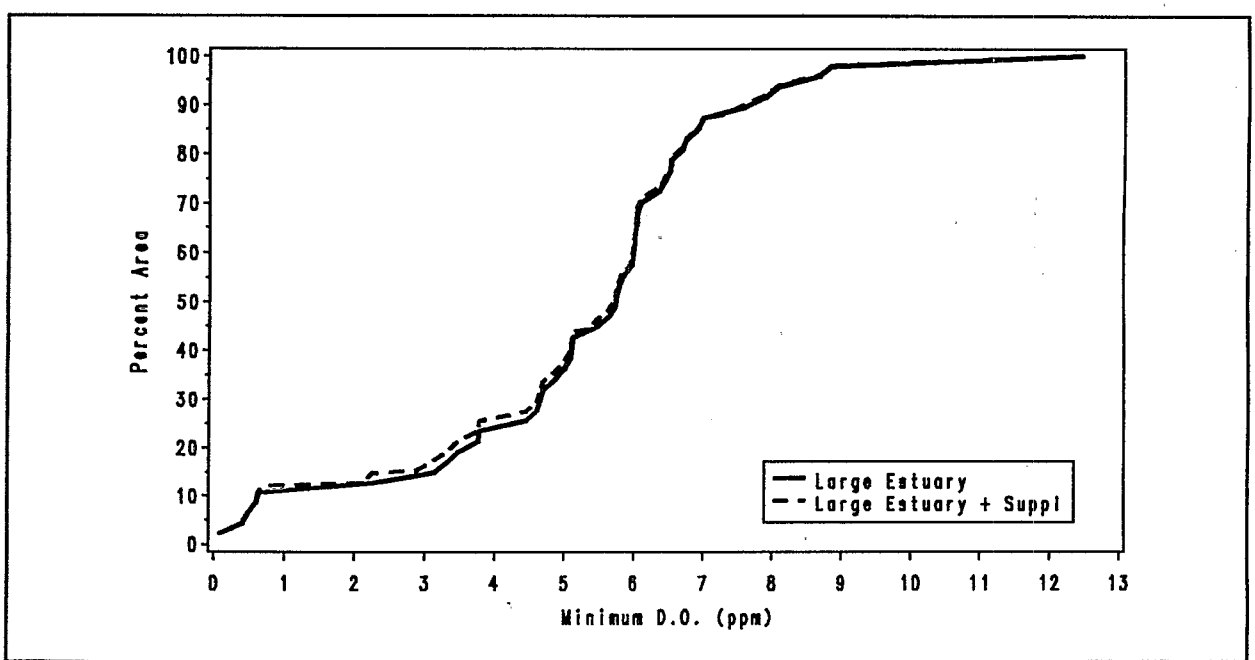

Figure 6.36 Cumulative distribution functions for minimum dissolved oxygen concentrations incorporating supplemental sampling for Louisianian Province.

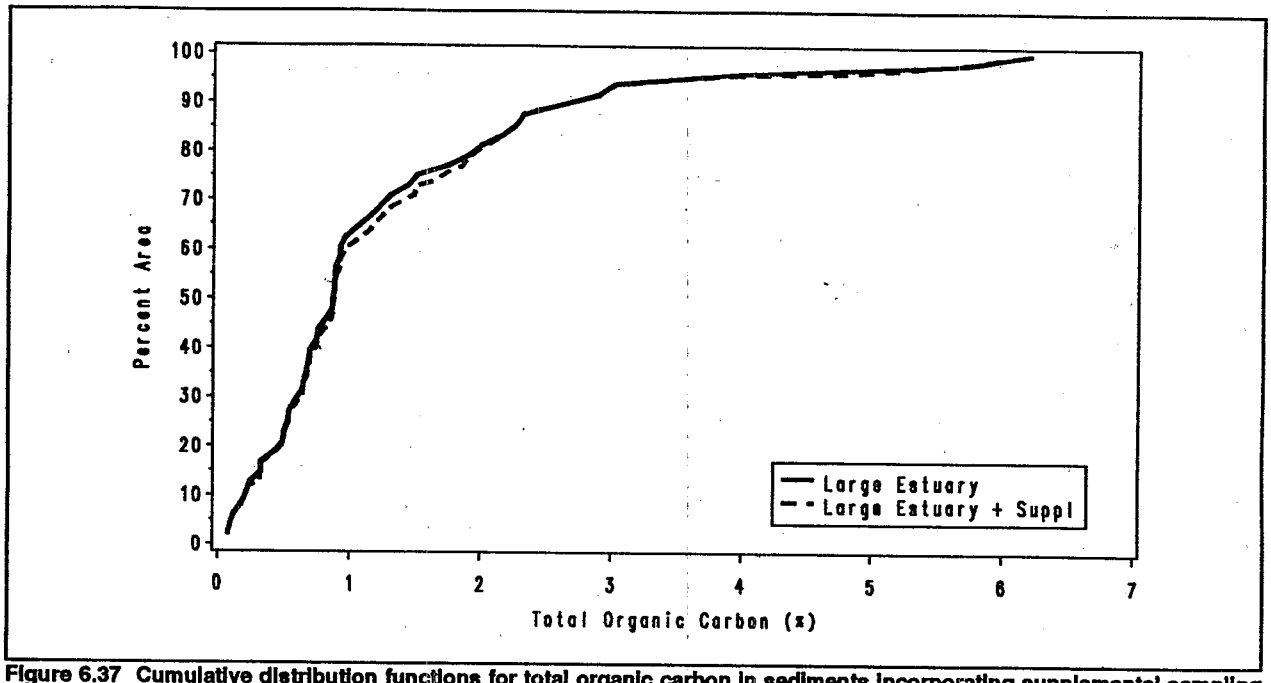

Figure 6.37 Cumulative distribution functions for total organic carbon in sediments incorporating supplemental sampling for Louisianian Province.

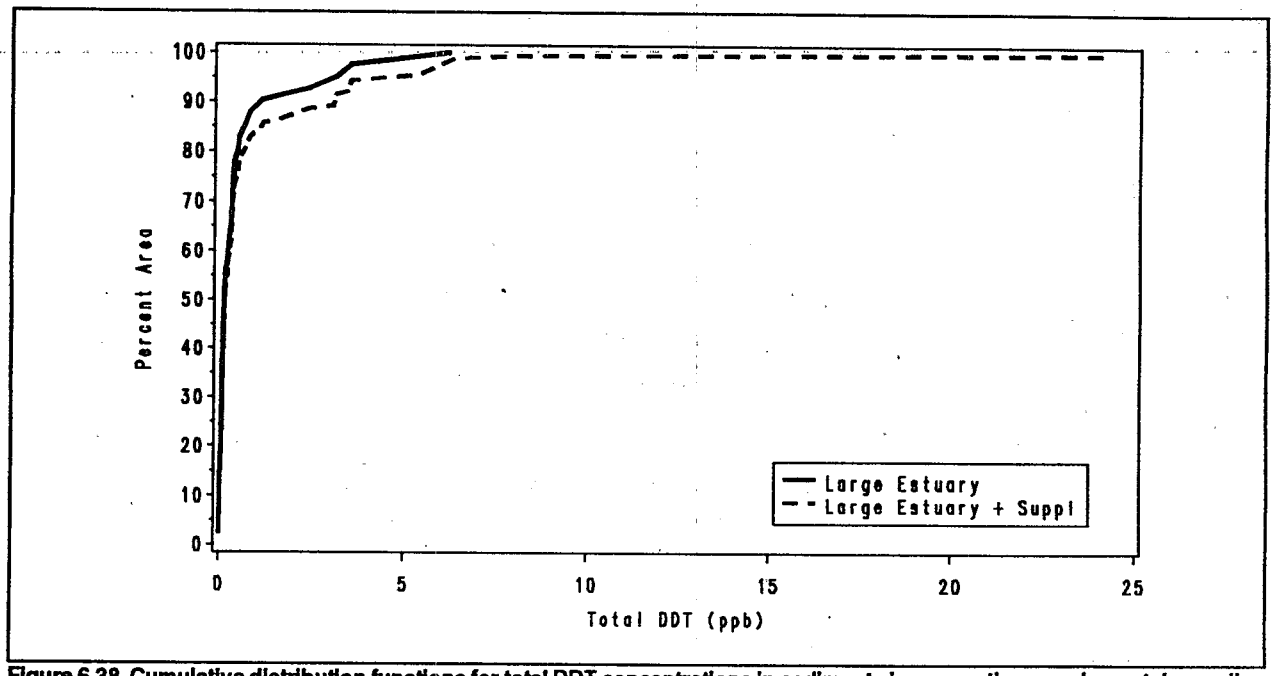

Figure 6.38 Cumulative distribution functions for total DDT concentrations in sediments incorporating supplemental sampling for Louisianian Province.

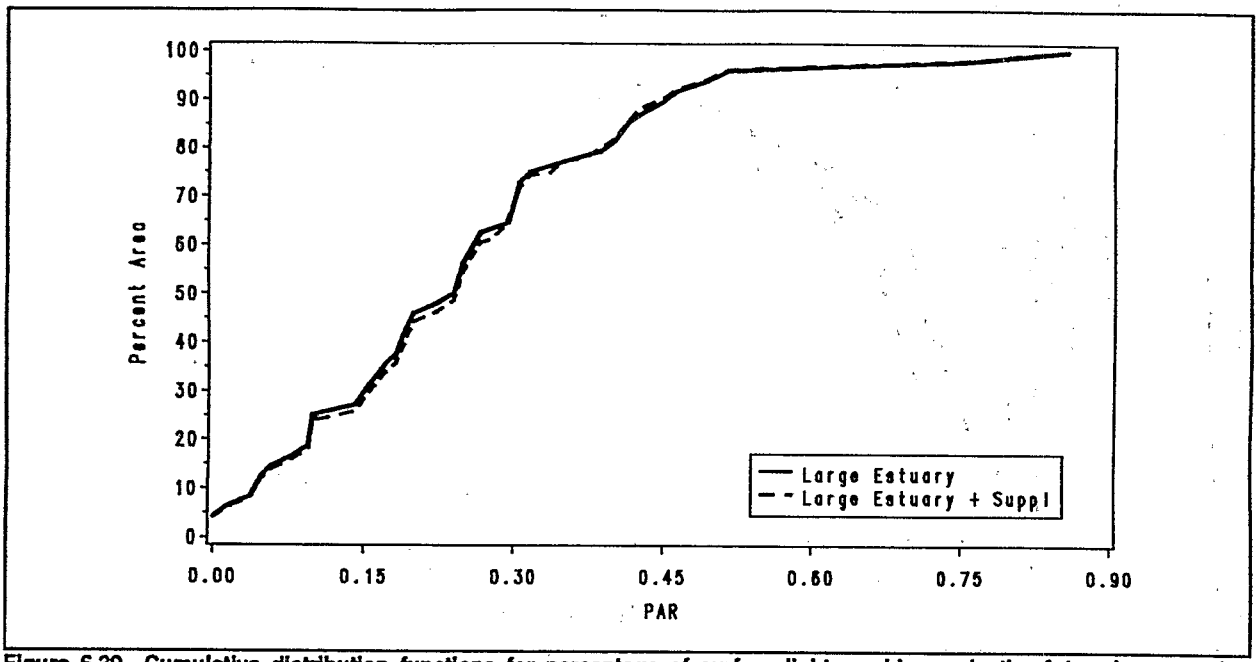

Figure 6.39 Cumulative distribution functions for percentage of surface light reaching a depth of 1 m incorporating supplemental sampling for Louisianian Province.

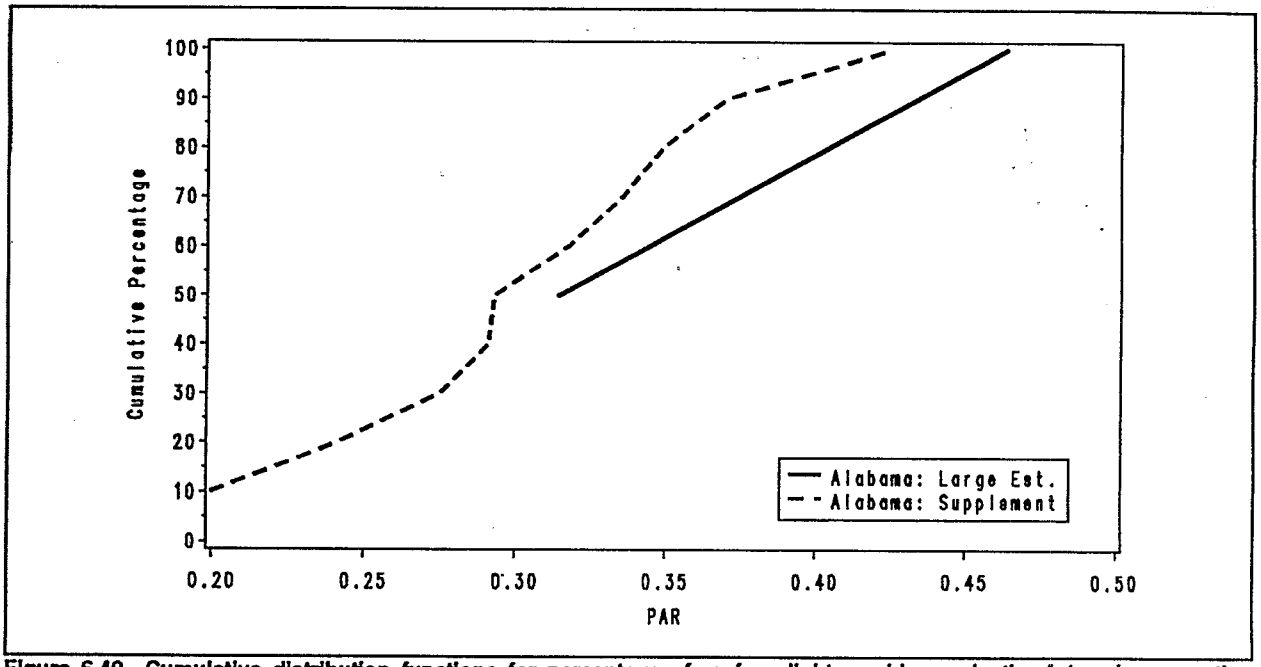

Figure 6.40 Cumulative distribution functions for percentage of surface light reaching a depth of 1 m incorporating supplemental sampling for Mobile Bay, AL.

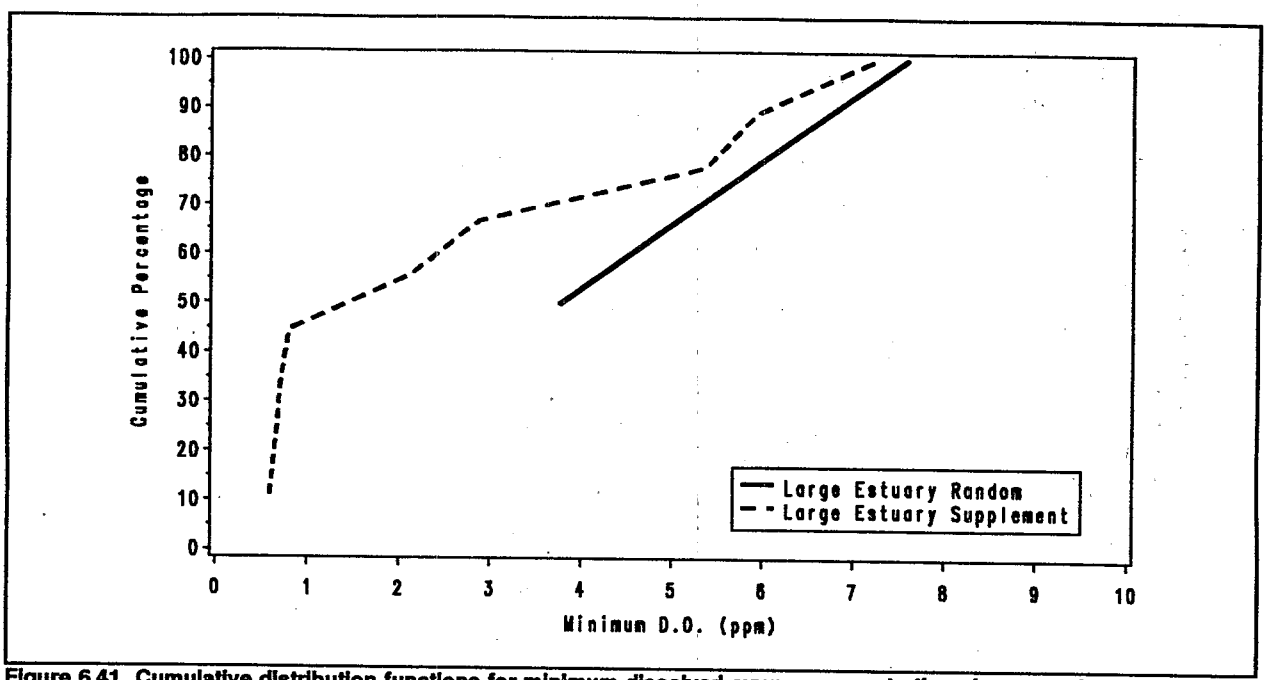

Figure 6.41 Cumulative distribution functions for minimum dissolved oxygen concentrations incorporating supplemental sampling for Mobile Bay, AL.

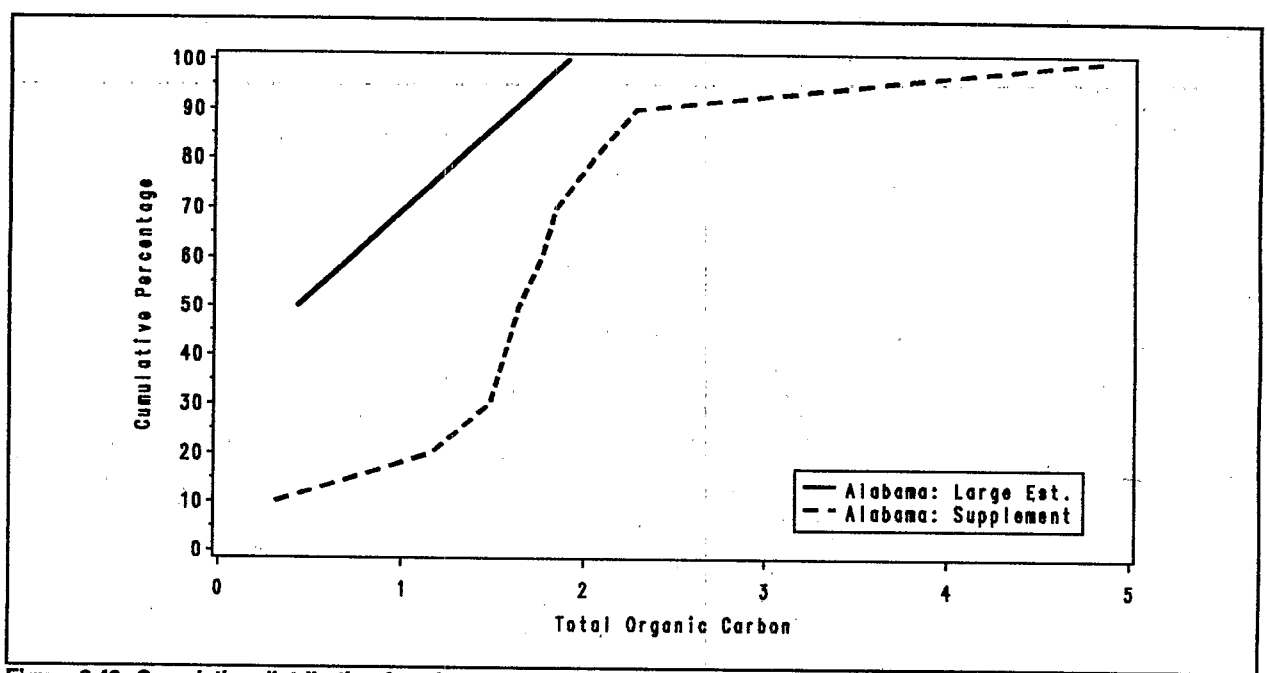

Figure 6.42 Cumulative distribution functions for total organic carbon in sediments incorporating supplemental sampling for Mobile Bay, AL.

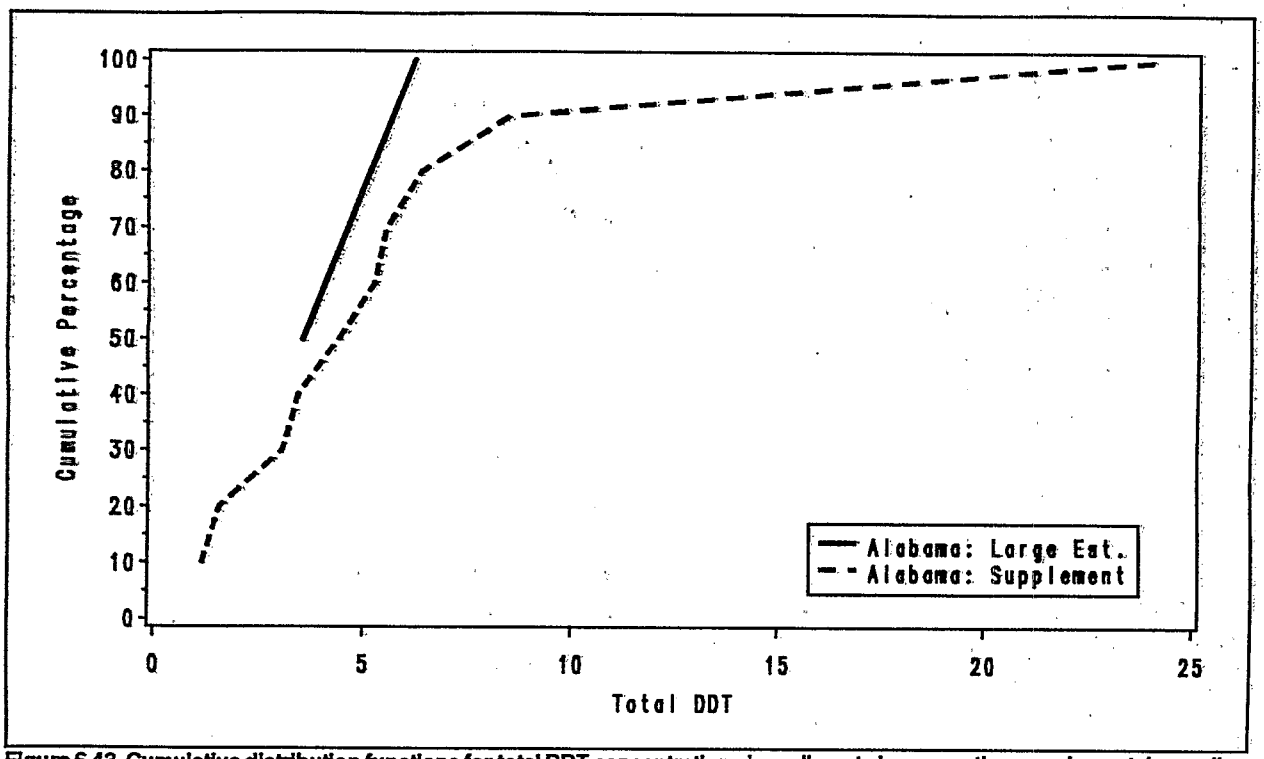

Figure 6.43 Cumulative distribution functions for total DDT concentrations in sediments incorporating supplemental sampling for Mobile Bay.

inverse of the distance in kilometers between the two random base sites. This weighing function allows every random site to impact on all other random sites unless a maximal influence distance is defined. For example, if site A is twice the distance to a site C as site B, the farther site will have 1/2 the spatial impact of the closer site. The hypothesis tested is, "The values are randomly distributed geographically." versus the alternative, "'Nearer sites have related values.". Three sets of spatial autocorrelative analyses were completed:

- Analysis examined the interdependency of all random base sites regardless of estuarine class,
- Analysis examining the interdependency of all sites within the large estuarine class (sites randomly located within hexagonal sampling space) with the assumption of no dependency existing after 20, 25, or 30 km except for bottom dissolved oxygen, bottom temperature, and degree of stratification which also included distances of 10 and 15 km,
- Analysis of five selected water bodies in the large estuarine class within the Louisianian Province (i.e., Galveston Bay, Lake Pontchartrain, Mobile Bay, Chandeleur Sound, and Mississippi Sound) to determine if patterns seen overall for the province were evident in individual systems.

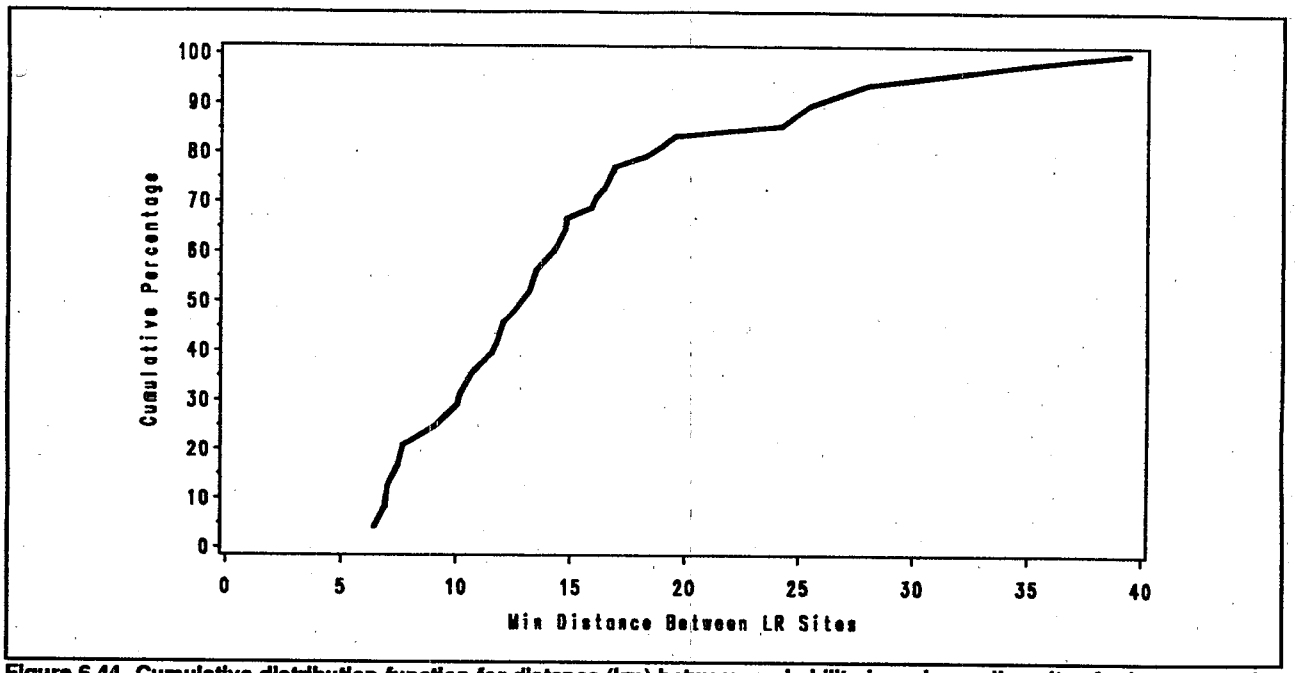

Figure 6.44 Cumulative distribution function for distance (km) between probability-based sampling sites for large estuaries (LR) in the Louisianian Province in 1991.

6.3.1 SPATIAL AUTOCORRELATION AMONG ALL PROBABILITY-BASED SITES

The results of the spatial autocorrelation analysis among all probability-based sites regardless of estuarine class in shown in Table 6.20. Several benthic response indicators showed significant spatial autocorrelation including: mean benthic abundance, mean number of benthic species, and percent of total abundance of polychaetes and amphipods. However, the benthic index showed no spatial autocorrelation. This lack of spatial dependency may be the result of the removal of the effects of salinity on biodiversity, a statistic related to abundance and number of species (both spatially dependent). No spatial dependency was seen in the mean abundance of fish per trawl possibly confirming the effects of finfish mobility. Regardless, the results with several response indicators (other than the calculated indices) suggest that spatial autocorrelation will have to be adjusted for in any long-term analyses.

As with response indicators, several habitat indicators portrayed spatial autocorrelation in the Louisianian Province. Water clarity, bottom salinity, and bottom temperature were all significantly spatially dependent. Sediment contaminant exposure indicators exhibited a heavy spatial dependency. Eighty five percent of alkanes, 87% of heavy metals, 80% of PAHs, and 70% of pesticides showed significant spatial dependency within the Louisianian Province in 1991 (Table 6.20).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	0.055	0.0042*
Benthic Species	0.110	<0.0001*
Percent Polychaetes	0.102	<0.0001*
Percent Amphipods	0.043	0.0150*
Benthic Index	0.027	0.0670
Fish Abundance	0.009	0.2260
Habitat Indicators		
Instantaneous DO	0.001	0.3238
Percent Light at 1 m	0.104	<0.0001*
Bottom Salinity	0.249	<0.0001*
Stratification	0.017	0.1357
Bottom Temperature	0.042	0.0179*
Exposure Indicators		
Alkanes		
Total Alkanes	0.037	0.0286*
C10	0.068	0.0008*
C11	-0.031	0.8034
C12	0.020	0.1121
C13	0.046	0.0114*
C14	0.058	0.0029*
C15	0.018	0.1261
C16	0.069	0.0007*
C17	0.054	0.0048*
C18	0.074	0.0003*
C19	0.084	0.0001*
C20	0.104	<0.0001*
C21	0.074	0.0003*
C22	0.061	0.0018*
C23	0.071	0.0005*
C24	0.049	0.0079*
C25	0.073	0.0004*
C26	0.088	<0.0001*
C27	0.054	0.0044*
C28	0.039	0.0223*
C29	0.020	0.1095
C30	0.059	0.0024*
C31	0.078	0.0002*
C32	0.069	0.0007*
C33	0.078	0.0002*
C34	0.065	0.0012*
Phytane	0.076	0.0002*
Pristane	0.068	0.0007*

Table 6.20 Spatial autocorrelation based on Moran's I for all probability-based sites within the Louisianian Province. (* p < 0.05 = spatial dependency exists).

Indicators	Moran's I	P-value
Exposure Indicators		
Pesticides		
Dieldrin	0.037	0.0270*
2,4'-DDT	0.001	0.3261
4,4'-DDT	0.061	0.0019*
Heavy Metals		
Aluminum	0.064	0.0014*
Antimony	0.139	<0.0001*
Arsenic	-0.026	0.7463
Cadmium	0.068	0.0009*
Chromium	0.090	<0.0001*
Copper	0.113	<0.0001*
Iron	0.094	<0.0001*
Lead	0.090	<0.0001*
Manganese	0.120	<0.0001*
Mercury	-0.007	0.4537
Nickel	0.127	<0.0001*
Selenium	0.040	0.0213*
Silver	0.033	0.0411*
Tin	0.113	<0.0001*
Zinc	0.093	<0.0001*
PAHS		
Total PAHs	0.085	0.0001*
Acenaphthene	0.047	0.0097*
Acenaphthylene	0.030	0.0535
Anthracene	0.041	0.0199*
Benzo(a)anthracene	0.028	0.0619
Benzo(a)pyrene	0.044	0.0143*
Benzo(b)fluoranthene	0.023	0.0910
Benzo(e)pyrene	0.033	0.0388*
Benzo(g,h,i)perylene	0.045	0.0127*
Benzo(k)fluoranthene	0.082	0.0001*
Biphenyl	0.041	0.0182*

Table 6.20 (Cont.) Spatial autocorrelation based on Moran's I for all probability-based sites within the Louisianian Province. (* p < 0.05 = spatial dependency exists).

Indicators	Moran's I	P-value
Exposure Indicators		
Chrysene	0.031	0.0460*
C1-Chrysene	0.008	0.2356
C2-Chrysene	0.007	0.2434
C3-Chrysene	0.009	0.2240
C4-Chrysene	0.007	0.2405
Dibenzo(a,h)anthracene	0.015	0.1552
Dibenzothio	0.061	0.0018*
C1-Dibenzothio	0.074	0.0003*
C2-Dibenzothio	0.087	<0.0001*
C3-Dibenzothio	0.076	0.0002*
Fluoranthene	0.038	0.0254*
Fluorene	0.067	0.0009*
C1-Fluorene	0.078	0.0002*
C2-Fluorene	0.090	<0.0001*
C3-Fluorene	0.106	<0.0001*
Naphthalene	0.049	0.0082*
C1-Naphthalene	0.067	0.0009*
C2-Naphthalene	0.071	0.0005*
C3-Naphthalene	0.063	0.0016*
C4-Naphthalene	0.070	0.0006*
Perylene	0.054	0.0044*
Phenanthrene	0.066	0.0010*
C1-Phenanthrene	0.077	0.0002*
C2-Phenanthrene	0.090	<0.0001*
C3-Phenanthrene	0.080	0.0001*
C4-Phenanthrene	0.025	0.0754
Pyrene	0.053	0.0052*
Ideno(1,2,3,c,d)Pyrene	0.053	0.0052*
1-Methylnaphthalene	0.072	0.0004*
1-Methylphenanthrene	0.063	0.0015*
2-Methylnaphthalene	0.062	0.0017*
2,3,5-Trimethylnaphthalene	0.061	0.0020*
2,6-Dimethylnaphthalene	0.070	0.0005*

Table 6.20 (Cont.) Spatial autocorrelation based on Moran's I for all probability-based sites within the Louisianian Province. (* < 0.05 = spatial dependency exists).

6.3.2 SPATIAL AUTOCORRELATION WITHIN THE LARGE ESTUARINE CLASS

Selected indicators were used to evaluate spatial dependency within the large estuarine class and to assess the distance at which this dependency becomes minimal. Several distances were used in this analysis as "cut-off" distances (i.e., distance beyond which no dependency is assumed to exist). In this analysis, a non-reflexive weight was used so that a value of 1 was assigned to the distance associated with the nearest neighbor to a site and 0 was assigned to all other distances to restrict the influence to only the closest site.

As the cut-off distance increases, and the spatial influence is still present, the test p-value should tend to decrease. If the distance goes beyond the real sphere of influence then unrelated values will be given a positive weight and the p-value will tend to increase. The benthic response indicators: benthic index, mean benthic abundance, and mean numbers of benthic species collected at a large estuarine site showed strong spatial dependence display a tendency to be most strongly related spatially to their nearest neighbor (Table 6.21). However, significant spatial autocorrelation for these response indicators exists to a distance of at least 30 km. As a result, corrections for spatial autocorrelation in long-term analyses for these indicators will have to include more than simple nearest-neighbor adjustments.

No spatial autocorrelation was observed for mean abundance of fish/trawl regardless of distance (Table 6.21).

Instantaneous bottom dissolved oxygen concentration's p-value decreased from 0.80 at 10 km to 0.67 at 30 km but was only 0.37 for the nearest neighbor test. Although never significant, the value at the nearest site appeared to be the most closely related (Table 6.21). The optimal distance for spatial dependency of stratification appeared to be about 20 km. P-values for bottom temperature, water clarity, and bottom salinity were still decreasing at 30 km suggesting a strong extended (> 30 km) spatial dependency. Within large estuaries, spatial dependencies were different for different heavy metals (Table 6.21). Arsenic, mercury, selenium, and silver showed no spatial dependency suggesting that these concentrations might simply represent point phenomena or, at worst, spatial dependencies of < 20 km. Aluminum, cadmium, chromium, copper, iron, lead, manganese, nickel, tin, and zinc showed increasing p-values with distance indicating spatial dependencies ranging farther than nearest neighbor. However, in all these spatial dependent metals, dependency was strongest on the site closest to the sampling point. Total PAHs showed a significant and consistent spatial dependency within the range of 0-30 km. Dieldrin and 4,4'-DDT were spatially dependent in large estuaries. However, for dieldrin, this dependency was stronger within the distance range 25-30 km than closer suggesting a patchy but related structure of occurrence for this pesticide. 2,4'-DDT was not spatially dependent.

Indicators	Distance (< km)	Moran's I	P-value
Response Indicators			
Benthic Abundance	20	0.283	0.0133*
	25	0.267	0.0044*
	30	0.274	0.0010*
	NN	0.278	0.0279*
Benthic Species	20	0.578	<0.0001*
	25	0.550	<0.0001*
	30	0.554	<0.0001*
	NN	0.504	<0.0001*
Benthic Index	20	0.236	0.0311*
	25	0.265	0.0047*
	30	0.245	0.0027*
	NN	0.325	0.0132*
Fish Abundance	20	0.023	0.4043
	25	0.011	0.4210
	30	0.014	0.3989
	NN	(a)[1]	
Habitat Indicators			
Instantaneous DO	10	-0.248	0.8023
	15	-0.139	0.7735
	20	-0.120	0.7978
	25	-0.061	0.6843
	30	-0.050	0.6698
	NN	0.038	0.3739
Percent Light at 1 m	20	0.247	0.0257*
	25	0.228	0.0122*
	30	0.187	0.0158*
	NN	0.393	0.0038*
Bottom Salinity	20	0.814	<0.0001*
	25	0.738	<0.0001*
	30	0.703	<0.0001*
	NN	0.684	<0.0001*
Stratification	10	-0.143	0.6827
	15	0.035	0.3958
	20	0.062	0.2936
	25	-0.002	0.4695
	30	-0.011	0.5053
	NN	-0.151	0.8221
Bottom Temperature	10	0.072	0.3853
	15	0.117	0.2306
	20	0.087	0.2316
	25	0.109	0.1306
	30	0.143	0.0476
	NN	0.106	0.2194

Table 6.21 Spatial autocorrelation based on Moran's I for all probability-based sites within the large estuarine class of the Louisianian Province. (* p < 0.05 = spatial dependency; NN = nearest neighbor).

Indicators	Distance (< km)	Moran's I	P-value
Exposure Indicators			
Total Alkanes	20	0.210	0.0479
	25	0.160	0.0542
	30	0.181	0.0187*
	NN	0.068	0.3022
Dieldrin	20	0.158	0.1021
	25	0.178	0.0378*
	30	0.186	0.0163*
	NN	0.070	0.2958
2,4'-DDT	20	0.076	0.2570
	25	0.078	0.2028
	30	0.053	0.2476
	NN	0.079	0.2776
4,4'-DDT	20	0.249	0.0250*
	25	0.251	0.0068*
	30	0.268	0.0012*
	NN	0.217	0.0655
Total PAHs	20	0.282	0.0135*
	25	0.230	0.0116*
	30	0.199	0.0115*
	NN	0.239	0.0492*
Aluminum	20	0.408	0.0007*
	25	0.381	0.0001*
	30	0.355	<0.0001*
	NN	0.476	<0.0001*
Antimony	20	0.273	0.0156*
	25	0.241	0.0087*
	30	0.221	0.0053*
	NN	0.187	0.0948
Arsenic	20	-0.099	0.7527
	25	-0.049	0.6450
	30	-0.071	0.7501
	NN	0.032	0.3875
Cadmium	20	0.287	0.0118*
	25	0.276	0.0033*
	30	0.211	0.0074*
	NN	0.278	0.0272*
Chromium	20	0.354	0.0027*
	25	0.321	0.0008*
	30	0.257	0.0016*
	NN	0.394	0.0035*
Copper	20	0.357	0.0026*
	25	0.336	0.0005*
	30	0.320	0.0001*
	NN	0.465	0.0008*

Table 6.21 Spatial autocorrelation based on Moran's I for all probability-based sites within the large estuarine class of the Louisianian Province.(* p < 0.05 = spatial dependency; NN = nearest neighbor).

Indicators	Distance (< km)	Moran's I	P-value
Exposure Indicators			
Iron	20	0.319	0.0061*
	25	0.308	0.0013*
	30	0.290	0.0005*
	NN	0.439	0.0014*
Lead	20	0.406	0.0007*
	25	0.363	0.0002*
	30	0.289	0.0005*
	NN	0.398	0.0033*
Manganese	20	0.493	0.0001*
	25	0.444	<0.0001*
	30	0.386	<0.0001*
	NN	0.453	0.0010*
Mercury	20	0.027	0.3895
	25	0.020	0.3868
	30	0.008	0.4204
	NN	0.031	0.3904
Nickel	20	0.380	0.0015*
	25	0.364	0.0002*
	30	0.347	<0.0001*
	NN	0.513	0.0002*
Selenium	20	0.134	0.1355
	25	0.127	0.0964
	30	0.093	0.1279
	NN	0.201	0.0791
Silver	20	0.155	0.1039
	25	0.114	0.1202
	30	0.097	0.1190
	NN	0.163	0.1235
Tin	20	0.371	0.0018*
	25	0.368	0.0002*
	30	0.349	<0.0001*
	NN	0.430	0.0017*
Zinc	20	0.405	0.0008*
	25	0.385	0.0001*
	30	0.332	0.0001*
	NN	0.356	0.0073*

Table 6.21 Spatial autocorrelation based on Moran's I for all probability-based sites within the large estuarine class of the Louisianian Province. (* $p < 0.05$ = spatial dependency; NN = nearest neighbor).

6.3.3 SPATIAL AUTOCORRELATION IN SELECTED LARGE ESTUARIES

Five individual large estuaries were examined to assess whether the observed prevalence of spatial dependence in the large estuarine class was consistently demonstrated by individual estuaries. Unfortunately, using all available sites (large estuaries and associated small systems), the number of observations was very small reducing the power of the test as well as making the normality assumption suspect. If significant spatial autocorrelations are shown within these estuaries, the relationship is strong and clear. However, if no relationship is seen, the results may represent a false negative due to the reduced sample size.

No significant spatial dependencies were found in any variable in the Galveston Bay, Lake Pontchartrain, or Chandeleur Sound (Tables 6.22 through 6.24). Because of the probability of false negatives, an intensive examination of Lake Pontchartrain has been planned for 1992 in which four times the number of 1991 sites will be sampled. In Mississippi Sound, total alkanes, tin, total PAHs, mean benthic abundance, and mean number of benthic species were spatially related (Table 6.25). Only bottom temperature was spatially dependent in Mobile Bay (Table 6.26).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	-0.617	0.5890
Benthic Species	-0.381	0.4096
Benthic Index	-0.393	0.4183
Fish Abundance	-0.671	0.6293
Habitat Indicators		
Instantaneous DO	-0.308	0.3554
Percent Light at 1 m	-0.310	0.3572
Bottom Salinity	-0.315	0.3609
Stratification	-0.311	0.3578
Bottom Temperature	-0.311	0.3578
Exposure Indicators		
Total Alkanes	-0.627	0.5969
Dieldrin	-0.636	0.6037
Total PAHs	-0.694	0.6461
Aluminum	-0.321	0.3652
Antimony	-0.388	0.4148
Arsenic	-0.454	0.4645
Cadmium	-0.390	0.4159
Chromium	-0.337	0.3768
Copper	-0.306	0.3544
Iron	-0.307	0.3547
Lead	-0.308	0.3559
Manganese	-0.695	0.6465
Mercury	-0.335	0.3755
Nickel	-0.466	0.4742
Silver	-0.350	0.3861
Tin	-0.403	0.4260
Zinc	-0.307	0.3547

Table 6.22 Spatial autocorrelation based on Moran's I for all probability-based sites within Galveston Bay, TX. ($p < 0.05$ = spatial dependency exists).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	-0.206	0.5118
Benthic Species	-0.102	0.3216
Percent Polychaetes	-0.212	0.5219
Percent Amphipods	-0.266	0.6220
Benthic Index	-0.054	0.2458
Fish Abundance	-0.155	0.4162
Habitat Indicators		
Instantaneous DO	-0.073	0.2742
Percent Light at 1 m	-0.292	0.6677
Bottom Salinity	-0.127	0.3653
Stratification	-0.042	0.2284
Bottom Temperature	-0.249	0.5922
Exposure Indicators		
Total Alkanes	-0.225	0.5477
Dieldrin	-0.163	0.4298
Total PAHs	-0.157	0.4188
Aluminum	-0.220	0.5370
Antimony	-0.311	0.6992
Arsenic	-0.068	0.2666
Cadmium	-0.070	0.2702
Chromium	-0.263	0.6176
Copper	-0.080	0.2855
Iron	-0.204	0.5071
Lead	-0.133	0.3759
Manganese	-0.173	0.4497
Mercury	-0.261	0.6127
Nickel	-0.168	0.4399
Silver	-0.234	0.5632
Tin	-0.154	0.4141
Zinc	-0.202	0.5042

Table 6.23 Spatial autocorrelation based on Moran's I for all probability-based sites within Lake Pontchartrain, LA. ($p < 0.05$ = spatial dependency exists).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	-0.111	0.3541
Benthic Species	-0.134	0.3902
Percent Polychaetes	-0.266	0.6096
Percent Amphipods	-0.142	0.4027
Benthic Index	-0.370	0.7638
Fish Abundance	-0.259	0.7166
Habitat Indicators		
Instantaneous DO	-0.260	0.5997
Percent Light at 1 m	0.021	0.1763
Bottom Salinity	-0.229	0.5493
Stratification	-0.247	0.5782
Bottom Temperature	-0.347	0.7317
Exposure Indicators		
Total Alkanes	-0.108	0.3491
Dieldrin	-0.087	0.3162
Total PAHs	-0.203	0.5042
Aluminum	-0.350	0.7359
Antimony	-0.320	0.6940
Arsenic	-0.308	0.6752
Cadmium	-0.279	0.6301
Chromium	-0.343	0.7264
Copper	-0.299	0.6625
Iron	-0.346	0.7315
Lead	-0.067	0.2875
Manganese	-0.172	0.4528
Mercury	-0.120	0.3678
Nickel	-0.350	0.7368
Silver	-0.114	0.3578
Tin	-0.302	0.6660
Zinc	-0.336	0.7166

Table 6.24 Spatial autocorrelation based on Moran's I for all probability-based sites within Chandeleur Sound, LA. ($p < 0.05$ = spatial dependency exists).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	0.231	0.0312*
Benthic Species	0.406	0.0027*
Percent Polychaetes	-0.099	0.4463
Percent Amphipods	-0.135	0.5219
Benthic Index	0.092	0.1284
Fish Abundance	-0.200	0.6523
Habitat Indicators		
Instantaneous DO	-0.356	0.8871
Percent Light at 1 m	0.131	0.0901
Bottom Salinity	-0.011	0.2754
Stratification	-0.123	0.4951
Bottom Temperature	0.032	0.2051
Eposure Indicators		
Total Alkanes	0.258	0.0226*
Dieldrin	0.022	0.2209
Total PAHs	0.216	0.0373*
Aluminum	0.144	0.0799
Antimony	-0.258	0.7561
Arsenic	-0.214	0.6799
Cadmium	-0.068	0.3824
Chromium	0.078	0.1447
Copper	0.097	0.1227
Iron	0.031	0.2079
Lead	0.158	0.0691
Manganese	-0.020	0.2905
Mercury	-0.090	0.4272
Nickel	0.049	0.1810
Silver	0.139	0.0836
Tin	0.202	0.0437*
Zinc	0.070	0.1540

Table 6.25 Spatial autocorrelation based on Moran's I for all probability-based sites within Mississippi Sound, MS/AL. (* $p < 0.05$ = spatial dependency exists).

Indicators	Moran's I	P-value
Response Indicators		
Benthic Abundance	-0.167	0.6787
Benthic Species	-0.034	0.3237
Benthic Index	-0.140	0.6106
Fish Abundance	0.001	0.2396
Habitat Indicators		
Instantaneous DO	-0.304	0.9227
Percent Light at 1 m	-0.037	0.3293
Bottom Salinity	-0.195	0.7458
Stratification	-0.292	0.9102
Bottom Temperature	0.203	0.0172*
Exposure Indicators		
Total Alkanes	-0.026	0.3240
Dieldrin	-0.061	0.4284
2,4'-DDT	-0.089	0.5176
4,4'-DDT	-0.145	0.6904
Total PAHs	-0.176	0.8122
Aluminum	-0.045	0.3783
Antimony	-0.057	0.4168
Arsenic	-0.071	0.4612
Cadmium	-0.073	0.4672
Chromium	-0.074	0.4712
Copper	-0.077	0.4783
Iron	-0.090	0.5223
Lead	-0.098	0.5465
Manganese	-0.099	0.5504
Mercury	-0.037	0.3541
Nickel	-0.084	0.5010
Silver	-0.069	0.4544
Tin	-0.106	0.5733
Zinc	-0.094	0.5360

Table 6.26 Spatial autocorrelation based on Moran's I for all probability-based sites within Mobile Bay, AL. (* $p < 0.05$ = spatial dependency exists).

6.4 NEED FOR REPLICATION OF BENTHIC GRABS

During the 1991 Louisianian Province Demonstration, replicates were used to potentially reduce site variation for all benthic response indicators (i.e., 3 grabs/site), RPD depth (3 measures per site), and fish response indicators (2 trawls/site at ITE stations and 2-3 trawls at sites exhibiting low abundance). A set of analyses were completed to assess the need for replication of benthic response indicators using total abundance and number of species as test cases.

A province-wide CDF for total benthic abundance is shown in Figure 6.45 depicting individual distributions for each replicate. With the exception of one outlier in grab 2 that extends abundance from about 900 to 1400 (i.e., one site), the CDFs for the replicates appear similar. This observation only means that the overall province-wide distribution does not change. It cannot be interpreted to mean that significant replicate differences at a site do not occur. Examination of the results of province-wide testing for significance of replicate differences for total abundance and the abundances of key benthic taxonomic groups showed that no replicate differences occurred in total abundance, but significant station differences were observed (Table 6.27). Of all the taxonomic groups examined, only amphipods showed a significant replicate effect. Significant station effects were observed for all taxonomic groups. Similarly no replicate effects were observed for total benthic abundance or taxonomic abundance in the three estuarine classes: large estuaries (Table 6.28), large tidal

rivers (Table 6.29) or small estuaries (Table 6.30). In large tidal rivers, station differences were not observed for the abundances of amphipods or gastropods suggesting that multiple sites would not be required to estimate these abundances for the large tidal river class.

Abundance only represents part of the assessment of the benthic community. However, if number of species also shows no replicate effect then the collection of multiple samples is not required to make province-wide or class-wide assessments. A province-wide CDF of number of benthic species by grab is shown in Figure 6.46 and depicts no differences in the number of benthic species due to replicate number.

This observation only means that the overall province-wide distribution of number of species does not change and cannot be interpreted to mean that significant replicate differences at a site do not occur. Examination of the results of province-wide testing for significance of replicate differences for total number of species and the number of species of key benthic taxonomic groups showed that no replicate differences occurred in total number of benthic species. However, significant station differences were observed (Table 6.31). Of all the taxonomic groups examined, only amphipods showed a significant replicate effect. Significant station effects were observed for all taxonomic groups. Similarly no replicate

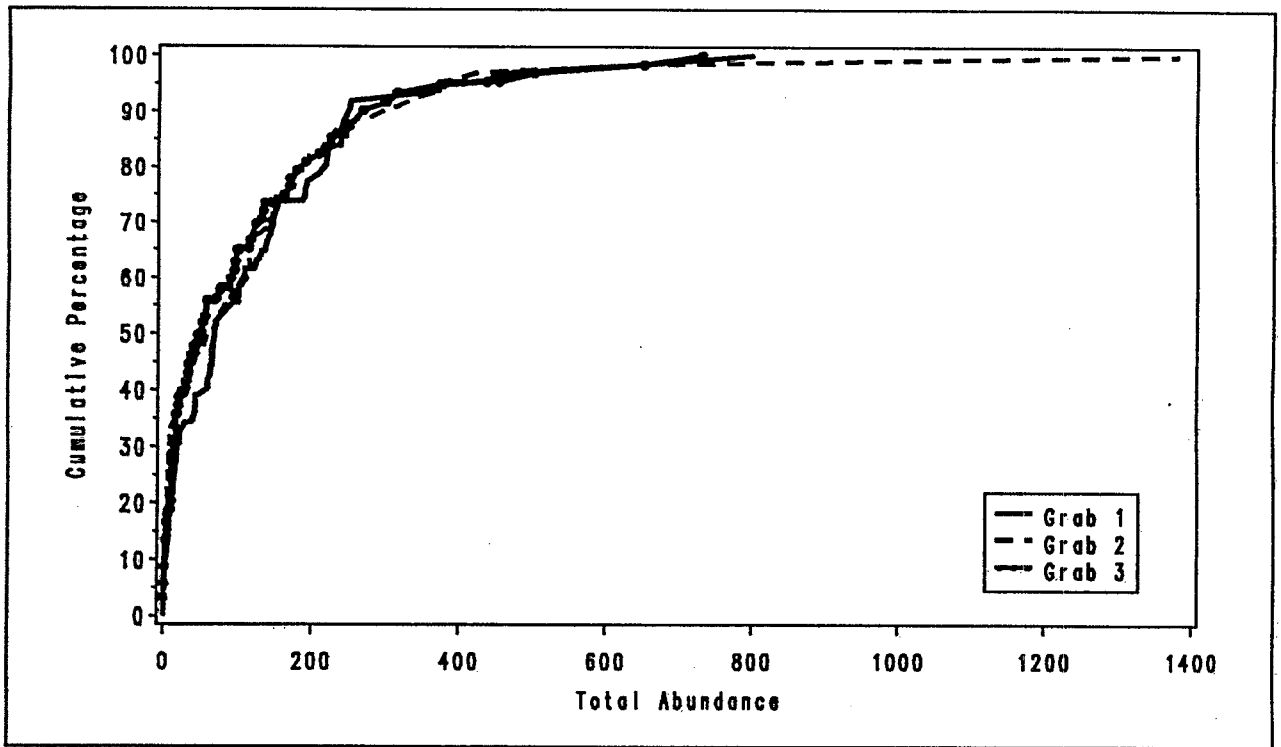

Figure 6.45 Cumulative distribution function for total benthic abundance associated with each benthic replicate for all probability-based sampling sites in the Louisianian Province in 1991.

effects were observed for total benthic abundance or generally for taxonomic abundance in the three estuarine classes: large estuaries (Table 6.32), large tidal rivers (Table 6.33) or small estuaries (Table 6.34). There were replicate differences among the grabs in large estuaries for the number of species of amphipods. In large tidal rivers, station differences were not observed for the abundances of amphipods or gastropods suggesting that multiple sites would not be required to estimate these abundances for the large tidal river class.

Evaluations of the redox potential discontinuity layer for each of the grabs showed no differences due to replicates but significant differences were observed for stations (Table 6.35). No replicate differences were seen for any of the three estuarine classes.

Benthic Indicator	Test Variable	P-Value
Total Abundance	Replicate Grab	0.621
	Station	<0.001*
Percent Abundance as:		
Amphipods	Replicate Grab	0.049*
	Station	<0.001*
Bivalves	Replicate Grab	0.367
	Station	0.001*
Capitellids	Replicate Grab	0.367
	Station	<0.001*
Decapods	Replicate Grab	0.783
	Station	<0.001*
Gastropods	Replicate Grab	0.404
	Station	<0.001*
Molluscs	Replicate Grabs	0.220
	Station	<0.001*
Polychaetes	Replicate Grabs	0.483
	Station	<0.001*
Spionids	Replicate Grabs	0.961
	Station	<0.001*
Tubificids	Replicate Grabs	0.576
	Station	<0.001*

Table 6.27 Results of ANOVA testing for differences in total benthic abundance and abundance by taxonomic group for benthic replicate grabs and stations for the Louisianian Province. (* $p < 0.05$ = significant difference due to test variable).

Benthic Indicator	Test Variable	P-Value
Total Abundance	Replicate Grab	0.406
	Station	<0.001*
Percent Abundance as:		
Amphipods	Replicate Grab	0.272
	Station	0.002*
Bivalves	Replicate Grab	0.432
	Station	0.001*
Capitellids	Replicate Grab	0.387
	Station	<0.001*
Decapods	Replicate Grab	0.366
	Station	0.004*
Gastropods	Replicate Grab	0.108
	Station	<0.001*
Molluscs	Replicate Grabs	0.140
	Station	<0.001*
Polychaetes	Replicate Grabs	0.542
	Station	<0.001*
Spionids	Replicate Grabs	0.980
	Station	<0.001*
Tubificids	Replicate Grabs	0.497
	Station	<0.001*

Table 6.28 Results of ANOVA testing for differences in total benthic abundance and abundance by taxonomic group for benthic replicate grabs and stations for the large estuarine class.(*$p < 0.05$ = significant difference due to test variable).

Benthic Indicator	Test Variable	P-Value
Total Abundance	Replicate Grab	0.638
	Station	<0.001*
Percent Abundance as:		
Amphipods	Replicate Grab	0.213
	Station	0.170
Bivalves	Replicate Grab	0.561
	Station	0.036*
Capitellids	Replicate Grab	0.582
	Station	<0.001*
Decapods	Replicate Grab	0.387
	Station	<0.001*
Gastropods	Replicate Grab	0.387
	Station	0.474
Molluscs	Replicate Grabs	0.554
	Station	0.036*
Polychaetes	Replicate Grabs	0.475
	Station	<0.001*
Spionids	Replicate Grabs	0.423
	Station	<0.001*
Tubificids	Replicate Grabs	0.597
	Station	<0.001*

Table 6.29 Results of ANOVA testing for differences in total benthic abundance and abundance by taxonomic group for benthic replicate grabs and stations for the large tidal river class. (* p < 0.05 = significant difference due to test variable).

Benthic Indicator	Test Variable	P-Value
Total Abundance	Replicate Grab	0.939
	Station	<0.001*
Percent Abundance as:		
Amphipods	Replicate Grab	0.194
	Station	<0.001*
Bivalves	Replicate Grab	0.406
	Station	<0.001*
Capitellids	Replicate Grab	0.868
	Station	<0.001*
Decapods	Replicate Grab	0.800
	Station	<0.001*
Gastropods	Replicate Grab	0.482
	Station	<0.001*
Molluscs	Replicate Grabs	0.614
	Station	<0.001*
Polychaetes	Replicate Grabs	0.824
	Station	<0.001*
Spionids	Replicate Grabs	0.398
	Station	<0.001*
Tubificids	Replicate Grabs	0.212
	Station	<0.001*

Table 6.30 Results of ANOVA testing for differences in total benthic abundance and abundance by taxonomic group for benthic replicate grabs and stations for the small estuary/small tidal river class. (* p < 0.05 = significant difference due to test variab

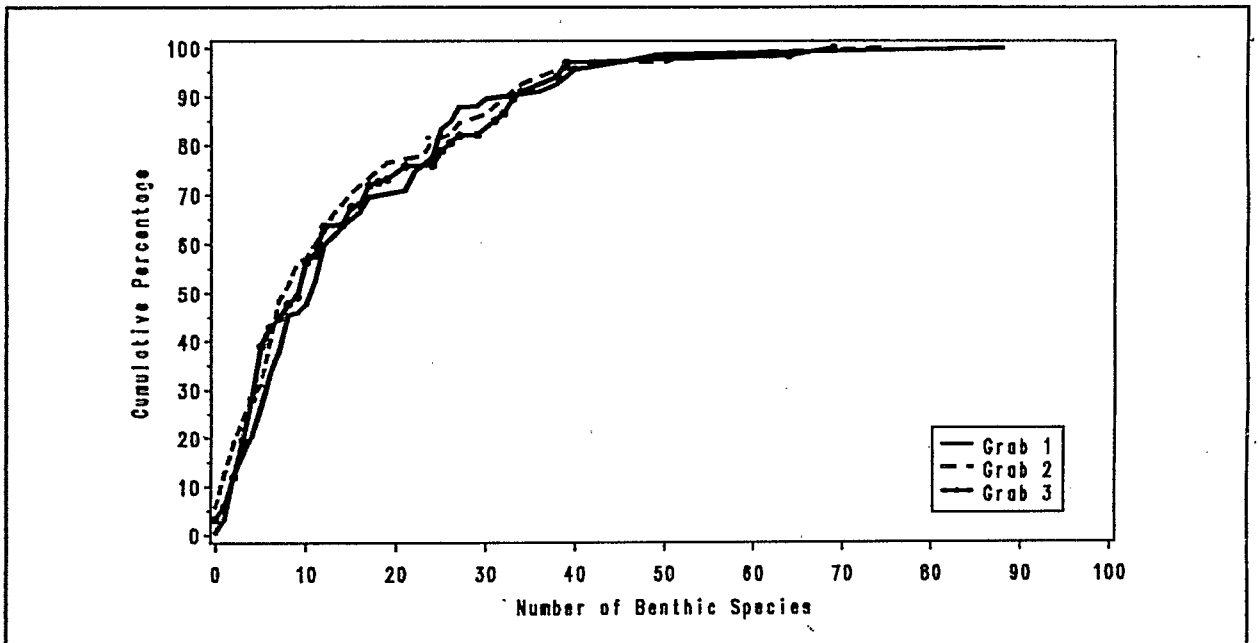

Figure 6.46 Cumulative distribution function for total number of benthic species associated with each benthic replicate for all probability-based sampling sites in the Louisianian Province in 1991.

Benthic Indicator	Test Variable	P-Value
Total Number of Species	Replicate Grab	0.574
	Station	<0.001*
Number of Species:		
Amphipods	Replicate Grab	0.019*
	Station	<0.001*
Bivalves	Replicate Grab	0.206
	Station	<0.001*
Capitellids	Replicate Grab	0.381
	Station	<0.001*
Decapods	Replicate Grab	0.386
	Station	<0.001*
Gastropods	Replicate Grab	0.834
	Station	<0.001*
Molluscs	Replicate Grabs	0.309
	Station	<0.001*
Polychaetes	Replicate Grabs	0.620
	Station	<0.001*
Spionids	Replicate Grabs	0.688
	Station	<0.001*
Tubificids	Replicate Grabs	0.977

Table 6.31 Results of ANOVA testing for differences in total number of benthic species and number of species by taxonomic group for benthic replicate grabs and stations for the Louisianian Province. (* p < 0.05 = significant difference due to test variab

Benthic Indicator	Test Variable	P-Value
Total Number of Species	Replicate Grab	0.091
	Station	<0.001*
Number of Species:		
Amphipods	Replicate Grab	0.014*
	Station	<0.001*
Bivalves	Replicate Grab	0.180
	Station	<0.001*
Capitellids	Replicate Grab	0.512
	Station	<0.001*
Decapods	Replicate Grab	0.774
	Station	<0.001*
Gastropods	Replicate Grab	0.414
	Station	<0.001*
Molluscs	Replicate Grabs	0.147
	Station	<0.001*
Polychaetes	Replicate Grabs	0.648
	Station	<0.001*
Spionids	Replicate Grabs	0.197
	Station	<0.001*
Tubificids	Replicate Grabs	0.632

Table 6.32 Results of ANOVA testing for differences in total number of benthic species and number of species by taxonomic group for benthic replicate grabs and stations for the large estuarine class. (* p < 0.05 = significant difference due to test vari

Benthic Indicator	Test Variable	P-Value
Total Number of Species	Replicate Grab	0.455
	Station	<0.001*
Number of Species:		
Amphipods	Replicate Grab	0.232
	Station	0.053
Bivalves	Replicate Grab	0.387
	Station	<0.001*
Capitellids	Replicate Grab	0.630
	Station	<0.001*
Decapods	Replicate Grab	0.387
	Station	<0.001*
Gastropods	Replicate Grab	0.387
	Station	0.474
Molluscs	Replicate Grabs	0.327
	Station	<0.001*
Polychaetes	Replicate Grabs	0.246
	Station	<0.001*
Spionids	Replicate Grabs	0.387
	Station	<0.001*
Tubificids	Replicate Grabs	0.573

Table 6.33 Results of ANOVA testing for differences in total number of benthic species and number of species by taxonomic group for benthic replicate grabs and stations for the large tidal river class. (* p < 0.05 = significant difference due to test vari

Benthic Indicator	Test Variable	P-Value
Total Number of Species	Replicate Grab	0.655
	Station	<0.001*
Number of Species:		
Amphipods	Replicate Grab	0.647
	Station	<0.001*
Bivalves	Replicate Grab	0.858
	Station	<0.001*
Capitellids	Replicate Grab	0.236
	Station	<0.001*
Decapods	Replicate Grab	0.205
	Station	0.006*
Gastropods	Replicate Grab	0.829
	Station	<0.001*
Molluscs	Replicate Grabs	0.874
	Station	<0.001*
Polychaetes	Replicate Grabs	0.249
	Station	<0.001*
Spionids	Replicate Grabs	0.751
	Station	<0.001*
Tubificids	Replicate Grabs	0.742

Table 6.34 Results of ANOVA testing for differences in total number of benthic species and number of species by taxonomic group for benthic replicate grabs and stations for the small estuary/small tidal river class. (* p < 0.05 = significant difference d

Estuarine Class	Test Variable	P-Value
Louisianian Province	Replicate Grab	0.232
	Station	<0.001*
Large Estuaries	Replicate Grab	0.566
	Station	<0.001*
Large Tidal Rivers	Replicate Grab	0.737
	Station	<0.003*
Small Estuaries/Small Tidal Rivers	Replicate Grab	0.322
	Station	<0.001*

Table 6.35 Results of ANOVA testing for differences in RPD depth (mm) for benthic replicate grabs and stations for the Louisianian Province and the three estuarine classes. (* $p < 0.05$ = significant difference due to test variable).

SECTION 7

CONCLUSIONS

This evaluation of indicators and design for the Louisianian Province is based on a single year of information; thus, it is subject to potential year-specific phenomena such as climate fluctuations (1991 was a high precipitation year), contaminant spills (a major oil spill occurred in Galveston Bay in late 1990), or year-class strengths (no deviations known). This assessment is preliminary and its findings should be confirmed by subsequent years of sampling in the Louisianian Province.

A companion report delineating a statistical summary of the 1991 results has been produced (Summers et al. 1993) and should be used if the reader is interested in the ecological status of the estuaries of the Louisianian Province. The following conclusions have been drawn from the monitoring data collected from the Louisianian Province in 1991 with regard to indicators and design:

Response Indicator Development

- An index of benthic community structure has been developed that effectively discriminated between sites of known hypoxia and sediment contamination and reference sites. The strength and validity of this index will be assessed using the 1992 monitoring data.

- A preliminary index of fish community structure has been developed that discriminated between sites of known hypoxia and sediment contamination and reference sites. While appearing statistical strong, this index produces considerable vague ecological conclusions. Significant additional effort is needed before this index is ready for general use.

Sensitivity of Indicators

- Benthic index values, benthic species diversity and number of species were sensitive indicators of ecological condition relating to hypoxia and sediment contamination.

- Number of finfish species and abundance/trawl were sensitive indicators of ecological condition in estuaries.

- Instantaneous and 24-hr continuous measures of bottom dissolved oxygen concentrations are indicative of hypoxic condition throughout the index period.

- Human use indicators and tissue contaminants in fish tissue were not indicative of ecological condition in estuaries where condition was defined as extent of hypoxia and sediment contamination.

- Degree of stratification and percent organic carbon content of sediments were habitat indicators that were strongly associated with ecological

condition.

- While mortalities in sediment bioassays averaged 15-20% higher in areas of hypoxia and sediment contamination than in reference sites, this difference was not statistically significant.

- Total alkanes, pesticides and heavy metals were significantly associated with sites having observed sediment contamination.

- Most PAHs and PCBs were not associated with observed sediment contamination.

- Most sediments that were contaminated had heavy metal contents exceeding the expected concentrations based on crustal aluminum.

- Significant longitudinal gradients (East-West) in the Louisianian Province existed for the number of fish species/trawl, minimum dissolved oxygen concentration, Secchi depth, acid volatile sulfides, total organic carbon in sediments, total and numerous specific alkanes, and several heavy metals.

Research Indicators

- Number of observed external fish pathologys/trawls were 2 to 3 times higher in regions of hypoxia and industrial contamination and a significant longitudinal gradient existed with western province fish having five times the pathologies observed in eastern province fish.

- The percent area occupied by splenic macrophage aggregates was 4 to 9 times greater in regions of hypoxia and sediment contamination than reference areas for pinfish and Atlantic croaker.

- The rate of vertebral deformities was an order of magnitude higher in western province estuaries than in eastern Louisianian Province with rates that were 3 to 7 times higher in areas of hypoxia and high industrial discharges.

- Selected blood chemistry compounds including c-reactive proteins were significantly higher in brown bullheads from heavily contaminated than in catfish from reference areas.

- Stable isotope and nutrient analysis in hypoxic areas indicated the high potential for eutrophic conditions resulting from algal production and decay.

Associations

- The benthic index was strongly associated with sediment contaminant levels and somewhat associated with dissolved oxygen concentrations, sediment toxicity, and habitat variation in RPD depth and salinity.

- When the benthic index was characterized as categories above or below the 4.1 criteria value, heavy metals were strongly associated with index classes in large and small estuaries; however, total alkanes were the primary contaminants associated with low index values in large tidal rivers.

- Presence of pesticides and selected PCBs, PAHs and heavy metals was associated with amphipod toxicity while the presence of heavy metals and pesticides was strongly associated with mysid toxicity.

- Significant associations exist between sediment contaminant concentrations and total organic carbon and acid volatile sulfides in the sediment.

Statistical Design

- Most response and exposure indicators showed no differences in distribution functions at the estuaries class level between index and randomly-placed sites.

- Paired comparisons showed many significant differences in response and exposure indicators between index and random sites in small estuaries and large tidal river segments.

- Significant spatial autocorrelation exists among values for benthic abundance, number of benthic species, water clarity, and bottom salinity.

- Significant spatial autocorrelation exists for most sediment contaminants.

- No significant differences were observed in benthic abundance or total number of species among three replicate benthic grabs for province-wide and estuaries class distributions.

- No differences were observed among three replicates of RPD depth for province-wide and estuarine class distributions.

- No differences were observed for finfish abundance or number of fish species in replicate trawls for province-wide or estuarine class distributions.

- No significant differences in the estimates of response and exposure indicators were observed at the large estuarine class-level between the base grid density and an enhanced density increasing the sample size by a factor of four; however, local or estuary-specific estimates were significantly different.

SECTION 8
REFERENCES

Agius, C. 1979. The role of melano-macrophage centers in iron storage in normal and diseased fish. J. Fish Dis. 2:337-343.

Agius, C. 1980. Phylogenetic development of melano-macrophage centers in fish. J. Zool., London 191:111-132.

Agius, C. and Roberts, R.J. 1981. Effects of starvation on the melano-macrophage centers in fish. J. Fish Biol. 19:161-169.

Altabet, M.A. and J.J. McCarthy. 1986. Vertical patterns in ^{15}N natural abundance in PON from the surface waters of several warm-core rings in the Sargasso Sea. J. of Mar. Res. 44:185-201.

Bell, S.S. and B.C. Coull. 1978. Field evidence that shrimp predation regulates meiofauna. Oecologia 35:141-148.

Bengtsson, A. and B. E. Bengtsson. 1983. A method to registrate spinal and vertebral anomalies in fourhorn sculpin, Myoxocephalus quadricornis L. (Pisces). Aquilo Ser. Zool.

Bengtsson, B.E. 1979. Biological variables, especially skeletal deformities in four fish for monitoring marine pollution. Philos. Trans. Soc. London. 286:457-464.

Bengtsson, B.E., A. Bengtsson and M. Himberg. 1985. Fish deformities and pollution in some Swedish waters. Ambio 14:32-35.

Bryant, V., D.S. McClusky, K. Roddie, and D.M. Newberry. 1984. Effect of temperature and salinity on the toxicity of chromium to three estuarine invertebrates (Corophium volutator, Macoma balthica, Nereis diversicolor). Marine Ecology - Progress Series. 20:137-149.

Carriker, M.R. 1967. Ecology of estuarine benthic invertebrates: A perspective. Pages 442-487. In: G.H. Lauff, ed. Estuaries, Publ. No. 83, 442-487. American Association for the Advancement of Science, Washington, D.C.

Chao, L.N. and J.A. Musick. 1977. Life history, feeding habitats, and functional morphology of juvenile scianid fishes in the York River estuary. Fish. Bull. 75:657-702.

Chapman, P.M. 1989. Current approaches to developing sediment quality criteria. Environmental Toxicology and Chemistry. 8:589-599.

Cifuentes, L.A., J. H. Sharp and M. L. Fogel 1988. Stable carbon and nitrogen isotope biogeochemistry in the Delaware estuary. Limnol. Oceanogr. 33:1102-1115.

Cloern, J.E. 1982. Does the benthos control phytoplankton biomass in South San Francisco Bay? Mar. Ecol. Prog. Serv. 9:191-202.

Coffin, R.B., L.A. Cifuentes, and P. Eldridge. 1992. The use of stable carbon isotopes to study microbial processes in estuaries. Stable Isotopes in Ecology (in press).

Cook, D. G. and R.O. Brinkhurst. 1973. Marine flora and fauna of the Northeastern United States, Annelida: Oligochaeta. NOAA Technical Report NMFS CIRC-374.

Ellis, A.E., Munro, A.L.S., and Roberts, R.J. 1976. Defense mechanisms in fish. I. A study of the phagocytic system and the fate of intraperitoneally injected particulate material in place (Pleuronectes platessa). J. Fish Biol. 8:67-78.

Ferguson, H.W. 1976. The relationship between ellipsoids and melano-macrophage centers in the spleen of turbot (Scopthalmus maximus). J. Comp. Path. 86:377-380.

Flint, R.W. and R. D. Kalke. 1985. Benthos structure and function in a south Texas estuary. Contributions in Marine Science. 28:33-53.

Fogel, M.L. and L.A. Cifuentes. 1992. Isotopic fractionation during primary production. In: M. Engel and S.A. Macko - Eds. Organic Geochemistry. Plenum Press (in press).

Fogel, M.L., L.A. Cifuentes, D.J. Velinski, and J.H. Sharp. 1992. The relationship of carbon availability in estuarine phytoplankton to isotopic composition. Mar. Ecol. Prog. Series (in press).

Fox, L.E. 1983. The removal of dissolved humic substances during estuarine mixing. Estuar. Coastal Mar. Sci. 16:431-440.

Fry, B. and E.B. Sherr. 1984. ^{13}C Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. Cont. Mar. Sci. 27:13-47.

Fry, B., S.A. Macko and J.C. Zieman. 1987. Review of Stable Isotopic Investigations of Food Webs in Seagrass Meadows, Florida Marine Research Publications.

Gaston, G.R., P.A. Rutledge, and M.L. Walther. 1985. The effects of hypoxia and brine on recolonization by macrobenthos off Cameron, Louisiana (USA). Contributions in Marine Science. 28:79-93.

Gaston, G.R. and J.C. Nasci. 1988. Trophic structure of macrobenthic communities in the Calcasieu Estuary, Louisiana. Estuaries. 11:201-211.

Grizzle, R.E. 1984. Pollution indicator species of macrobenthos in a coastal lagoon. Marine Ecology - Progress Series. 18:191-200.

Grossman, E.O. 1984. Carbon isotopic fractionation in live benthic foraminifera-comparison with inorganic precipitate studies. Geochim. Cosmochim. Acta 48:1505-1512.

Hamilton, S.J., P.M. Mehrle, F.L. Mayer and J. R. Jones. 1981. Mechanical properties of bone in channel catfish as affected by vitamin C and toxaphene. Trans. Am. Fish. Soc. 110:718-724.

Harper, D.E., L.D. McKinney, R.R. Salzer and R.J. Case. 1981. The occurrence of hypoxic bottom water off the upper Texas coast and its effects on the benthic biota. Contributions in Marine Science. 24:53-79.

Hedges, J.I. and D.C. Mann. 1979. The characterization of plant tissues by their lignin oxidation products. Geochim. Cosmochim. Acta 43:1803-1807.

Holland, J.S., N.J. Maciolek, and C.H. Oppenheimer. 1973. Galveston Bay benthic community structure as an indicator of water quality. Contributions in Marine Science. 17:169-188.

Holland, A.F., A.T. Shaughnessy, L.C. Scott, V.A. Dickens, J. Gerritsen, J.A. Ranasinghe. 1989. Long-term benthic monitoring and assessment program for the Maryland portion of Chesapeake Bay: Interpretive report. CBRM-LTB/EST-2. Prepared for Maryland Department of Natural Resources, Power Plant Research Program. Versar, Inc., ESM Operations, Columbia, MD.

Holligan, P.M., R.P. Harris, R.C.Newell, D.S. Harbour, R.N. Head, E.A.S. Linley, M.I. Lucas, P.R.G. Tranter and C.M. Weekley. 1984. Vertical distribution and partitioning of organic carbon in mixed, frontal and stratified waters of the English Channel. Mar. Ecol. Prog. Ser. 14:111-127.

Jurot, J.A., M.A. Poirrier, and T.M. Soniat. 1983. Effects of saltwater intrusion from the Inner Harbor Navigation Canal on the benthos of Lake Pontchartrain, Louisiana. Gulf Research Reports. 7:247-254.

Karr, J.R. and D.R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management. 5:55-68.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Special Publication 5, Illinois Natural History Survey, Champaign, IL.

Kemp, W.M. and W.R. Boynton. 1980. Influence of biological and physical processes on dissolved oxygen dynamics in an estuarine system: Implications for measurements of community metabolism. Estuarine and Coastal Marine Science 11:407-431.

Knapp, C.M., D.R. Marmorek, J.P. Baker, K.W. Thornton, J.M. Klopatek, and D.F. Charles. 1990. The indicator development strategy for the Environmental Monitoring Assessment Program. EPA 600/3-91/023. U.S. EPA Office of Research and Development, Washington, D.C.

Lee, S.H. and J.A. Fuhrman. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. Appl. Environ. Microbiol. 53:1298-1303.

Long, E.R. and L.G. Morgan. 1990. The potential for biological effects of sediment-sorbed contaminants tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52. US Department of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service, Rockville, MD.

Luna, L.G. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edition. McGraw-Hill, New York.

Macko, S.A. 1981. Stable nitrogen isotope ratiós as tracers of organic geochemical processes. Ph.D. thesis, Univ. of Texas at Austin. 181 p.

Mariotti, A., C. Lancelot, and G. Billen. 1984. Natural isotopic composition of nitrogen as a tracer of origin for suspended organic matter in the Scheldt estuary. Geochim Cosmochim. Acta 48:549-555.

Mayer, F.L., Jr., P.M. Mehrle, Jr. and W.P. Dwyer. 1977. Toxaphene: Chronic toxicity to fathead minnows and channel catfish. Ecol. Res. Ser. No. EPA-600/3-77-069. U.S. Environmental Protection Agency, Duluth, MN.

McClusky, D.S., V. Bryant and R. Campbell. 1986. The effects of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. Oceanography and Marine Biology Annual Review. 24:481-520.

McManus, J.W. and D. Pauly. 1990. Measuring ecological stress: variations on a theme by R.M. Warwick. Marine Biology. 106:305-308.

Mehrle, P.M., Jr., T.A. Haines, S.J. Hamilton, J.L. Ludke, F.L. Mayer, Jr., and M.A. Ribick. 1982. Relationship between body contaminants and bone development in east-coast striped bass. Trans. Am. Fish. Soc. 111:231-241.

Messer, J.J. 1990. EMAP indicator concepts. Chapter 2. In, C.T. Hunsaker and D.E. Carpenter (eds) Ecological indicators for the Environmental Monitoring and Assessment Program. EPA 600/3-90/060. U.S. Environmental Protection Agency, Office of Research and Development, Research Triangle Park, NC.

NOAA (National Oceanic and Atmospheric Administration). 1987. NOAA estuarine and coastal ocean science framework. U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Estuarine Program Office, Washington, D.C.

NOAA. 1988. Federal plan for ocean pollution research development, and monitoring: Fiscal years 1988-1991. Prepared by the National Ocean Pollution Program Office for the National Ocean Pollution Policy Board, Rockville, MD.

Officer, C.B., T.J. Smayda, and R. Mann. 1982. Benthic filter feeding: A natural eutrophication control. Mar. Ecol. Prog. Ser. 9:203-210.

Overton, W.S., D.L. Stevens, and D. White. 1991. Design report for EMAP, Environmental Monitoring and Assessment Program. Document in Review. U.S. EPA, Environmental Research Laboratory, Corvallis, OR.

Pearson, T.H. and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review. 16:229-311.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish. EPA/444/4-89-001. U.S. Environmental Protection Agency, Assessment and Watershed Protection Division, Washington, D.C.

Rabalais, N.N. 1990. Biological communities of the south Texas continental shelf. American Zoologist. 30:77-87.

Rakocinski, C., R.W. Heard, T. Simons and D. Gledhill. 1991. Macroinvertebrate associations from beaches of selected barrier islands in the Northern Gulf of Mexico: important environmental relationships. Bulletin of Marine Science. 48:689-701.

Reish, D.J. 1986. Benthic invertebrates as indicators of marine pollution: 35 years of study. Oceans 86. 3:885-888.

Rhoads, D.C. 1974. Organism-sediment relations on the muddy sea floor. Oceanogr. Mar. Biol. Ann. Rev. 12:263:300.

Rosenberg, R. 1977. Benthic macrofaunal dynamics, production, and dispersion in an oxygen-deficient estuary of West Sweden. Journal of Experimental Marine Biology and Ecology. 26:107-133.

Rygg, B. 1986. Heavy-metal pollution and log-normal distribution of individuals among species in benthic communities. Marine Pollution Bulletin. 17:31-36.

SAS Institute, Inc. 1989. SAS/STAT® User's Guide, Version 6, Fourth Edition, Volume 1. SAS Institute, Inc.:Cary, NC. 943 pp.

Sharp, J.H., Culberson, C.H., and Church, T.M. 1982. The chemistry of the Delaware Estuary. General considerations. Limnol. Oceanogr. 27:1015-1028.

Summers, J.K. and V.D. Engle. 1992. Evaluation of sampling strategies to characterize dissolved oxygen conditions in northern Gulf of Mexico estuaries. Environmental Monitoring and Assessment. In press.

Summers, J.K., J.M. Macauley, and P.T. Heitmuller. 1992. Environmental Monitoring and Assessment Program - Estuaries Component: Louisianian Province 1991 Demonstration Field Activities Report. EPA/ERL-GB No. SR-188. U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL.

Summers, J.K., J.M. Macauley, P.T. Heitmuller, V.D. Engle, A.M. Adams, G.T. Brooks. 1993. Annual Statistical Summary: EMAP-E Louisianian Province. 1991. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Gulf Breeze, FL EPA/600/R-93/001.

Thurman, E.M. 1985. Organic Geochemistry of Natural Waters. Martinus Nijhoff/Dr. W. Junk Publishers. 497 pp.

Tsutsumi, H., T. Kikuchi, M. Tanaka, T. Higashi, K. Imasaka, and M. Miyazaki. 1991. Benthic faunal succession in a cove organically polluted by fish farming. Marine Pollution Bulletin. 23:23-238.

Weisberg, S.B., J.B. Frithsen, A.F. Holland, J.F. Paul, K.J. Scott, J.K. Summers, H.T. Wilson, R. Valente, D.G. Heimbuch, J. Gerritsen, S.C. Schimmel, and R.W. Latimer. 1992. EMAP-Estuaries Virginian Province 1990 Demonstration Project Report. EPA/600/R-92/100. U.S. Environmental Protection Agency. Environmental Research Laboratory, Narragansett, RI.

Williams, B.K. 1983. Some observations on the use of discriminant analysis in ecology. Ecology. 64:1283-1291.

Wolke, R.E., Murchelano, R.A., Dickstein, C.D., and George, C.J. 1985. Preliminary evaluation of the use of macrophage aggregates (MA) as fish health monitors. Bull. Environ. Contam. Toxicol. 35:222-227.

U.S. GOVERNMENT PRINTING OFFICE: 1994 - 550-001/80333