EMAP/NS&T Pilot Studies in the Carolinian Province: Indicator Testing and Evaluation in the Southeastern Estuaries

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Notice

EMAP/NS&T Pilot Studies in the Carolinian Province: Indicator Testing and Evaluation in the Southeastern Estuaries

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SUMMARY

Indicator development studies were conducted during a pilot year program designed to evaluate existing EMAP indicators as well as to identify and develop new indicators of environmental quality for southeastern Atlantic estuaries. EMAP sampling activities were conducted at 24 stations throughout the Carolinian Province. Hydrolab Datasonde 3s were deployed in situ for $\geq$ 23 hr for water quality data (temperature, salinity, pH, dissolved oxygen, and depth). Sediment grabs were collected for infaunal community analyses. The top 2 cm of sediments from multiple grabs were composited and used for a variety of analyses (sediment contaminants, toxicity, sediment characterization). A limited suite of metals and PAHs were determined from sediment samples. Trawls were conducted for fish and shellfish community analyses.

The 24 stations were grouped into three categories on the basis of dissolved oxygen (DO) and sediment contaminant criteria. Nine "Reference" stations had little to no evidence of elevated sediment contaminants or DO stress. Nine "Degraded" stations were identified that were characterized by elevated sediment contaminants or low DO conditions. The remaining six stations were categorized as "Unknown" or "Uncertain" because they were clearly metal enriched so could not really be considered reference sites, but did not quite fit more stringent criteria for classification as degraded. These sites may represent potentially degraded sites that are experiencing early or low levels of stress. A three tiered classification scheme may aide in the development of bioindicator strategies that facilitate discrimination between areas experiencing early or chronic stress as well as those acutely impacted. Therefore, based on this scheme the efficacy of indicators for assessing habitat condition was evaluated.

Some indicators, such as dissolved oxygen, sediment contaminants, and benthic index have functioned as valuable core indicators in the Virginian and Louisianian Provinces. Studies in the Carolinian Province supported their continued use. Many estuarine environments experience fluctuations in habitat parameters such as dissolved oxygen and pH as well as salinity. Dissolved oxygen cycles, driven in part by tidal and diurnal processes, were frequently observed at reference as well as degraded sites. However degraded sites were characterized by greater magnitudes of fluctuations and increased frequency of the cycles. These conditions present a high potential for oxygen reperfusion damage. Although water quality parameters based on minima or mean values may help to identify some stressful conditions, they may underestimate the potential impacts of cyclical patterns. Relative criteria (based on range or rate functions) that rely less on absolute values were developed for DO and pH that may be more biologically relevant for cyclical systems.

Sediments are the primary sink for contaminants that are introduced into estuarine ecosystems, and are an important indicator of potential pollutant exposure. Exceedances of Long and Morgan ER-M values were observed at only three of the degraded sites, but all degraded sites (and some of the uncertain sites) had one or more exceedances of ER-L concentrations. When the sediment metals data were evaluated based on Al-normalization procedures, enrichment of multiple metals were observed at degraded and uncertain sites. Sediment criteria are established for each contaminant, but there are presently no provisions for

classifying stations with lower but enriched concentrations of multiple contaminants. Two approaches for making assessments based on multiple contaminants were described. Sites with 3 contaminants that exceeded ER-L values or 6 metal contaminants that were enriched based on Al normalization were classified as degraded. Sediment criteria based on the summed proportional metal concentrations (i.e. metal concentration divided by ER-L or ER-M concentration) were also presented.

Laboratory toxicity tests have been used in EMAP and other monitoring programs as indicators of potential impacts on the biota and as indirect indicators of contaminant bioavailability. The amphipod (*Ampelisca abdita*) assay was the primary test of potential toxicity for the Virginian and Louisianian Provinces. Amphipod toxicity, mysid toxicity, and Microtox assays were evaluated in this study. A new candidate indicator was also developed. In this assay, the effects of sediments on growth of juvenile seed clams (*Mercenaria mercenaria*) were evaluated. Although efforts are made during pilot year programs to sample in some of the most degraded sites (validated by high contaminant levels), amphipod and mysid toxicity assays did not discriminate these sites from reference sites. Microtox was more successful at discriminating between reference sites and degraded sites with an error rate of less than 10%. Although the seed clam assay was conducted at a limited number of sites, it also demonstrated a high potential for discriminating between reference and degraded conditions.

The abundances of fish and shellfish in the trawls did not provide an acceptable approach for discriminating reference from degraded sites. In fact, the results suggest that abundances and diversity are often high in even the most degraded sites. It is difficult to correlate the conditions at a site with mobile populations. Trawls are best conducted to collect specimens for contaminant analysis or disease status. The incidence of diseases in organisms caught in trawls is relatively rare, but still appeared to be a valuable indicator. It is recommended that the disease status of shrimps and crabs should be tallied as well as fish. In the southeast, the invertebrate species are common components of trawls, and both diseases (shell disease in blue crabs and cotton disease in shrimp) are relatively easy to identify by field crews. Moreover they are important commercial species.

Studies were also conducted in which organisms were deployed *in situ* at some of the sites. Hatchery-reared oysters (*Crassostrea virginica*) were deployed at sites in the Charleston Harbor area, and the effects on growth, bioaccumulation of contaminants, and disease status were evaluated. Oysters deployed at degraded sites had significantly slower growth rates, greater bioaccumulation of metal contaminants, and increased incidence of Dermo (a bivalve disease caused by a protistan parasite). Although this approach may not always be feasible for large scale monitoring programs, it is certainly amenable for regional studies. Furthermore, it is particularly valuable as a means of validating how well laboratory toxicity assays represent field conditions, i.e. whether the effects observed in laboratory assays are likely to reflect reduced fitness of field populations.

The assemblages of organisms associated with the benthos were described. Simple parameters, abundance and species richness, demonstrated some capacity for discriminating

between reference and degraded sites. These data will be combined with 1994 data to develop a benthic index based on functionality that is expected to have greater powers of discrimination.

Mummichog (*Fundulus heteroclitus*) eggs were also deployed at various sites in SC and GA in order to evaluate the effects on reproductive success. No significant differences were observed in the response variables (embryo survival, hatching rates, length and weight of hatchlings).

The pilot year studies served to identify indicators that can discriminate reference from degraded sites in southeastern estuaries. Many of the methods and approaches used in the Virginian and Louisianian Provinces were used successfully in the Carolinian Province. The utility of some indicators (DO and pH) was extended by considering range criteria as well as absolute minima. The amphipod sediment toxicity assay was found to be the weakest component. It is uncertain if the failure of the *Ampelisca abdita* assays was due to the perceived less toxic nature of southeastern sediments or low sensitivity of this species. No single assay is likely to ever be ideal, so at least two types should be employed to eliminate potential errors due to false positives and false negatives. An effort was made to use some criteria for the identification of sites that suffer from chronic stress as well as acute conditions. Microtox, seed clam assays, multiple metals, and range/rate criteria were discussed toward this aim, and there are certainly others that warrant development. Some means of incorporating the significance of multiple stressors into assessments of habitat condition must be developed.

Acknowledgments

Authors' Note

At the time of the publication of these studies as a NOAA document, the conclusions and recommendations of this study have changed in a few cases as we have continued to develop and evaluate the indicators. The seed clam assay has gone through substantial methods development and the definitive protocols are different from the methods described in this report. We have continued to work towards the development of toxicity criteria for Microtox (particularly given the strong sediment bias associated with the test), and have developed relative as well as absolute criteria that are not reported here. Although the contaminant analyses were conducted in a careful and conscientious manner, there is insufficient QA/QC documentation to confirm the reliability of these data. Therefore, these data should only be used to make relative station-to-station comparisons, not absolute statements. Similarities between sediment metal concentrations at some sites that were also evaluated during the 1994 Demonstration Project (analyses conducted at Texas A&M) indicate that the data are reasonable estimates.

CHAPTER I. INTRODUCTION

FRAMEWORK FOR INDICATOR DEVELOPMENT

Estuaries provide critical feeding, spawning, and nursery habitats for numerous species, including commercially and recreationally important fish, shellfish, and waterfowl. These important ecosystems are also the repositories for pollutants associated with urbanization through industrial discharges, watershed drainage, and aerial fallout. Natural hydrodynamic and chemical processes facilitate retention of contaminants. The most acute effects are restricted to limited areas such as those adjacent to the "end of the pipe" or in depositional zones. However, the chronic effects of elevated contaminants may occur over a broader region and are manifested as subtle effects on fitness components such as growth, reproduction, and disease resistance. The net response to an array of multiple stressors represents tradeoffs between various energetic components, as energy allocated for normal metabolic processes is reallocated for activation of compensatory mechanisms. Moreover, the ability of organisms to tolerate environmental insults is often exacerbated by natural fluctuations in salinity, dissolved oxygen concentrations, etc., and by other pressures such as overharvesting and habitat loss.

The incidence and scale of coastal environmental problems have increased over the past several decades. The public is increasingly concerned that the coastal resources upon which they rely for food, recreation, and economic livelihood remain sustainable (Morganthau, 1988; Toufexis, 1988). Monitoring and assessment information covering a range of spatial (national, regional, system-specific) and temporal (long- and short-term) scales that characterize ecosystem status is vital to the protection and restoration of estuarine and coastal ecosystems (Wolfe et al., 1987; NRC, 1990).

In recognition of the need for better environmental surveillance, EPA planned and implemented EMAP (Environmental Monitoring and Assessment Program). EMAP is a comprehensive nationwide program designed to: (1) estimate the status and trends in condition of the nation's ecological resources on a regional basis; (2) identify associations between human-induced stress and ecological condition; and (3) provide periodic statistical summaries and interpretative reports on status and trends to environmental managers and the public. Questions that EMAP has been designed to answer include: What is the current extent of ecological resources and how are they distributed geographically? What proportions of these resources are in acceptable condition? What proportions are degrading or improving, in what regions, and at what rates? Are changes in ecological resources correlated with pollution exposure and other environmental stressors? Are degraded resources improving in response to pollution control or other environmental management actions?

EMAP was implemented in the Virginian Province (northeast region) in 1990 and in the Louisianian Province (Gulf of Mexico region) in 1991. Extension into the Carolinian Province

(southeast region) began during 1993 with the pilot studies described in this report, and full implementation is slated for the summer of 1994. In the process of ecological indicator development for the Carolinian Province, we have the advantage of the experiences and information from the other near coastal provinces. The approach in the Carolinian Province is based on the indicator framework previously developed in the Virginian and Louisianian Provinces. EMAP-Estuaries does not have the resources to monitor all of the ecological parameters of concern. Therefore, the limited resources available must be focused on the system attributes that are of greatest concern ecologically, and best address program objectives. Indicator data should be comparable with those from other provinces and contribute to a national assessment of the environmental condition of estuarine resources. The environmental conditions of Carolinian Province estuaries differ in many ways from those of the Virginian and Louisianian Provinces. Contaminant inputs into Carolinian Province estuaries have historically been less than in other regions. The depths and tidal variations may also be different enough to require methodological modifications. Therefore, more sensitive or innovative indicators may be required to accurately estimate the extent of anthropogenic impacts on southeastern estuarine systems.

Other goals of indicator development and assessment components are to extend the ability to discriminate between anthropogenic stress and natural stress, and to expand the interpretive power or methodologies for the identification of those systems that are showing signs of chronic or early stress. The mutual advantages of EMAP for addressing needs of other users (such as local or state management agencies) provide an impetus to collect data in a manner that will maximize the potential information. Results from the Virginian and Louisianian studies indicate that the present methodologies can be used to effectively discriminate between highly degraded and undegraded areas. It is not so clear that the present methodologies are effective for identifying those areas which are experiencing less severe, more chronic stress, which if unmitigated may progress towards more severe status. From a management point of view these systems may be more responsive to early intervention, perhaps less costly, strategies.

The selection of indicators is an ongoing process. The tiered model previously used for indicator selection strategy continues to be used as the operational model (Figure 1) (Summers et al., 1990). In this model candidate indicators that are consistent with EMAP goals are evaluated by research activities designed to determine their effectiveness at discriminating between degraded and undegraded environmental conditions. Those that are promising at the research level are then elevated to developmental status. At this stage indicators are evaluated more critically with regard to the feasibility of regional-scale application and value of the data. Finally, those indicators that meet the criteria for regional-scale implementation and demonstrate significant power to discriminate between degraded and undegraded sites are designated as core indicators. Indicators that are selected and developed should:

- Relate to ecological condition in a way that can be quantified and interpreted.
- Apply across a range of habitats.
- Be a concern of and valued by society.

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- Regional data interpretable within conceptual model

- Provides new, important insights not available from existing programs

- Cost in proportion to value of insights

- Responsive to stressors on a regional scale

- Methods believed feasible on a regional scale

CORE

DEVELOPMENTAL

- Important within the conceptual model

- Responsiveness demonstrated in lab or small-scale field study

- Low incremental cost

RESEARCH

CANDIDATE

- Not responsible to stressors of concern

- Redundant with superior measures

- Not measurable on an EMAP frame

- Temporarily unstable withing the index period

REJECTED

Figure 1. Model of evaluation criteria used for indicator selection strategy, from Summers et al., 1990.

Furthermore, indicators should possess the following attributes:

- Quantifiable in a standardized manner with a high degree of repeatability.
- Balanced sensitivity, i.e. be sufficiently sensitive to enable identification of stressful conditions, but not hypersensitive to natural environmental variables or sampling methodologies.
- Methods that can be applied on a regional scale, incorporating local modifications.
- Balanced costs, i.e. minimal incremental costs but high insight value.

Parameters that serve as indicators of ecological condition have been organized into various categories. Some confusion results from the fact that the nomenclature has gone through various modifications and the Virginian and Louisianian Provinces are using slightly different category schemes (Summers et al., 1993; Macauley et al., 1994; Schimmel et al., 1994). The scheme described below is based on that used by the Louisianian Province, but the older terminology and how it relates to the current terms will also be defined briefly. Regardless of the categories, the indices and data are collected in a consistent manner.

- **Habitat Indicators.** Habitat indicators describe the physical and chemical conditions of sample sites, and provide basic information about the overall environmental setting. Examples include depth, salinity, temperature, and sediment characteristics (such as grain size, percent water, percent silt/clays, total organic carbon), pH, water clarity, etc. Habitat indicators are frequently used to normalize exposure and response indicators across natural environmental gradients.
[*The Habitat indicator category has been used in older schemes and in both provinces, although the parameters have varied occasionally. For example, the Virginian Province places total organic carbon in the category of Abiotic Condition Indicators (i.e. Exposure Indicator) whereas the Louisianian Province keeps total organic carbon as a Habitat Indicator.*]

- **Exposure Indicators.** Exposure indicators provide measures of the magnitude and extent of pollution exposure. Measures of potential pollutant exposure include physical, chemical, and biological parameters that quantify pollution exposure, habitat degradation, or other causes of degraded ecological condition. Measurements related to this category include dissolved oxygen concentrations, sediment toxicity, and sediment contaminant concentrations.
[*Exposure indicator corresponds to the category referred to as Abiotic Condition Indicator (used most recently by the Virginian Province).*]

- **Biotic Condition Indicators.** Biotic Condition indicators are characteristics of the environment that provide quantitative evidence of the status of ecological resources and biotic integrity. These measurements quantify the integrated response of ecological resources to individual and multiple stressors. Measurements related to this category are benthic community parameters, fish and shellfish community parameters, incidences of gross pathology or disease, and tissue concentrations of contaminants.
[*Biotic Condition Indicator corresponds to the category previously referred to as Response Indicators, and is presently used in both the Louisianian and Virginian Province.*]

•   **Stressor Indicators.**   Stressor indicators are economic, social, engineering, and landscape measures that can be used to estimate pollutant loadings to coastal waters and identify their sources. Examples include land use patterns, point source discharge estimates, freshwater inflows, and pesticide use along a watershed. These parameters are not measurable as part of the annual EMAP sampling efforts but represent data derived from other agencies.

Associations between biotic condition indicators and exposure indicators serve to validate conclusions and increase the likelihood of appropriate site classification. Statistical analyses should be conducted to identify significant correlations. Significant correlations should not be used to imply causations, but can be used by researchers to develop and test hypotheses regarding causations. It is important that parameters from each category are measured so that a comprehensive characterization is used for site classification. Valid site classification is essential to the development of regional-scale assessments regarding the status of estuarine resources.

OVERVIEW OF PILOT YEAR INDICATORS

Indicator development studies were conducted during the pilot year to evaluate existing indicators as well as to identify and develop new indicators of environmental quality for southeastern Atlantic estuaries. Major tasks were:
•   Assessment of the degree to which the indicators of ecological condition used in the Virginian and Louisianian apply to the presumed less-polluted southeastern estuaries.
•   Refinement and adaptation of sampling methods used by EMAP in the Virginian and Louisianian Provinces so that they are appropriate for southeastern estuaries.
•   Identification and preliminary evaluation of "new" environmental quality indicators that are appropriate for the unique physical settings and presumed low levels of degradation characteristic of southeastern estuaries.
•   Identification of a suite of indicators for sampling during the 1994 Carolinian Province Demonstration Project.

The indicators that were measured during pilot year studies are listed in Table 1. Their status as core, research, developmental, or candidate is also noted. Twenty-four stations were sampled throughout the Carolinian Province during the summer of 1993 (Figure 2). A variety of habitat types were sampled, sandy as well as silty from various salinity regimes (Table 2). Sampling strategies during pilot year studies deviate from the random sampling design used during full implementation of EMAP.   The sites were chosen based on a priori assumptions of site classification. Presumed degraded sites were identified based on previous data, or potential sources of contaminant inputs. Selection of reference sites was based on the apparent absence of pollutant inputs.

EMAP sampling activities were conducted at each station. The methods were adapted from those described in the Field Operations Manual of the Louisianian Province (Macauley,

Table 1. List of indicators evaluated during pilot year studies in the Carolinian Province, summer 1993. Their status as Core (C), Developmental (D), Research (R), or Candidate (Ca) is noted.

INDICATOR CATEGORY	SPECIFIC INDICATOR	STATUS
HABITAT	Salinity	C
	Temperature	C
	pH	C
	Depth	C
	Sediment Characteristics	C
EXPOSURE	Dissolved Oxygen Concentrations	C
	Sediment Contaminants	D
	Amphipod Toxicity	D
	Microtox®	R
	Seed Clam Toxicity	Ca
BIOTIC CONDITION	Benthic Species Composition	C
	Fish and Shellfish Assemblages	D
	Gross Pathology of Fish and Shellfish	D
	Oyster Growth, Contaminant Bioaccumulation, Disease Status	Ca
	Fundulus Reproductive Success	Ca

Figure 2. Map of sites sampled during pilot year studies (summer, 1993) in the Carolinian
Province. The site numbers correspond to the numbers in Table 2.

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Table 2. List of stations sampled in the Carolinian Province during pilot year studies conducted during the summer of 1993. Latitude and longitude coordinates are expressed as degrees:minutes:hundredths. Key habitat characteristics (mean depth and salinity range) are also listed. The numbers preceeding the station name correspond to the station numbers in Figure 2.

STATION	STATION CODE	STATE	N. LAT.	W. LON.	DEPTH (m)	SALINITY RANGE
1. Slocum Creek	SLO	NC	34:00:10	75:51:14	0.8	8.9 - 9.6
2. Clubfoot Creek	CFT	NC	34:02:19	77:39:06	0.8	19.4 - 19.9
3. Whiskey Creek	WKY	NC	34:84:91	77:55:18	0.5	28.5 - 35.4
4. Masonborough Inlet	MAS	NC	34:04:37	77:55:26	0.5	30.0 - 35.9
5. Brickyard Creek	BKY	SC	32:50:09	80:00:21	2.6	10.3 - 20.9
6. Detyens (Wando R.)	DEY	SC	32:55:34	79:49:59	3.0	19.0 - 21.6
7. Drayton (Ashley R.)	DYT	SC	32:51:00	80:02:96	1.9	11.5 - 19.2
8. Fort Johnson	FTJ	SC	32:45:14	79:54:17	8.4	22.1 - 31.4
9. Breech Inlet	INL	SC	32:46:55	79:48:85	3.5	24.6 - 35.4
10. Koppers (Ashley R.)	KOP	SC	32:49:03	79:57:44	5.0	18.9 - 23.2
11. Plum Island	PLM	SC	32:45:82	79:56:52	8.5	24.7 - 29.3
12. Shipyard Creek	SPY	SC	32:50:16	79:56:37	1.5	15.1 - 22.0
13. Lighthouse Creek	LTH	SC	32:41:80	79:53:65	0.5	31.6 - 32.2
14. South Santee River	SAN	SC	33:09:22	79:19:62	3.0	10.6 - 22.7
15. Sampit River	SAM	SC	33:21:31	79:17:11	5.0	12.4 - 18.8
16. South Edisto River	SED	SC	32:37:21	80:25:37	5.7	5.0 - 16.6
17. Savannah River	SAV	GA	32:08:07	81:08:08	5.3	1.2 - 5.2
18. Ogeechee River	OGE	GA	31:55:61	81:15:33	4.2	3.2 - 18.1
19. Brunswick River	BRU	GA	31:11:36	81:31:31	1.5	27.0 - 28.3
20. Sapelo Island	SAP	GA	31:25:00	81:17:54	6.0	27.0 - 30.0
21. Nassau River	NAS	FL	30:34:75	81:38:51	5.5	15.1 - 18.5
22. Ortega River	ORT	FL	30:16:08	81:43:38	0.5	3.6 - 6.1
23. Titusville (Indian R.)	TIV	FL	28:41:62	80:48:79	6.9	30.9 - 31.6
24. Eau Gallie (Indian R.)	EAU	FL	28:07:59	80:37:02	1.5	27.9 - 28.3

∞

1993), and the basic components are briefly described as follows. Hydrolab Datasonde 3s were continuously deployed in situ for $\geq$ 23 hr for water quality data (temperature, salinity, pH, dissolved oxygen, and depth). Sediment grabs were collected for infaunal community analyses. The top 2 cm of sediments from multiple grabs were composited and used for a variety of analyses (sediment contaminants, toxicity, sediment characterization). A limited suite of metals and PAHs were determined from sediment samples. Trawls were conducted for fish and shellfish community analyses.

After completion of the sampling and data collection, the final classification of the sites and subsequent evaluation of the indicators were based on a posteriori assessment of dissolved oxygen and contaminant data (sediment contaminant results and dissolved oxygen data will be discussed in more detail in the chapters that follow). Since the classification of sites is necessary before the efficacy of indicators can be tested, and is a recurrent theme throughout, it is appropriate to describe the rationale and classification at this point. In most cases the a posteriori classification agreed with the a priori assumptions.

The criteria that were used to classify sites as reference or degraded are listed in Table 3. The first criterion is exceedance of one or more ER-M values (Long and Morgan, 1990). The ER-M concentrations are defined as probable effects concentrations, those that are likely to cause acute as well as chronic effects. Long and Morgan (1990) also defined ER-L values or possible effects concentrations which may cause chronic effects or even acute effects with sensitive organisms. Although sediments containing only one contaminant exceeding ER-M criteria could have a high probability of causing toxicity, the effects of a single exceedence of an ER-L value is less predictable. However, multiple occurrences of contaminants exceeding ER-L values increase the potential for toxicity due to additive or synergistic effects. Therefore, in order to take into account the potential effects of multiple contaminants, a site was classified as contaminated if sediments contained three or more contaminants that exceeded ER-L values.

Sediment metal concentrations were also evaluated using aluminum normalization procedures (Windom et al., 1989; Hanson et al., 1993). These analyses indicated a more widespread pattern of metal enrichment at virtually all degraded sites. Because many of these concentrations fell below ER-L values, the presence of six or more metal contaminants that were enriched was arbitrarily chosen as a criterion for classification as degraded.

Criteria for sites experiencing anoxic or hypoxic conditions are also listed, and are consistent with dissolved oxygen indicators used in the Virginian and Louisianian Provinces. A less stringent criterion of < 5 mg/L was not applied at this phase (although it will be re-introduced later). It became apparent in preliminary studies conducted in creeks in the Charleston Harbor area that DO levels of < 5 mg/L occurred on a routine basis. Dissolved oxygen will be discussed more extensively in Chapter 3, where some new approaches for evaluating the potential physiological significance of cyclical patterns are introduced.

On the basis of the criteria listed in Table 3, the stations were classified as reference, degraded, or uncertain (Table 4). The first nine stations listed represent the reference sites (R),

validated by the lack of elevated contaminants or evidence of DO stress. The last nine stations represent degraded stations (D) based on the above criteria. The six stations listed in the middle represent stations that are clearly metal enriched, but do not quite fit more stringent criteria (Long and Morgan values) for classification as degraded, and can not really be considered reference sites. These sites will be referred to as unknowns or uncertains (U) and may represent potentially degraded sites that are experiencing early or low levels of stress. A three tiered classification scheme may aide in the development of bioindicator strategies that facilitate discrimination between areas experiencing early or chronic stress as well as those acutely impacted. Therefore, based on this scheme the efficacy of indicators for assessing habitat condition was evaluated.

Table 3. Sediment contaminant and dissolved oxygen criteria used to classify stations that were sampled during 1993 pilot year studies in the Carolinian Province as degraded.

■ Sediment Contaminants
- one or more contaminants exceeded ER-M values
- three or more contaminants exceeded ER-L values
- six or more elevated metals (based on Al normalization)

■ Dissolved Oxygen
- < 0.3 mg/L at any time during sampling
- < 1.0 mg/L 10 percent of time
- < 2.0 mg/L 20 percent of time

In the chapters that follow, the indicators listed in Table 1 will be discussed, and the findings will be evaluated with regard to indicator robustness. A robust indicator should possess those attributes described earlier (i.e. balanced sensitivity, applicability over a range of habitat types, and high reliability for discriminating between reference and degraded sites, etc.). From these studies, ecological indicators for assessing the environmental quality of southeastern estuarine ecosystems and coastal regions will be identified.

Table 4. Classification of stations (based on contaminant and dissolved oxygen criteria listed in Table 3) sampled during pilot year studies in the Carolinian Province, summer 1993. The first nine stations are Reference (R) sites, the next six stations are of Uncertain (U) status, status, and the last nine stations are Degraded (D).

	STATION	> ER-L	> ER-M	ELEVATED METALS
R	MAS			
	INL			
	FTJ			
	DYT			
	LTH			
	OGE	BaP		Zn
	SAP			
	NAS			
	TIV	Fle, Chy		
U	WKY			Cr, Pb, Cu, Zn
	DEY			Pb, Cu, Zn
	BKY	BaP		Cr, Cd, Pb, Cu, Zn
	PLM			Cr, Pb, Cu, Zn
	BRU	BaP		Cr, Pb, Cu, Zn
	EAU			Cr, Pb, Cu, Zn, Mn
D	CFT	As, BaP		Cr, Cd, Pb, Cu, Zn, As
	SLO	Cd, Pb, Zn		Cr, Cd, Pb, Cu, Zn, Mn
	SAM	As		Cr, Pb, Cu, Zn, Mn, As
	SAN	Pyr	BaA	
	SPY	As, BaP, Chy, Pyr		Cr, Pb, Cu, Zn, Mn, As
	KOP	As, Ant, Chy, Fla	BaA, BaP, Pyr	Cr, Pb, Cu, Zn, Mn, As
	SED*	Fle, Chy		
	SAV*	As, BaP, Pyr		Cr, Pb, Cu, Zn, Mn, As
	ORT	Cu, BaP, Fla, Pyr	BaA, Phe, Pb, Zn	Cr, Pb, Cd, Cu, Zn

* Stations that are degraded based on Dissolved Oxygen criteria listed in Table 3.

CHAPTER 2. HABITAT INDICATORS

INTRODUCTION

Water quality parameters and sediment characteristics provide important information about the environmental setting of the sites. These parameters are often very important for normalizing other indicators to environmental gradients. Grain size, percent silt-clays and organic content of sediments, salinity, and temperature are important environmental parameters that affect the distribution and species composition of biota (Remane and Schlieper, 1971). Other environmental variables such as pH, sulfides, and redox potential also affect numerous physical and biological processes, such as contaminant availability and the ability of organisms to compensate physiologically. Interactions between variables can result in effects that may be predictable or stochastic.

Most of the habitat variables are unknowns until after sample collection and analyses are completed. Many of them vary in a fairly predictable manner with salinity, and salinity can be verified at the time of sampling. Therefore, an effort was made to sample in a range of habitat types, so low salinity (< 15 ppt) as well as high salinity (> 15 ppt) sites in both degraded and reference stations were selected.

METHODS

Salinity, temperature, pH, and water depth are measured readily with the Hydrolab Datasonde 3s. The deployment and mooring systems used in the Louisianian Province were used successfully during 1993 pilot year studies at deep sites (Figure 3). In more shallow water environments (approximately 3 meters), a pole deployment system was also developed and used successfully in these systems, in both high current as well as slower flow regimes (Figure 3). Datasondes were deployed for a minimum of 23 hours at all sites and for $\geq$ 24 hours at 20 of 24 sites. A LoFlow membrane was used for the DO probe as recommended for measurements in waters characterized by high sediment loads and variable flow regimes.

Sediments were collected using a 1/25-m^2 stainless steel Young-modified Van Veen grab sampler. Samples for % silt-clays were taken as a 50-ml core from the first grab sample. The other sediment characteristics were performed with samples taken from the sediment composite, which was composed of the top 2 cm of approximately 8 to 10 grabs.

Sediment characteristics (water content, % silt-clays, and grain size) were determined using standard EMAP protocols (EMAP, Laboratory Methods Manual, 1993). Minor modifications with regards to replication were incorporated. Although EMAP protocols require only 1 replication, 3 replicates were analyzed, and the means and standard deviations were computed. The percent water content of sediments was calculated as a loss in weight after

A

Shallow Deployment

Pinger

DataSonde 3

B

Float

Float

Deep Deployment

Pinger

Concrete Weight

Figure 3. Datasonde Deployment Methods. Two methods for deployment of Datasondes are shown for: (A) shallow water habitats, and (B) deep water habitats. In both cases, the Datasonde is first secured inside a protective PVC tube, and is equipped with a pinger for echo-location if needed.

drying, and the values were corrected for salt content. These dried sediment samples were then ashed in a muffle furnace at 500°C for 4 hours. Percent total organics were then computed as the loss in wet after ashing divided by dry weight. Means and standard deviations were computed for the 3 replicates.

For silt-clay analyses, sediment samples were first dispersed with sodium hexametaphosphate and sieved through a 63-um screen. Sediments retained on the screen were dried and weighed, and a 40-ml subsample of the filtrate was then dried and used to estimate % silt-clays. Although EMAP only requires one 40 ml subsample, 3 subsamples were taken and averaged. Because these all came from the same sediment sample, they represent pseudo-replicates. Therefore the average number was used to estimate % silt-clay, but no standard deviations were computed.

Grain size distributions were determined according to EMAP protocols. In these methods, the sand fraction (> 63 um) is not separated further for stations with silt-clay values greater than 80%, and a full pipette analysis (Plumb, 1981) is not performed for stations with silt-clays of < 20%. Therefore, silt-clay percentages are used to determine which method of grain size analyses is conducted. However, the sample for silt-clays was different from the other sediment characteristic samples, because it was taken as a core from the undisturbed grab, rather than from the composite sample. This created a potential for errors regarding the choice of method for grain size analysis. At the conclusion of the analyses, the median quartiles were calculated for each station.

RESULTS and DISCUSSION

Salinity

Mean salinities ranged from 2.7 - 32.5 ‰ over the range of the province during 1993 pilot year studies (Table 5). Some stations exhibited relatively narrow salinity ranges, while others showed wide variations over a 24 hour cycle (Figure 4). Although there is a lack of high salinity stations in the degraded category, there are a few lower salinity sites in the reference category that can be examined carefully as signals of salinity effects. This is an unavoidable problem, due to a tendency for greater pollutant inputs in lower salinity areas. When considering overall trends, salinity must be carefully scrutinized, and the ability of indicators to operate over a wide range of salinities is clearly an important attribute.

Measurements of the water column profile at the time of sampling indicated very little salinity stratification (Table 5). The most stratified site was Sampit River which had a surface salinity of 14.9 ‰ that increased to 18.1 ‰ near bottom (Table 5). The low incidence of stratification can be explained in part by the low number of deep sites.

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Table 5. Salinity characteristics of stations sampled during Pilot Year Studies in the Carolinian Province, summer 1993. Data were taken during Continuous (≥ 23 hr) deployment of Datasonde 3s, and during Instantaneous water column profile measurements taken at the time of sampling. Blanks in the "Surface" column indicate that the site was shallow (≤ 0.5 m) so only a single reading was taken. Stars (*) indicate that no instantaneous profile measurements were taken.

	CONTINUOUS DEPLOYMENT	INSTANTANEOUS PROFILE		
STATION	Mean ± SD	Surface	Bottom	Depth
MAS	32.5 ± 1.62		31.7	0.3
INL	26.3 ± 2.36	35.9	36.0	3.4
FTJ	25.9 ± 2.15	*	*	*
DYT	14.5 ± 2.67	12.3	12.2	2.1
LTH	32.1 ± 0.30		31.3	0.4
OGE	11.9 ± 4.72	11.5	15.8	3.8
SAP	28.3 ± 0.81	28.1	29.3	5.8
NAS	16.5 ± 1.00	14.8	14.7	5.2
TIV	31.2 ± 0.22	30.4	30.4	1.0
WKY	31.4 ± 1.64		30.5	0.2
DEY	20.1 ± 0.95	22.4	22.0	2.9
BKY	17.9 ± 3.51	19.2	19.2	2.7
PLM	26.9 ± 1.13	24.5	24.7	1.3
BRU	7.8 ± 0.58	27.9	28.4	1.5
EAU	28.1 ± 0.12	19.5	27.8	1.5
CFT	19.7 ± 0.14		20.1	0.4
SLO	8.9 ± 0.47	8.1	8.1	0.8
SAM	14.8 ± 1.79	14.9	18.1	5.2
SAN	16.6 ± 2.76	9.6	12.9	2.7
SPY	18.2 ± 2.77	16.0	16.0	1.2
KOP	20.9 ± 1.19	24.3	25.0	4.5
SED	10.6 ± 2.46	14.9	15.7	6.0
SAV	2.7 ± 0.76	4.4	5.1	3.7
ORT	4.5 ± 0.73		3.4	0.5

Temperature

Temperatures ranged from 26.1 - 33.8°C over the range of the province, consistent with the purposes of the summer collection period (Table 6). During this period, the temperature variation is minimal and consistently high, representing the period of most significant temperature stress. There was very little temperature stratification, with surface to bottom temperatures varying by only 2 - 3 degrees centigrade.

pH

Mean pH values ranged from 6.98 to 8.17 (Table 7). During the course of the deployment period, there were numerous excursions in the acidic range. Estuarine and coastal waters have a relatively high alkalinity and usually have high buffering capacity. In unpolluted waters, pH is controlled by carbon dioxide and bicarbonate ions. The pH of seawater tends to vary with salinity, and all of the acidic pH values (< 7.0) occurred at the stations with salinities < 18 ‰ salinity. There was also a strong tidal signal in pH patterns.

When the stations were evaluated based on the groupings in Table 4 (reference, degraded, unknown), the mean pH for each class of sites was 7.57, 7.40, 7.41 respectively. Although this small mean variation would suggest little in the way of differences between various classes of sites, there are other characteristics of these data that warrant scrutiny. The minimum and maximum pH values for each station are shown in Figure 5. These data are replotted showing the range deviations (maximum minus minimum) to further emphasize the range differences among sites (Figure 6). A reference line is drawn at 0.5 as a potential criteria for comparing reference and degraded sites. The range deviations at reference sites were 0.15 - 0.61 pH units (11% exceeding 0.50), compared to 0.20 - 0.56 for unknown sites (50% exceeding 0.50), and 0.18 - 1.3 for degraded sites (67% exceeding 0.50). Although minimal pH values do correlate with minimal salinity ($r^2=0.51$), there is no relationship between salinity range deviations (plotted in Figure 7) and pH range deviations ($r^2= 0.02$). Degraded sites such as SLO and ORT had narrow salinity ranges, but high pH range deviations. Reference sites such as INL and OGE had high salinity ranges, but low pH range deviations. Therefore, although minimum pH values are controlled by salinity, variables that are based on pH ranges may be less susceptible to salinity bias.

Those sites with low pHs that remain in the basic range and show less fluctuations may be less stressful than those sites characterized by wide fluctuations in pH. Stable conditions promote physiological adaptation to ambient conditions, whereas unstable fluctuations may result in stress. Innovative approaches to interpreting the pH data and its physiological significance are warranted. Unlike oceanic waters that have pHs above 8 and tend to be well buffered, many southeastern estuarine waters have pHs in the low 7 range and below and may be particularly susceptible to perturbation. pH shifts are one of the most important components affecting bioavailability of contaminants as well as enzyme function.

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Figure 4. Minimum and maximum salinities from continuous Datasonde records taken at sites sampled in the Carolinian Province during pilot year studies, summer 1993.

Figure 5. Minimum and maximum pHs from continuous Datasonde records taken at sites sampled in the Carolinian Province during pilot year studies, summer 1993.

Table 6. Temperature characteristics of stations sampled during Pilot Year Studies in the Carolinian Province, summer 1993. Data were taken during Continuous ($\geq$ 23 hr) deployment of Datasonde 3s, and during Instantaneous water column profile measurements taken at the time of sampling. Blanks in the "Surface" column indicate that the site was shallow ($\leq$ 0.5 m) so only a single reading was taken. Stars (*) indicate that no instantaneous profile measurements were taken.

| STATION | CONTINUOUS DEPLOYMENT | INSTANTANEOUS PROFILE | | |
	Mean $\pm$ SD	Surface	Bottom	Depth
MAS	27.45 $\pm$ 1.19		28.23	0.3
INL	30.14 $\pm$ 0.63	30.50	30.52	3.4
FTJ	29.02 $\pm$ 0.97	*	*	*
DYT	31.59 $\pm$ 0.34	30.80	31.00	2.1
LTH	29.93 $\pm$ 0.53		29.47	0.4
OGE	28.46 $\pm$ 0.37	28.46	28.17	3.8
SAP	29.20 $\pm$ 0.24	29.07	28.83	5.8
NAS	29.76 $\pm$ 0.25	29.86	29.66	5.2
TIV	28.72 $\pm$ 0.54	28.62	28.62	1.0
WKY	27.58 $\pm$ 0.84		28.60	0.2
DEY	31.90 $\pm$ 0.46	29.30	31.51	2.9
BKY	31.29 $\pm$ 0.66	30.50	30.80	2.7
PLM	30.82 $\pm$ 0.83	31.70	31.80	1.3
BRU	29.77 $\pm$ 0.64	30.12	29.92	1.5
EAU	29.92 $\pm$ 0.26	28.60	30.08	1.5
CFT	29.58 $\pm$ 0.97		30.42	0.4
SLO	29.8 $\pm$ 0.41	29.33	29.65	0.8
SAM	27.63 $\pm$ 0.39	26.73	27.15	5.2
SAN	26.92 $\pm$ 0.69	27.13	26.46	2.7
SPY	29.51 $\pm$ 0.37	29.89	29.23	1.2
KOP	30.92 $\pm$ 0.51	30.39	30.42	4.5
SED	29.07 $\pm$ 0.60	29.70	29.51	6.0
SAV	27.00 $\pm$ 0.18	28.10	27.24	3.7
ORT	31.42 $\pm$ 0.68		29.94	0.5

Table 7. The pH characteristics of stations sampled during Pilot Year Studies in the Carolinian Province, summer 1993. Data were taken during Continuous ($\geq$ 23 hr) deployment of Datasonde 3s, and during Instantaneous water column profile measurements taken at the time of sampling. Blanks in the "Surface" column indicate that the site was shallow ($\leq$ 0.5 m) so only a single reading was taken. Stars (*) indicate that no instantaneous profile measurements were taken.

STATION	CONTINUOUS DEPLOYMENT Mean + SD	INSTANTANEOUS PROFILE Surface	Bottom	Depth
MAS	7.78 ± 0.11		7.76	0.3
INL	7.93 ± 0.12	7.94	7.96	3.4
FTJ	7.71 ± 0.16	*	*	*
DYT	7.23 ± 0.07	7.30	7.17	2.1
LTH	7.65 ± 0.15	7.72	7.72	0.4
OGE	7.00 ± 0.05	6.92	6.90	3.8
SAP	7.62 ± 0.06	7.41	7.47	5.8
NAS	7.02 ± 0.04	6.91	6.88	5.2
TIV	8.17 ± 0.05	8.14	8.14	1.0
WKY	7.78 ± 0.14		7.52	0.2
DEY	7.31 ± 0.10	6.88	7.17	2.9
BKY	7.05 ± 0.09	6.63	6.84	2.7
PLM	7.16 ± 0.13	7.75	7.75	1.3
BRU	7.13 ± 0.05	7.20	7.20	1.5
EAU	7.47 ± 0.06	7.78	7.44	1.5
CFT	7.78 ± 0.11		7.99	0.4
SLO	7.65 ± 0.42	8.08	8.06	0.8
SAM	7.21 ± 0.15	7.25	7.36	5.2
SAN	7.33 ± 0.18	7.34	7.12	2.7
SPY	7.52 ± 0.10	7.80	7.49	1.2
KOP	7.23 ± 0.12	7.02	7.08	4.5
SED	7.09 ± 0.14	7.51	7.39	6.0
SAV	6.98 ± 0.05	7.09	6.93	3.7
ORT	7.89 ± 0.27		7.90	0.5

Figure 6. The pH range deviations (maximum pH - minimum pH) for sites sampled in the Carolinian Province during pilot year studies, summer 1993.

Figure 7. The salinity range deviations (maximum salinity - minimum salinity) for sites sampled in the Carolinian Province during pilot year studies, summer 1993.

The significance of these changes in pH is unknown. Low pH conditions may be caused by natural as well as anthropogenic factors. For this indicator, it is important to remember that even small variations can translate into major problems for the biota. In mammals, changes in blood pH of only a few tenths of a pH unit can result in severe physiological effects or even death (Klaassen et al., 1986). Although, there is a substantial body of literature on the physiological effects of acidified fresh water systems, there are very few studies on the effects of pH in marine or estuarine systems. Acidic pHs will affect regulation of ions (particularly Na) and osmoregulation processes, so ionoregulatory failure can be a common cause of mortalities (Milligan and Wood, 1982). Recruitment failure in fish from acidified lakes has been attributed to the production of less viable offspring, and to adverse effects on early life stages (Weiner et al., 1986; Gunn and Noakes, 1987). Studies have indicated that episodic or intermittent exposures are as stressful (and in some cases more stressful) than continuous exposures (Siddens et al., 1986). Optimal enzymatic activity is highly pH dependent so alterations in environmental pH that affect blood pH levels may affect a variety of enzyme systems. There are many issues that we presently do not understand. Are there absolute minima or critical durations of exposure to lower pHs that result in stress? Are fluctuations in pH values or the rate of pH change stressful? One of the most critical issues is how do these potentially stressful pH conditions actually affect blood pH levels? The effects of environmental pH changes on blood or physiological pHs deserves serious evaluation.

Sediment Characteristics

Sediment parameters (% silt-clays, organics, grain size) provide important means of correcting for variations in habitat type with respect to other indicators. The available surface area for sorption of contaminants is considered to be a function of grain size, so sandy sediments have a lower tendency for accumulation of contaminants than muddy sediments. Silt-clay content of sediments is often a determining factor in the composition of the benthic community, and sediment composition factors such as aluminum concentration are also important in the evaluation of the sediment chemistry data. Organic matter is an important component of estuaries, controlling food availability and nutrient recycling, as well as in modulation of binding and bioavailability of contaminants.

The results of sediment analyses are listed in Table 8, and graphically depicted in Figure 8. Reference sites as a whole were generally sandier than degraded sites. Although for purposes of indicator testing, it would be desirable to sample reference and degraded sites with various sediment regimes, this problem is difficult to circumvent. Degradation of habitat quality by anthropogenic factors typically results from depositional processes. Therefore, siltier sediments are typical of depositional zones, whereas sandier sediments are most often found in well-flushed or scoured habitats typical of non-depositional areas. As noted in the Methods section, the silt-clay sample was different from the one used for grain size. In most cases, grain size and % silt-clays yield similar patterns, but there were a few discrepancies. In two cases (FTJ and WKY), the sample taken for silt-clays was siltier than the one used for grain size.

In the Louisianian Province, total organic carbon (TOC) could be used to discriminate between reference and degraded sites (Summers et al., 1993). Total sediment organics, but not TOC, were measured during the 1993 pilot year studies, but even this crude measure was effective at discriminating between reference and degraded sites (Figure 8C). High organics may reflect high inputs. They may also provide some information regarding the status of food webs and nutrient cycles, since alterations in bacterial components may reduce the efficiency and rate of detrital decomposition and nutrient cycling.

Grain size data indicated that the degraded sites were frequently associated with depositional processes, as does the cruder measure, % silt-clays. Grain size analyses are substantially more time consuming than are the methods for % silt-clays, and the pipette analyses performed with silty sediments are also prone to error. Therefore, silt-clay values represent a less costly indicator of this aspect of the habitat characteristics. Total organics, similar to results in other studies with total organic carbon, are also a good indicator of overall ecosystem health. Many of the components discussed in this chapter will be reintroduced in later chapters as standardizing factors.

Table 8. Sediment characteristics of stations sampled during Pilot Year studies in the Carolinian Province, summer 1993.

STATION	% SILT-CLAYS	MEDIAN GRAIN SIZE (phi)	% ORGANICS
MAS	0.90	2.52	0.85
INL	2.69	2.83	1.75
FTJ	82.71	< 4.0	4.32
DYT	0.68	1.21	1.28
LTH	2.95	2.29	0.74
OGE	0.23	1.31	0.24
SAP	17.13	2.84	3.74
NAS	9.16	1.21	0.99
TIV	3.53	2.86	1.34
WKY	51.94	2.92	7.49
DEY	4.64	2.80	2.75
BKY	29.04	6.01	8.39
PLM	44.71	4.41	7.93
BRU	50.41	5.57	10.88
EAU	84.96	7.09	19.16
CFT	68.36	5.67	19.59
SLO	53.95	7.42	21.36
SAM	82.76	> 9.0	17.59
SAN	4.42	1.31	3.63
SPY	93.33	7.98	13.66
KOP	90.18	8.11	14.95
SED	6.94	2.46	4.76
SAV	88.25	> 9.0	15.07
ORT	81.94	7.36	27.04

A % Silt-Clay

B Median Grain Size

C % Total Organics

Reference: MAS INL FTJ DYT LTH OGE SAP NAS TIV

Unknown: WKY DEY BKY PLM BRU EAU

Degraded: CFT SLO SAM SAN SPY KOP SED SAV ORT

Figure 8. Sediment characteristics for sites sampled in the Carolinian Province during pilot year studies, summer 1993.

* - Median quartile was < 4.0 phi.

** - Median quartile was > 9.0 phi.

CHAPTER 3. EXPOSURE INDICATORS

INTRODUCTION

Exposure indicators are physical, chemical, and biological measurements that indicate pollutant exposure and habitat degradation. The exposure indicators that were evaluated during pilot year studies were dissolved oxygen, sediment contaminants, and four toxicity assays (amphipod toxicity, mysid toxicity, Microtox®, and seed clam growth). Dissolved oxygen and sediment contaminants continue to be important core indicators. EMAP has traditionally used the amphipod, *Ampelisca abdita*, as the test organism in 10 day acute toxicity assays. In addition to EMAP, this assay has been used for impact assessment studies in Puget Sound (Dinnel, 1990), San Francisco Bay (Long et al., 1990), and New York Harbor (Scott et al., 1990). Tests were also conducted using a mysid crustacean, *Mysidopsis bahia*, in 4 day acute toxicity assays. Microtox® is a recently developed assay that utilizes bacteria in a sublethal response assay. It had not been used in other EMAP provinces, but the increase in use of this test in other assessment programs suggested that it may also be valuable to EMAP. Studies were also initiated regarding the use of a juvenile clam (*Mercenaria mercenaria*) growth assay.

One of the most important physical components of aquatic environments is the dissolved oxygen concentration. Oxidative processes affect many physical-chemical processes such as pH and sediment binding affinities of contaminants, as well as biological responses. Dissolved oxygen measurements reflect the integrated effects of physiological and ecological processes to nutrient and contaminant inputs. Dissolved oxygen levels are of fundamental importance to the maintenance of balanced indigenous populations of estuarine biota because of the essential requirement of oxygen and variations in adaptive mechanisms. When oxygen levels are low, mobile species have the ability to migrate away from undesirable conditions. Sessile or low mobility organisms may not have that option, and have in many cases evolved mechanisms to cope with short term excursions of low DO or cyclical DO patterns. Therefore estuaries can function as refugia for those organisms that can adapt to variable DO patterns.

Low DO conditions can be the result of both natural and anthropogenic factors. Natural sources of elevated nutrients and high productivity, particularly in shallow tidal systems, facilitate the development of habitats dominated by cyclical DO patterns (Holland, Charleston Harbor Study, work in progress). In perturbed systems, anthropogenic inputs of nutrients (via wastewater discharges, agricultural and stormwater runoff) may also cause cyclical DO patterns. Therefore the critical question is how can anthropogenic effects be distinguished from natural variations?

Aquatic pollutants rapidly adsorb onto suspended particles which settle to the bottom, or are absorbed by the biota. Pollutants absorbed by the biota are cycled as wastes or detritus which also contribute pollutants as well as organic materials to the bottom sediments. Therefore,

sediments function as the primary sink for contaminants, so they provide a more reliable estimate of anthropogenic enrichment and the most direct measure of exposure potential. Concentrations of contaminants in the water column tend to underestimate exposure potential. Although sediment contaminants provide estimates of potential exposure risks for the biota, the realized risks to estuarine populations are controlled by factors that affect contaminant availability. The bioavailability of sediment-associated contaminants is a dynamic component that is affected by a variety of physical and chemical processes. Therefore sediment contaminant concentrations provide valuable information for predicting exposure potential, but bioavailability and interaction issues frequently do not enable statements regarding actual impacts on the biota.

Laboratory toxicity tests have been used as indicators of potential impacts on the biota and as indirect indicators of contaminant bioavailability. There are inherent limitations. Experiments conducted in closed laboratory systems under standard laboratory conditions can not possibly mimic field conditions, particularly the physical and chemical fluctuations in environmental variables (salinity, flow regimes, depth, etc.) that characterize estuarine environments. Laboratory toxicity tests commonly use an acute toxicity endpoint that does not adequately reflect the chronic toxicity potential. The choice of test organisms is critical. Ideally, test organisms should be closely associated with the sediments, and be sensitive enough to reflect toxicity without being hypersensitive to test conditions (sediment type, ammonia concentrations, etc.). Regardless of these problems, toxicity testing with sediments from a variety of locations still provides the most powerful means to evaluate relative toxicities of different stations. To guard against misinterpretation of results, associations between toxicity tests and contaminants are needed to appropriately identify a habitat as degraded. High toxicity with no evidence of contamination should be suspect. It suggests that animals may be hypersensitive to variables that occur as artifacts in the laboratory setting (such as ammonia, sulfides, crowding, etc.). The reverse situation (low toxicity with evidence of high contaminant loadings) must also be scrutinized more carefully. This situation could result from low bioavailability of contaminants, or may indicate that indicator organisms possess heightened detoxification responses such as metallothioneins, multi-xenobiotic transport mechanisms, or mixed function oxidases.

The amphipod (*Ampelisca abdita*) assay was the primary test of potential toxicity for the Virginian and Louisianian Provinces. Historically, inputs into southeastern estuaries are presumed to be less in comparison to the northeast and Gulf of Mexico, so the degree of degradation may be less. Therefore an important question that was addressed in this study was whether the *Ampelisca abdita* assay is sufficiently sensitive to discriminate degraded sites from reference sites in this region? Recently concerns about hypersensitivity to ammonia levels and other experimental conditions have also been raised. Therefore, *A. abdita* assays were compared to other measures of toxicity as well as sediment contaminant concentrations. Multiple toxicity tests should always be conducted when possible because no single test is likely to apply to all conditions. This is particularly important during the indicator development phase, and should continue at some level during all phases to insure the generation of valid results.

Microtox® was used as the assay for parallel evaluations. This test has also been used extensively for toxicological evaluations in aquatic environments, and standard methods have been developed. Microtox measures the effects of sediments or pore waters on light production

by the photoluminescent bacteria *Photobacterium phosphoreum*, so it is a sublethal assay. Light is emitted as a result of a metabolic pathway that is intrinsically linked to cellular respiration, so disruption of normal cellular metabolism causes a decrease in light production.

Growth of juvenile clams, *Mercenaria mercenaria*, was used as a sublethal indicator of toxicity in an assay developed during this study as a new candidate indicator. Newly metamorphosed bivalves exhibit very rapid growth, so it should be possible to observe effects on growth in a relatively short time frame. Seed clams possess a number of attributes. They are infaunal, crawling through the sediments and feeding at the surface-water interface. They are readily cultured (approximately 8 weeks from fertilization) so experiments can be conducted with a well defined population.

METHODS

Dissolved Oxygen Measurements

During 1993 pilot year studies, instantaneous readings as well as 24 hr continuous records of DO were taken at each site using a Hydrolab Datasonde 3. This instrument employs a polarographic-type sensor which was fitted with a LoFlow membrane. For instantaneous measurements, profiles of water column measurements were taken at approximately 0.5 m intervals for sites less than 3 m, and at 1.0 intervals for sites greater than 3 m. For continuous records, measurements were taken at 30 minute intervals for $\geq$ 23 hours. The goal was $\geq$ 24 hour readings, but this was not possible at a few sites due to weather conditions. Each instrument was calibrated either the night or morning before deployment. Just prior to conducting the instantaneous profile or deployment of a unit for continuous records, an on-board quality control (QC) check was performed. For the QC check, water from the site was collected in a bucket and then brought to saturation using a small pump or by brisk stirring. Readings for both the profiling unit and the deployed unit had to agree within an acceptable range (10%), and had to read > 90% saturation. If this criteria was not met, then a third instrument was introduced and the two that agreed were used. Therefore, this procedure insured that the instruments were capable of reading near saturation as well as validating the reliability of the measurements.

Other ancillary dissolved oxygen data were also collected. Continuous readings were taken for three of the sites (FTJ, BKY, KOP) over a 14 day period. Also, DO patterns of seven tidal creeks in the Charleston area were monitored (Long Creek, Adam's Creek, Hobcaw Creek, Ledenwah Creek, New Market Creek, Parrot Creek, and Yacht Club Creek). In these studies Datasondes were deployed for $\geq$ 3 days (7 day deployments were most commonly used), and for some sites (particularly Long Creek and Parrot Creek) Datasondes were deployed repeatedly.

There were some cases in which the Datasondes were exposed during low tides, but other parameters such as salinity and depth can be used to identify invalid records. These records were

removed from the data set before any statistical analyses were conducted. Means and standard deviations were calculated and time series plots were generated.

Sediment Contaminant Analyses

A limited suite of metal and PAH contaminants were determined from sediment samples. The analytes are listed in Table 9. Samples for metal analyses were homogenized, dried, and pulverized. One gram of dried sample was then digested using nitric and hydrochloric acids in a microwave oven according to National Bureau of Standards methods (Kingston and Jassie, 1986), and filtered. The metal concentrations of the filtrate were then analyzed by sequential ICP (Perkin Elmer Plasma 40 ICP), using USGS method I1472-85. Four aliquots from each sample were analyzed and averaged, and results are reported as *ug*/g dry weight. Standard sediments were not available at the time of analysis, so the concentrations reported should be used in a relative manner only.

For PAH analyses, sediment samples were extracted with a mixture (25ml) of methanol, isopropanol, and acetone (2:1:1). A 15% solution of the filtered extract was then passed through a 500 mg C18 extraction column or Empore disc that had been cleaned by successive rinses with methylene chloride, hexane, ethyl ether, methanol, and 15% extraction solvent. After drying, the C18 column was eluted with methylene chloride and brought up to 1.0 ml. The sample was then analyzed on a Tracor model 540 gas chromatograph with a nonpolar (J&W DB-5) capillary column and a flame ionization detector. Standard sediments were analyzed and the rates of recovery are listed in Table 13 along with the results.

Table 9. Metals and PAHs measured in sediments collected in the Carolinian Province during pilot year studies, summer 1993.

Sediment Contaminants

PAHs		Metals	
Anthracene	Anth	Aluminum	Al
Fluorene	Fluo	Arsenic	As
Phenanthrene	Phen	Cadmium	Cd
Benzo (a) anthracene	B(a)A	Chromium	Cr
Benzo (a) pyrene	B(a)P	Copper	Cu
Chrysene	Chry	Iron	Fe
Fluoranthene	Fl-an	Lead	Pb
Pyrene	Pyr	Manganese	Mn
Benzo (b) fluoranthene	B(b)Fl	Zinc	Zn
Benzo (k) fluoranthene	B(k)Fl		

Sediment Toxicity Assays

Ampelisca abdita. The *Ampelisca abdita* assays were conducted by TRAC Labs (Gulf Breeze, FL) using standard techniques (Swartz et al., 1985; ASTM, 1990). Basically, adult

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amphipods are incubated with sediments for 10 days under static test conditions, and the effects on survivorship are evaluated. All samples were tested within 6 weeks of collection.

Mysidopsis bahia. Investigators at Gulf Breeze have been conducting tests with an endemic mysid, *Mysidopsis bahia*, as an alternative bioassay organism (Summers et al., 1993). Mysid contact with the sediments is frequent but not continuous as in the amphipod assays. In the mysid tests, mortalities were recorded after 4 days of exposure to test sediments.

Microtox®. Solid-phase tests were conducted by Dr. Phil Ross of The Citadel, according to standardized protocols with the Microtox model 500 (Bulich, 1979; Ross et al., 1991; Microbics, 1992). A 0.3 g sediment sample was used to make a series of 12 dilutions ranging from 0.01% to 10% sediment, and incubated with the bacteria (*Photobacterium phosphoreum*) for 20 minutes. Therefore the test organisms come in direct contact with sediment associated contaminants in an aqueous suspension. A column filter is then used to separate the liquid phase containing the bacteria from the sediment, and their post-exposure light output is measured. The data from the analyzer is captured directly by the Microtox data system, and Gamma (% effect) is calculated. A linear regression model is used to calculate EC_{50} (the sediment concentration that reduces light production by 50%).

Seed Clam Assay. On the day before initiation of an experiment, sediments were wet sieved through a 500 um screen, and the sediments as well as the water were distributed to test beakers (approximately 100 ml of sediment in 500 ml suspension with gentle aeration, 3 - 6 replicates). The sediment suspension was allowed to settle overnight and clams were added the next day. Juvenile clams (*Mercenaria mercenaria*) were size selected using 750 and 1000 um sieves in series, and only those seed clams captured on the 750 um screen were used. Seed clams were incubated with the sediments for 7 days (approximately 100 clams per beaker), and no additional food was added. At the end of the exposure period, clams from each test beaker were removed and subdivided into replicate groups of 30 - 50 clams, dried, and weighed on an analytical balance. The results were expressed as dry weight per clam, and the effects on growth rates were compared. This assay was used in two sets of experiments with 9 sediment samples that were 2 to 3 months old.

RESULTS and DISCUSSION

Dissolved Oxygen (DO)

Mean DO concentrations during continuous deployments are listed in Table 10, and the minimum and maximum values are shown in Figure 9. The mean DO concentration that corresponded to 100% saturation was 6.9 mg/L (median value = 6.8). The range of values that corresponded to 100% saturation was 6.5 at full salinity sites to 7.6 at the lowest salinity sites. Two stations, Savannah River (SAV) and South Edisto (SED), were identified as DO stressed (based on the criteria listed in Table 3 in Chapter 1). These are stringent criteria that identify

Table 10. Dissolved oxygen characteristics of stations sampled during Pilot Year Studies in the Carolinian Province, summer 1993. Data taken during continuous ($\geq$ 23 hr) deployment of Datasonde 3s, and during instantaneous water column profile measurements taken at the time of sampling. Blanks in the "Surface" column indicate that the site was shallow ($\leq$ 0.5 m) so only a single reading was taken. Stars (*) indicate that no instantaneous profile measurements were taken.

	CONTINUOUS DEPLOYMENT	INSTANTANEOUS PROFILE		
STATION	Mean + SD	Surface	Bottom	Depth
MAS	5.43 $\pm$ 0.99		6.11	0.3
INL	4.92 $\pm$ 0.90	6.46	6.85	3.4
FTJ	6.35 $\pm$ 0.83	*	*	*
DYT	4.23 $\pm$ 0.77	3.46	4.20	2.1
LTH	5.40 $\pm$ 0.55	5.42	4.96	0.4
OGE	4.41 $\pm$ 0.76	5.13	4.39	3.8
SAP	5.55 $\pm$ 0.53	4.95	4.47	5.8
NAS	5.17 $\pm$ 0.26	5.32	5.09	5.2
TIV	5.41 $\pm$ 0.73	6.02	5.34	1.0
WKY	4.80 $\pm$ 1.04		4.35	0.2
DEY	5.04 $\pm$ 0.82	7.49	4.89	2.9
BKY	3.35 $\pm$ 0.35	2.50	2.39	2.7
PLM	4.89 $\pm$ 1.10	6.26	6.28	1.3
BRU	3.09 $\pm$ 0.32	3.15	3.09	1.5
EAU	3.57 $\pm$ 0.47	6.04	3.77	1.5
CFT	6.53 $\pm$ 0.94		8.01	0.4
SLO	4.49 $\pm$ 2.92	8.07	8.04	0.8
SAM	2.90 $\pm$ 0.61	5.70	5.37	5.2
SAN	6.34 $\pm$ 0.74	8.53	7.25	2.7
SPY	5.31 $\pm$ 1.05	7.53	6.05	1.2
KOP	3.05 $\pm$ 0.68	2.90	2.92	4.5
SED	4.20 $\pm$ 2.55	7.26	6.12	6.0
SAV	0.34 $\pm$ 1.13	5.03	4.31	3.7
ORT	7.11 $\pm$ 1.27		6.03	0.5

Figure 9. Minimum and maximum dissolved oxygen readings from continuous Datasonde records taken at sites sampled in the Carolinian Province during pilot year studies, summer 1993.

anoxic conditions. The value of 5 mg/L as an indicator of hypoxic conditions has been frequently referred to when considering water quality criteria. All sites had minima below 5 mg/L (Figure 9). Six sites (3 unknown sites and 3 degraded sites) had DO concentrations that never exceeded 5 mg/L, so these sites may suffer from chronic hypoxia. Evidence of supersaturation, probably due to eutrophication, was observed at some of the degraded sites, particularly Plum Island (PLM), Slocum Creek (SLO), and Ortega River (ORT), all known to receive substantial inputs of treated wastewater from sewage treatment plants.

There were only two cases, Brickyard Creek (BKY) and Sapelo (SAP), in which the instantaneous measurements provided minimum estimates that approximated those found during continuous deployments (Figure 9, Table 10). Since instantaneous measurements are taken during the day at the time of sampling, which did not necessarily coincide with low tide, minimal concentrations would rarely be detected using this approach. Even supersaturated values may not be detected since they tend to occur during late afternoon or early evening. No evidence of stratification was evident during instantaneous measurements, which is in part a function of the relative shallow depths of all stations (less than 10 m), and the well-mixed nature of the estuaries.

At many sites, the DO patterns exhibited a strong tidal periodicity such that minimum DO values were observed during low tides, but rose to normoxic levels during high tides (Figure 10). In some cases there was also a diurnal component, such that the most hypoxic conditions were observed when low tides occurred during late night or early morning periods. The records from FTJ shown in Figure 10 demonstrate the tidal component as well as a subtle diurnal pattern (lowest daily minima were associated with early morning lows). The DO concentrations during midday or afternoon lows represent a balance between decreases due to tidal effects and increases due to oxygen release during photosynthesis. The patterns of DO in shallow tidal creeks also showed similar cyclical patterns. The pattern observed at Long Creek (Figure 11) was typical of reference sites, characterized by minima associated with night time or early morning low tides. When low tides occurred during the day, high productivity in shallow creeks resulted in maintenance or increases of DO concentrations. From both the creek data and EMAP station data, there was a tendency in degraded sites for a pattern of cyclical DO that showed strong tidal variations, during both nighttime and daytime low tides (Figure 12). At reference sites, a pattern of lowest DO measurements during late night or early morning low tides, but not during daytime low tides was observed.

The patterns of the longer term (2 week) deployments did not generally change the conclusions established from the 24 hour deployment. Differences in magnitude will occur due to seasonal as well as tidal differences. The records indicate that after 6 days in situ, a degradation of the DO measurements occurs, attributable to fouling of the DO sensor membrane. Therefore, for longer term deployments, it is recommended that the Datasondes be serviced every 5 - 7 days.

Although normoxic and anoxic conditions, the extremes, can be used to effectively discriminate between reference and degraded sites, the potential impacts of cyclical patterns or periodic hypoxia are more difficult to interpret. Chronic moderate hypoxia may not be as

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FORT JOHNSON

Figure 10. Dissolved oxygen patterns measured during continuous datasonde deployment at Fort Johnson, SC.

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LONG CREEK

━━━ DO (mg/l) − − − DEPTH (m)

Figure 11. Dissolved oxygen patterns measured during continuous datasonde deployment at Long Creek, SC.

Figure 12. Dissolved oxygen patterns measured during continuous datasonde deployment at Sampit River, SC.

Figure 13. A. The dissolved oxygen range deviations (maximum dissolved oxygen - mimimum dissolved oxygen) for sites sampled in the Carolinian Province during pilot year studies, summer 1993. B. The dissolved oxygen change rate (the difference between morning minimum and next maximum, divided by time) for sites sampled in the Carolinian Province during pilot year studies, summer 1993.

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Table 11. DO range deviation values (maximum minus minimum), and DO change rate (the difference between morning minimum and next maximum, divided by time). A DO Range Deviation > 3.5 was regarded as stressful, and a DO Change Rate > .50 was regarded as stressful. When both DO range and change rate were below these criteria, the predicted DO stress was considered low (L). When both DO range and change rate were above these criteria, high (H) DO stress was predicted. When one parameter exceeded the criteria but not both, DO stress was predicted as moderate (M).

STATION	DO RANGE DEVIATION	DO CHANGE RATE	PREDICTED DO STRESS
MAS	3.5	0.23	L
INL	3.0	0.74	M
FTJ	2.8	0.35	L
DYT	3.4	0.51	L
LTH	3.0	0.40	L
OGE	2.4	0.31	L
SAP	2.2	0.24	L
NAS	1.0	0.15	L
TIV	2.3	0.50	L
WKY	4.3	0.37	M
DEY	3.0	0.56	M
BKY*	1.2	0.24	L
PLM	6.4	1.39	H
BRU*	1.6	0.54	M
EAU*	2.1	0.13	L
CFT	3.6	0.80	H
SLO	9.6	1.60	H
SAM*	3.0	0.23	L
SAN	4.2	0.92	H
SPY	4.1	0.67	H
KOP	3.5	0.50	L
SED+	7.5	13.10	H
SAV*+	4.5	2.50	H
ORT	4.0	0.56	H

+ DO stressed based on criteria listed in Table 3.
* DO stressed because DO was always < 5.0 mg/L

damaging as cyclical hypoxia because the extended stable conditions would favor adaptational responses such as increases in respiratory pigments, behavioral changes, or biochemical changes that increase efficiency. It is now recognized from mammalian toxicology that cyclical DO levels characterized by periods of hypoxia and then return to normoxia can cause severe cell and organ damage (McCord, 1985). During hypoxia, reduced electron carriers that are capable of forming superoxide radicals accumulate. On reperfusion, the reintroduction of oxygen results in increases in oxygen radicals that cause serious localized tissue damage (Dawson et al., 1993). It is also important to remember that even the successful activation of compensatory mechanisms by animals reduces their optima and places them "on the edge." Therefore habitats characterized by moderate hypoxia or cyclical DO patterns may be highly susceptible to anthropogenic perturbation.

On this basis, some simplistic approaches for evaluating the effects of cyclical DO patterns were developed which may stimulate some thought in this area. One is a simple range deviation, and the other is DO change rate, since a steeper gradient is expected to be more damaging than a more gradual change. Therefore, DO change rates of habitats exacerbated by anthropogenic stress are expected to be higher than those of habitats that are naturally cyclical. In general, both range and rate criteria discriminated between reference and degraded sites (Figure 13; Table 11). The robustness of these variables were evaluated with regard to their potential bias from salinity. The correlation coefficients (r^2) between range or rate criteria and mean salinity or salinity range are shown in Table 12. These results indicate that the computed DO variables are not related to salinity variables.

Table 12. Correlation coefficients (r^2) between salinity and dissolved oxygen parameters.

	DO Range Deviation	DO Change Rate
Mean Salinity	0.14	0.11
Salinity Range	0.00	0.07

Therefore interpretative analyses that can be used to consider cyclical patterns as well as absolute criteria may be developed. For example, at Whiskey Creek (WKY), the range was somewhat high but the rate suggested that the stressful conditions may be alleviated by the slow transition time. On the other hand, Clubfoot Creek (CFT) had only a slightly elevated range, but a rapid transition rate. Sites with low ranges and rates were predicted as low DO stressed (L), sites with high ranges and high rates were categorized as high DO stressed (H), and sites with conflicting range and rate values were listed as moderate DO stressed (M). The results of this exercise are also listed in Table 11. It is intriguing to consider that those that are listed as moderate are within compensatory ranges of resident organisms, and the ability of estuarine organisms to tolerate these conditions may give them a competitive edge over potential predators. However, these kinds of systems may be readily perturbed if additional insults occur. A battery of DO criteria such as minimum < 2.0 mg/L, maximum < 5.0 mg/L, maximum > 8.0

mg/L, range deviation > 3.5 mg/L, change rate > 0.5 mg/L/hr could be used so that sites that are positive for 2 or more of these criteria would be classified as stressed.

An important issue is how the organisms are affected. The magnitude and duration of hypoxic conditions affect the ability of organisms to tolerate exposures to low dissolved oxygen. Catastrophic fish kills occur during anoxia, or when very sensitive species are exposed to hypoxia. Many mobile fish and benthic invertebrates exhibit behavioral changes and simply migrate away from undesirable conditions. Infaunal polycheates have been observed to move to sediment surfaces, where they may be susceptible to predation. There are also species specific differences in the ability of organisms to tolerate hypoxic conditions (Stickle et al., 1989). The effects of extended periods of hypoxia and anoxia on survivorship have been previously documented, but the effects of cyclical exposures are not so clear (Seliger et al., 1985; Winn and Knott, 1992; Baker and Mann, 1992). The responses of animals exposed to hypoxia or anoxia followed by normoxic conditions vary. Responses range from a transient oxygen debt phenomenon followed by a return to normal metabolic rates, to irreversible effects on metabolism (Stickle et al., 1989). Changes in biochemical components such as the oxygen transporting pigment hemocyanin may occur. However increases in hemocyanin have been reported in some studies and decreases in others (Baden et al., 1990; DeFur et al., 1990; Engel et al., 1993). Therefore, the relationship between a response and physiological condition must be recognized for valid identification of populations under stress.

Changes in dissolved oxygen concentrations are clearly the result of natural as well as anthropogenic factors. Diurnal fluctuations of oxygen concentrations are a well documented characteristic of estuaries (Day et al., 1989). It is almost certain that inhabitants of estuaries have evolved adaptive mechanisms to compensate for short term or cyclical changes. For example, during anaerobic respiration, molluscs use succinate and propionate pathways instead of lactate, which are more efficient and generate more ATPs than the classical glycolysis system (deZwann and Thillart, 1985). However, factors that exacerbate DO patterns and alter their duration and magnitude may result in DO patterns that are beyond the compensatory mechanisms. Increased nutrient or contaminant inputs, or modification of water flow patterns may alter the range of DO concentrations or subject animals to more frequent DO cycles. Increases in cycle frequency, such as the case when DO drops during day time as well as night time low tides, will increase stress. Therefore, we should not be lulled into ignoring cyclical DO phenomenon simply because they also occur in non-perturbed systems. The effects of anthropogenic perturbations may range from periodic catastrophic mortalities to more chronic effects on growth and reproduction.

Sediment Contaminants

Determination of contaminant concentrations that are likely to have adverse effects on the biota is still an emerging science. EPA, for example, is in the process of developing sediment

Table 13. The concentrations of metal contaminants measured in sediments from sites sampled in the Carolinian Province during pilot studies, summer 1993. ER-L and ER-M values (Long and Morgan, 1990), and NOEL and PEL values (MacDonald, 1993) are also listed. Concentrations are ppm (ug/g dry weight).

STATION	Al	Cr	Cu	Fe	Pb	Zn	Cd	As	Mn
MAS	1632.44	6.18	1.80	1321.34	3.70	8.12	0.17	11.84	13.10
INL	1631.42	7.24	2.28	4274.62	3.86	10.76	0.22	6.30	100.98
FTJ	3322.74	10.54	5.28	5895.98	5.64	18.38	0.26	16.62	74.70
DYT	638.84	4.28	1.38	496.24	1.40	4.46	0.12	12.16	4.80
LTH	743.20	6.52	1.94	1244.98	2.36	5.02	0.35	20.14	14.00
OGE	218.20	2.94	1.00	428.56	2.08	26.16	0.20	18.60	3.60
SAP	2965.20	7.30	3.00	3451.32	3.94	10.50	0.30	19.20	45.10
NAS	1492.70	4.48	1.20	1053.36	1.66	5.12	0.10	10.44	13.80
TIV	1154.72	5.32	0.94	565.78	1.64	3.54	0.40	11.44	6.06
WKY	3283.48	22.26	35.20	7869.10	11.42	49.30	0.36	18.14	63.98
DEY	3154.36	11.22	54.56	4722.20	31.78	123.32	0.36	17.36	51.94
BKY	3344.06	18.82	18.66	7918.76	15.68	46.20	0.72	27.50	68.30
PLM	3351.28	14.52	11.14	8017.12	10.50	39.32	0.30	18.72	123.16
BRU	3332.90	79.10	13.46	7866.94	10.64	52.44	0.37	18.14	112.34
EAU	3299.80	32.54	50.38	7850.30	53.68	100.70	0.48	14.83	307.22
CFT	3337.44	18.08	15.14	7928.30	20.40	47.64	0.71	39.92	78.12
SLO	3032.92	55.78	32.20	7203.12	67.12	130.48	8.94	17.64	278.36
SAM	3327.84	26.66	21.52	7909.68	14.28	71.24	0.54	36.20	572.04
SAN	3352.94	9.62	5.02	6499.52	4.94	16.14	0.21	9.58	107.94
SPY	3288.58	47.80	29.58	7830.74	20.86	72.58	0.59	33.30	318.40
KOP	3305.08	32.66	40.38	7819.72	29.00	95.48	0.51	53.38	280.56
SED	3385.04	12.26	4.30	5586.76	6.34	17.26	0.33	20.58	64.16
SAV	3347.44	25.10	11.13	7829.20	14.40	64.72	0.51	37.46	723.10
ORT	3468.42	24.52	72.68	8241.90	171.26	444.58	2.13	16.74	94.70

Long & Morgan									
ER-L		80	70		35	120	5	33	
ER-M		145	390		110	270	9	85	
MacDonald									
NOEL		33	28		21	68	1	8	
PEL		240	170		160	300	7.5	64	

Table 14. The concentration of PAHs measured in sediments collected from sites in the Carolinian Province during pilot year studies, summer 1993. Sediment criteria, ER-Ls and ER-Ms (Long and Morgan,1990), and NOELs and PELs (MacDonald, 1993) are listed for reference. The percent recoveries of analytes from standard reference material (SRM) are also listed. Concentrations are ppb (ng/g dry weight).

STATION	Anth	Fluo	Phen	B(a)A	B(a)P	Chry	Fl-an	Pyr	B(b)Fl	B(k)Fl
MAS										
INL									772	2580
FTJ										
DYT										
LTH										
OGE					958					2700
SAP										
NAS									1700	1700
TIV		476				808				
WKY										
DEY										
BKY					1210				1060	
PLM										
BRU					1220		405			1170
EAU										
CFT					850					2410
SLO	423	476		1980			635		3480	3450
SAM									1650	2350
SAN				2670				445		
SPY					2020	744		744		672
KOP	359			4970	3050	1290	1140	8960	4290	4140
SED		406			828					3030
SAV					1380		522	541		
ORT			299	1880	1550		1430	2110	2340	3860

| Recovery SR | 61 | 97 | 78 | 42 | NA | 129 | 49 | 61 | 16 | 154 |

Long & Morgan										
ER-L	85	35	225	230	400	400	600	350		
ER-M	960	640	1380	1600	2500	2800	3600	2200		
MacDonald										
NOEL	85	18	140	160	230	220	380	290		
PEL	740	460	1200	1300	1700	1700	3200	1900		

quality criteria. Long and Morgan (1990) and MacDonald (1993) have produced important studies regarding the development of sediment criteria based on biological effects. Long and Morgan defined ER-L and ER-M concentrations. ER-Ls (low effects range) are those concentrations which may cause chronic toxicity and possibly acute toxicity for sensitive species. ER-Ms (median effects range) are those concentrations that are expected to cause acute toxicity. MacDonald defined NOELs as the upper limit of the no effects range of contaminant concentrations, and PELs or probable effects levels as the lower limit of the range of concentrations that are usually or always associated with adverse biological effects. Within the possible effects range (delineated by the NOEL and PEL), adverse biological effects are possible, but less reliable to predict. These criteria are included at the bottoms of Table 13 (which shows the metal concentrations at all sites), and Table 14 (which shows the concentrations of PAHs analyzed). Exceedances of ER-Ms (and possibly upper ER-L ranges) are expected to result in major mortalities of the biota, with obvious effects on communities. Degraded sites contained a few exceedances of metals, and had high concentrations of numerous PAHs.

Trace metals are naturally associated with the silt and clay fractions of detrital sediments, so finer grain sediments will inherently have higher concentrations of metals than sands. Normalization to aluminum is recognized as one of the most useful means of adjusting for natural variability (Bruland et al., 1974; Goldberg et al., 1979; Windom et al., 1989). In our studies, Al normalization and 95% prediction intervals were generated using previously described methods (Windom et al., 1989; Schropp et al., 1990). Studies from Schropp et al. (1990) use a logarithmic transformation for Al concentrations, but other studies express Al concentration as percent (Windom et al., 1989; Hanson et al., 1993). In both approaches, only the reference sites are used to generate Al normalized plots and then the unknown and degraded sites are plotted. Our analyses were based on a small sample size of 9 reference sites with aluminum concentrations that ranged from 218 to 3468 ug/g. Although the concentrations of Al were underestimated (later analyses with standard sediments yielded approximately 10% recovery of Al), the results of these analyses are still useful because they can be evaluated in a relative context.

Our results were then compared to those based on more extensive data bases. The slopes and intercepts of Al-normalized analyses from our study and other southeastern studies as well as results from the Louisianian Province are shown in Table 15. Our regression parameters based on log-log or ln-ln transformations are generally lower, but are at least similar in magnitude, and in some cases show reasonable agreement. In all studies, the cadmium to aluminum relationship is weak or non-significant. Log-log regressions compared favorably with Schropp's analyses, especially for Cd, Cu, and Zn. The regression for As was not significant. Windom et al. (1989) found no As / Al relationship for sediments collected from SC and GA, but a significant relationship for sediments collected from FL (similar to that reported by Hanson et al., 1993, and to 1991 and 1992 data from the Louisianian Province). Notice that log-log or ln-ln transformation of pilot study data resulted in regression parameters that showed better agreement with other studies than when the analyses were based on % Al. This discrepancy based on % Al is probably due to the low recovery of Al, and suggests that using logarithmic

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Table 15. The regression statistics for metals analyzed by MRRI during pilot year studies (summer, 1993) are listed as well as those from similar studies for comparison. Results from three methods of aluminum normalization are shown. A. Results from log-log transformations of metal data. B. Results from ln-lntransformations of metal data. C. Results from data with Al expressed as percent. *Regressions were not significant and no parameters were reported.

A.   Log Al

Metal	MRRI slope	intercept	r^2	Schropp et al., 1990 slope	intercept	r^2
As	-0.08	1.38	0.03	0.63	-1.80	0.71
Cd	0.29	-0.63	0.30	0.29	-2.20	0.45
Cu	0.48	-1.22	0.52	0.48	-1.20	0.73
Cr	0.38	-0.41	0.74	0.55	-1.10	0.74
Pb	0.38	-0.75	0.42	0.73	-2.10	0.90
Zn	0.79	-1.60	0.69	0.71	-1.80	0.88
Mn	1.13	0.33	0.63			

B.   Ln Al

Metal	MRRI slope	intercept	r^2	Louisianian Province 1991 slope	intercept	r^2	Louisianian Province 1992 slope	intercept	r^2
As	-0.08	3.18	0.03	0.57	1.02	0.61	0.63	0.87	0.67
Cd	0.09	-2.16	0.02	0.46	-2.60	0.38	0.60	-2.70	0.46
Cu	0.48	-2.81	0.52	0.79	1.14	0.80	0.78	1.14	0.79
Cr	0.38	-0.95	0.74	0.61	2.90	0.84	0.66	2.71	0.77
Pb	0.38	-1.72	0.42	0.70	1.75	0.90	0.67	1.62	0.93
Zn	0.79	-3.79	0.69	0.92	2.27	0.85	0.78	2.86	0.80
Mn	1.13	-5.23	0.63						

C.   % Al

Metal	MRRI slope	intercept	r^2	Windom et al., 1989 GA/SC slope	intercept	r^2	Windom et al., 1989 FL slope	intercept	r^2	Hanson et al., 1993 slope	intercept	r^2
As	3.50	13.5	0.00	ns*			7.50	-0.07	0.77	1.50	0.03	0.61
Cd	0.15	0.21	0.02	ns*			ns*			0.02	0.05	0.14
Cu	11.27	0.33	0.73	1.80	-1.40	0.64	2.50	2.20	0.61	2.00	-0.22	0.78
Cr	18.20	3.30	0.72	ns*			9.50	4.00	0.81	8.10	0.98	0.64
Pb	11.60	1.14	0.70	0.35	1.50	0.62	3.20	2.30	0.69	3.00	3.40	0.76
Zn	44.45	0.69	0.77	12.0	-8.00	0.70	12.0	1.00	0.83	12.0	0.86	0.72
Mn	217.6	-2.68	0.41	55.0	57.0	0.61	46.0	27.0	0.50	90.0	22.0	0.24

Table 16. Proportional metal concentrations (metal concentration divided by Long and Morgan ER-L values) for 5 metal contaminants measured in sediments from sites sampled in the Carolinian Province during pilot year studies, summer 1993. The proportions are summed in the last column for possible use as an index of multiple contaminant effects. Site classifications are Reference (R), Unknown (U), and Degraded (D) as described in Table 4.

	STATION	Proportional Metal Concentration (PMC)					Summed PMC
		Cd	Cr	Cu	Pb	Zn	ER-L
	MAS	0.034	0.077	0.026	0.106	0.068	0.310
	INL	0.044	0.091	0.033	0.110	0.090	0.367
	FTJ	0.052	0.132	0.075	0.161	0.153	0.573
	DYT	0.024	0.054	0.020	0.040	0.037	0.174
R	LTH	0.070	0.082	0.028	0.067	0.042	0.288
	OGE	0.040	0.037	0.014	0.059	0.218	0.368
	SAP	0.060	0.091	0.043	0.113	0.088	0.394
	NAS	0.020	0.056	0.017	0.047	0.043	0.183
	TIV	0.080	0.067	0.013	0.047	0.030	0.236
	WKY	0.072	0.278	0.503	0.326	0.411	1.590
	DEY	0.072	0.140	0.779	0.908	1.028	2.927
U	BKY	0.144	0.235	0.267	0.448	0.385	1.479
	PLM	0.060	0.182	0.159	0.300	0.328	1.028
	BRU	0.074	0.989	0.192	0.304	0.437	1.996
	EAU	0.096	0.407	0.720	1.534	0.839	3.595
	CFT	0.142	0.226	0.216	0.583	0.397	1.564
	SLO	1.788	0.697	0.460	1.918	1.087	5.950
	SAM	0.108	0.333	0.307	0.408	0.594	1.750
	SAN	0.042	0.120	0.072	0.141	0.135	0.510
D	SPY	0.118	0.598	0.423	0.596	0.605	2.339
	KOP	0.102	0.408	0.577	0.829	0.796	2.711
	SED	0.066	0.153	0.061	0.181	0.144	0.606
	SAV	0.102	0.314	0.159	0.411	0.539	1.526
	ORT	0.426	0.307	1.038	4.893	3.705	10.369

Table 17. Proportional metal concentrations (metal concentration divided by Long and Morgan ER-M values) for 5 metal contaminants measured in sediments from sites sampled in the Carolinian Province during pilot year studies, summer 1993. The proportions are summed in the last column for possible use as an index of multiple contaminant effects. Site classifications are Reference (R), Unknown (U), and Degraded (D) as described in Table 4.

| | STATION | Proportional Metal Concentration (PMC) | | | | | Summed PMC |
		Cd	Cr	Cu	Pb	Zn	ER-M
R	MAS	0.019	0.043	0.005	0.034	0.030	0.130
	INL	0.024	0.050	0.006	0.035	0.040	0.155
	FTJ	0.029	0.073	0.014	0.051	0.068	0.234
	DYT	0.013	0.030	0.004	0.013	0.017	0.076
	LTH	0.039	0.045	0.005	0.021	0.019	0.129
	OGE	0.022	0.020	0.003	0.019	0.097	0.161
	SAP	0.033	0.050	0.008	0.036	0.039	0.166
	NAS	0.011	0.031	0.003	0.015	0.019	0.079
	TIV	0.044	0.037	0.002	0.015	0.013	0.112
	WKY	0.040	0.154	0.090	0.104	0.183	0.570
	DEY	0.040	0.077	0.140	0.289	0.457	1.003
U	BKY	0.080	0.130	0.048	0.143	0.171	0.571
	PLM	0.033	0.100	0.029	0.095	0.146	0.403
	BRU	0.041	0.546	0.035	0.097	0.194	0.912
	EAU	0.053	0.224	0.129	0.488	0.373	1.268
	CFT	0.079	0.125	0.039	0.185	0.176	0.604
	SLO	0.993	0.385	0.083	0.610	0.483	2.554
	SAM	0.060	0.184	0.055	0.130	0.264	0.693
	SAN	0.023	0.066	0.013	0.045	0.060	0.207
D	SPY	0.066	0.330	0.076	0.190	0.269	0.930
	KOP	0.057	0.225	0.104	0.264	0.354	1.003
	SED	0.037	0.085	0.011	0.058	0.064	0.254
	SAV	0.057	0.173	0.029	0.131	0.240	0.629
	ORT	0.237	0.169	0.186	1.557	1.647	3.796

transformations will tolerate data inaccuracies better than percent data, although most of the recent studies report the percent approach.

Considering all metal contaminants, there are few exceedances of ER-Ms or PELs, or ER-Ls. There is clear evidence that some areas are metal-enriched, but do not exceed sediment criteria defined by Long and Morgan (1990). This was most commonly observed for Cu, Cr, Pb, and Zn, and suggests more wide-spread anthropogenic impact. Sediment criteria are established for each contaminant, but there are no provisions for classifying stations with lower but enriched concentrations of multiple contaminants. Elevations in only a single contaminant are rare, and there are at present no schemes that consider the effects of multiple contaminants. Although additivity is often assumed, synergism and even antagonism may occur. In Chapter 1, arbitrary approaches for making assessments based on multiple contaminants were described in which sites with 3 contaminants that exceeded ER-L values or 6 contaminants that were enriched based on Al normalization were classified as degraded. A more quantitative approach would be desirable.

A quantitative approach was developed based on the proportional contributions of five metal contaminants: Cu, Cr, Pb, Cd, and Zn. These metals were chosen because they are recognized as toxic, but others such as Hg (not measured in this study) would also be appropriate to include. The measured concentrations were divided by the ER-L value (proportional metal concentration, PMC) and then the proportions were summed over the five metals (Table 16). The same computations were also made using ER-M values (Table 17). Sediment criteria based on the summed proportional metal concentrations can be developed for multiple contaminants. For example, a criteria of > 1.0 would clearly discriminate reference sites from sites potentially degraded by metal contamination. More stringent criteria such as 1.5 or 2 would suggest that enough metals may be present to elicit ER-L responses. Another way of using this method would be to refer to the presence of 1 or more ER-L or ER-M equivalents. Obviously this model assumes additivity. A similar scheme could be applied to PAH data.

Polycyclic aromatic hydrocarbons occur naturally as products of fossil fuels such as oil and coal. Low molecular weight PAHs are generally associated with petroleum, whereas the high molecular weight PAHs are combustion products. The first three PAHs in Table 14 (anthracene, fluorene, phenanthrene) are low molecular weight PAHs while the others are high molecular weight PAHs. The major mode of toxicity for low molecular weight PAHs is believed to be due to interference with cellular membrane function and membrane associated enzyme systems (Neff, 1984). Higher weight PAHs are frequently found to be very carcinogenic. Tumorigenic, teratogenic, and mutagenic effects as well as immunosuppressive effects have been demonstrated (Eisler, 1987; Faisal and Huggett, 1993). Metabolic transformations can occur that result in intermediates that may also be highly toxic. Cytochrome P-450 mixed function oxidases can detoxify some PAHS, but others result in the production of even more toxic compounds (Neff, 1984). Toxicity generally increases as molecular weight increases. Benzo(a)pyrene and Benzo(k) fluoranthrene (both high molecular weight PAHs) were the most commonly observed PAH contaminants in the sediment samples.

Contaminant enrichment is clearly demonstrable, but the critical issue of what concentrations affect the biota is less readily determined. A myriad of factors can affect the outcome. Salinity, pH and temperature, as well as bioavailability and detoxification mechanisms, affect toxicity so that multiple contaminant effects are very difficult to model. These issues require cellular and physiological indicators that will enable recognition of populations under stress. Studies with metal binding proteins, mixed function oxidases, and neurotoxicity studies suggest that we may be able to use cellular indices as indicators of metal exposures, PAH exposures, or organophosphate pesticide exposures respectively (Engel and Roesijadi, 1987; Sanders, 1990; Collier et al., 1992; Stegeman, 1992; Bocquene et al., 1993). These are emerging technologies that warrant further development. A model of environmental monitoring that begins with screening the biota for signs of stress may facilitate reductions in costly contaminant screenings at all sites, with the emphasis on detailed analyses of specific sites.

Toxicity Assays

Although sediment analyses can suggest presence of contaminants, the potential for adverse effects is not readily predictable, because the bioavailability of pollutants to organisms is the result of complex physical and biological interactions. Therefore sediment bioassays are regarded as the most direct measure of the potential for contaminant-induced effects on the biota. Bioassays using whole sediment exposures were conducted with four tests, two acute toxicity assays (amphipod and mysid toxicity) and two sublethal assays (Microtox® and seed clam growth).

Sediment bioassays using the amphipod *Ampelisca abdita* have been part of the core program in the Virginian and Louisianian Provinces. Although efforts are made during pilot year programs to sample in some of the most degraded sites (validated by high contaminant levels), the amphipod toxicity assays (with *A. abdita*) did not discriminate these sites from reference sites (Figure 14). Toxicity was observed only at two of our most pristine sites, and was believed to be due to the sandy nature of the sediments, and possibly ammonia problems. There is some concern that contaminant levels in southeastern estuaries may not be high enough to cause amphipod toxicity. It's ability to discriminate between site categories in the Louisianian Province has also been questioned (Summers et al., 1993). This acute toxicity assay may be sufficient only for extremely contaminated conditions.

Mysid assays also did not discriminate between reference and degraded sites. Figure 15 indicates that no sediments collected during the pilot year studies were toxic to *M. bahia*. There is no evidence that this species is more sensitive, and the shorter duration of the test may not be adequate for causing mortality, especially if they undergo behavioral modifications that result in avoidance or reduction in sediment contact.

Microtox assays, based on the attenuation of light production by the photoluminescent bacteria *Photobacterium phosphoreum*, were conducted as a research component during the

Ampelisca abdita

Figure 14. Results of amphipod toxicity assays (*Ampelisca abdita*) conducted with sediments sampled from sites in the Carolinian Province during pilot year studies, summer 1993. * Sediment samples were lost, so no tests were conducted.

Mysidopsis bahia

Figure 15. Results of mysid toxicity assays (*Mysidopsis bahia*) conducted with sediments sampled from sites in the Carolinian Province during pilot year studies, summer 1993. * Sediment samples were lost, so no tests were conducted.

1993 pilot studies. The results indicated that it holds potential as a toxicity assay. Microtox was more successful at discriminating between reference sites and degraded sites than the amphipod toxicity assay (Figure 16A). If EC_{50} = 2% is arbitrarily chosen as a reference line, then the error rate for discriminating reference and degraded sites is approximately 12.5% (compared to a 100% error rate for the amphipod toxicity assays). Recently, Microbics corporation recommended correcting for percent water, and the results when the EC_{50}s were corrected for this factor are shown in Figure 16B. This method converts the EC_{50} value to a dry weight result. This step removes the error associated with water content, and to some degree for sediment type, since siltier sediments will have more water than sands. An EC_{50} = 1% was arbitrarily chosen as a reference line for water corrected values. One degraded site and one reference site would be incorrectly classified and the error rate would then be 8.3%.

The Microtox assay has a number of advantages, including the requirement for a small sediment sample (100 ml compared to 4000 ml for the amphipod assay) and rapidity (assays are completed in a matter of hours). However, the actual amount of sediment used in the assays is very small (0.3 g), so proper homogenization becomes critical and could represent a substantial source of error. The large sample procedure that uses 7 g should be explored and multiple replicates must be conducted. There is also some concern that Microtox may be sensitive to sediment characteristics. On the basis of log transformations of the data, significant correlations were found for % silt-clays (r^2 = 0.60), Al (r^2 = 0.60), and % organics (r^2 = 0.55). The potential for biases due to effects of sediment characteristics should be evaluated more rigorously.

One potential disadvantage of a very short-term assay is that a hormetic response may occur. Hormesis refers to a transient response to altered conditions, which is typically an overshoot or undershoot response. Transient stimulation of some type of response is often observed with organic pollutants and low concentrations of metals (Laughlin et al., 1981; Stebbing, 1981). Although there was no evidence of hormesis, enhanced light production was observed at several sandy sites (LTH, OGE, DEY).

Seed Clam Toxicity

During 1993 pilot studies, a new sublethal assay was developed based on growth of young seed clams (*Mercenaria mercenaria*). This assay was conducted with a limited number of sites and with aged sediments, but in every case, degraded sediments resulted in significantly slower growth rates than with controls (Table 18). The results from the seed clam assays corresponded closely to the results with the Microtox assays. This assay has a number of advantages including requirement for a relatively small sediment sample volume (500 - 1000 ml), and the fact that the endpoint (growth) is a chronic rather than acute index. Seed clams are particularly useful because they have endogenously high rates of growth, so adverse effects would be readily detected. They are readily reared in the laboratory or are often available for a small cost through aquaculture hatcheries.

Figure 16. Results of Microtox assays conducted with sediments sampled from sites in the Carolinian Province during pilot year studies, summer 1993. A. Microtox EC-50s uncorrected for water content. B. Microtox EC-50s corrected for water content.

Table 18. Result of the effects of sediments collected from sites in the Carolinian Province during 1993 pilot year studies on seed clam (*Mercenaria mercenaria*) growth. Juvenile clams (1 mm) were incubated with the sediments for 7 days, and then the effects on total dry weight were evaluated. Site classifications are Reference (R), Unknown (U), and Degraded (D).

Seed Clam Growth			
Station	Class	Growth Rate (ug/day)	% Controls
Breech Inlet	R	20.8 ± 4.2	
Sampit River	D	13.0 ± 1.4	62.5%
Shipyard Creek	D	15.8 ± 0.6	76.0%
Plum Island	U	13.2 ± 3.7	63.5%
Nassau River	R	13.9 ± 3.5	
Ortega River	D	8.8 ± 4.1	52.9%
Eau Gallie	U	14.3 ± 5.1	102.9%
South Edisto	D	9.2 ± 2.0	66.2%
South Santee	D	10.0 ± 2.6	71.9%

Conclusions

Although the data are limited, Table 19 summarizes the outcome of the different toxicity assays as well as whether or not contaminant enrichment was observed. Confidence in any of these indicators is reinforced by associations between them. Three sites (SAN, KOP, ORT) had contaminants that exceeded ER-M values, but showed no toxicity to the *A. abdita*. The two sites that did show toxicity with *A. abdita* were Lighthouse Creek (LTH) and Ogeechee (OGE), reference sites that showed no evidence of contamination or dissolved oxygen stress. It is interesting that these sites were the least toxic based on the Microtox assay, and actually showed stimulation of light production. This would suggest that some inherent component is toxic to *A. abdita*, but stimulatory to *P. phosphoreum*. The toxic effects of ammonia have been recognized as a potential problem in amphipod toxicity assays, but is not believed to be as important with the Microtox assays. Comparative studies with other toxicity assays using fish and Daphnia indicate that the Microtox assay is less sensitive to ammonia than most (Qureshi et al., 1982). The decreased sensitivity to ammonia may be related to pH effects. Optimal pH conditions for Microtox are 6.5 - 7.0, whereas most amphipod toxicity assays are conducted at 7.5 - 8.0. The lower pH conditions of the Microtox test would favor the formation of ionized ammonia rather

Table 19. Associations between the four toxicity tests and degree of contamination. Site classifications are Reference (R), Unknown (U), and Degraded (D) as described in Table 4. X indicates significant toxicity. Blanks indicate no toxicity. Shaded areas indicate tests were not conducted for those sites.

	Station	Seed Clam Toxicity	Microtox	Amphipod Toxicity	Mysid Toxicity
R	MAS	▓			
	INL				
	FTJ	▓	X		
	DYT	▓			
	LTH	▓		X	
	OGE	▓		X	
	SAP	▓			
	NAS				
	TIV	▓			
U	WKY	▓	X		
	DEY	▓	X		
	BKY	▓	X		
	PLM	X	X		
	BRU	▓	X		
	EAU		X		
D	CFT	▓			
	SLO	▓	X		
	SAM	X	X		
	SAN	X	X		
	SPY	X	X		
	KOP	▓	X		
	SED	X	X		
	SAV	▓	X		
	ORT	▓	X		

than the more toxic unionized form, whereas the higher pHs of the amphipod tests would increase the likelihood of toxic ammonia effects. Therefore, it is possible that conflicting results such as that seen with Lighthouse Creek in which high amphipod mortality occurs in the absence of contamination in conjunction with low Microtox toxicity, may represent ammonia effects. It should be noted that contaminant effects may be more pronounced in the Microtox assay because in general lower pHs tend to cause desorption of contaminants from sediment particles and increase bioavailability.

Therefore Microtox® was more effective at discriminating between reference and degraded sites than either the amphipod or mysid assays. Some concerns such as effects of aluminum, sediment sample size, and appropriate use of control sediments should be examined. In general, the assays conducted with seed clams agreed with the results from the Microtox assays. In both of these assays, there were good associations between sediment contaminants and toxicity.

Development of tools for recognition of chronic toxicity potential are needed. Identification of DO patterns and sediment contaminant profiles that may cause long- term stress, and some means of accounting for multiple contaminants are essential. Toxicity assays that use a sublethal endpoint and provide some estimate of chronic toxicity potential must be encouraged. Chronic toxicity causes adverse effects on growth and reproduction, and gradual declines in populations. Our present tools do enable discrimination between the extremes, but are not as effective for the identification of areas showing more subtle symptoms. Indicators of chronic toxicity are essential to the development of long-term proactive monitoring strategies. Often, chronic effects can be reversed, so areas experiencing chronic stress have a higher potential for remediation success. The costs are likely to be less than that required for cleanup of a heavily degraded area, and the likelihood of restoration success will be higher than in those systems that have progressed to the a more extreme stage. The importance of estuaries must be recognized, and there must be commitment to the preservation of their diversity and function. Long-term biomonitoring programs that use chronic bioindicators to monitor their status and trends are required.

CHAPTER 4. BIOTIC CONDITION INDICATORS

INTRODUCTION

The exposure indicators described in the previous chapter provide important tools for the evaluation of exposure and potential toxicity, and are necessary for making comparative statements concerning samples taken from different stations. The final, perhaps most critical question to be considered is whether the indigenous biota are showing evidence of stress. The effects on the organisms that comprise the ecosystems will represent the net sum of contaminant exposure, bioavailability, and interactions between natural and anthropogenic stressors. For example, contaminant levels in a system characterized by salinity, pH, and dissolved oxygen regimes that are stable and high may not be affected, but the same level of contaminants in a system characterized by low dissolved oxygen or acidic conditions may have severe effects on the biota. The results of biotic condition evaluations serve to confirm the potential for toxicity identified from exposure indicators. Moreover, biotic condition indicators should provide some estimate of the degree of degradation.

Indicators of biotic condition should reflect the responses of the organisms to environmental perturbation. These indicators include measurements of the kinds and abundances of the biota present as well as their health and condition. Environmental perturbations may affect the biota in a variety of ways, ranging from no effects to severe acute effects and an extremely depauperate community, characterized by severe reductions in numbers of species and/or abundances. Within this range, a continuum of responses may occur so that anthropogenic perturbations may result in:
- no effects
- conditions that cause physiological effects and induction of homeostatic responses which effectively ameliorate the insults so there are no net adverse effects
- conditions that overwhelm homeostatic mechanisms resulting in chronic stress (adverse effects on growth and reproduction)
- loss of sensitive species or early life stages
- increases in disease and malformations
- loss of critical species and/or tolerant species
- depauperate community

During pilot year studies, four biotic condition indicators were evaluated: benthic assemblages, fish and shellfish assemblages, deployed oyster physiology, and fish reproductive success. An index of benthic community condition is a core EMAP indicator that continues to function as a robust indicator of environmental status. Under extreme conditions (pristine or highly degraded), simple diversity and richness indices will discriminate between reference and degraded sites, but it has been established that the power of this index is extended when measures of functionality are used in multivariate analyses. Trawls were conducted at each station and data were collected regarding species assemblages as well as disease status. Both benthic and trawl parameters have been evaluated for the Virginian and Louisianian Provinces as well as the Carolinian Province.

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The remaining biotic condition indicators involve the use of deployed organisms *in situ* at various sites. Hatchery reared oysters (*Crassostrea virginica*) were deployed at sites in the Charleston Harbor area and the effects on growth, bioaccumulation of contaminants, and disease status were evaluated. Bivalves have been used in various biomonitoring programs because of a variety of attributes such as their widespread distribution, sessile or sedentary lifestyle, and their ability to bioconcentrate contaminants (Phillips, 1980; Farrington, 1983). In programs such as Mussel Watch and NOAA Status and Trends, the contaminant concentrations of individuals that resided at specified sites were measured. These programs have provided valuable information regarding distributions of contaminants in bivalves, but have encountered problems in the interpretation of the data. Attributing the variations in tissue concentrations to pollution impacts has been difficult because factors such as age and reproductive status affect normal levels. Moreover, it is frequently not known if the physiological status of the organisms is compromised, or if the concentrations are within the compensatory ranges. Marine organisms have a range of detoxification mechanisms that can reduce the impact of contaminant exposures. Distinguishing tissue burdens that translate into toxic effects from normal variations in concentrations or tolerable burdens continues to present a major problem. One approach to dealing with these issues is the use of deployed organisms (Salazar and Salazar, 1994), and the use of common stock increases the power of this approach.

Deployed bivalves have not been considered appropriate for broad-scale EMAP sampling because of the uncertainties associated with habitat effects on growth and bioaccumulation, and duration of deployment (Hunsaker and Carpenter, 1990). However with hatchery-reared bivalves, all animals are of the same age and have the same exposure history so deployed animals are more likely to possess similar baseline contaminant concentrations, and the use of juvenile animals circumvents the problems associated with variation due to reproductive status. Moreover, juveniles have endogenously high growth rates (that may override many environmental variables) so effects on growth could be detected in a short time frame. They also have more rapid rates of bioaccumulation of contaminants, and tend to be relatively sensitive to pollutant stress (Boyden, 1974). Therefore, the use of hatchery reared organisms deployed *in situ* provides a valuable means of making valid comparisons in tissue contaminant concentrations, and also identifying when organisms are stressed by habitat conditions.

In the fish studies, conducted by Dr. Ron Kneib (University of Georgia Marine Institute, Sapelo Island), the potential of using reproductive success of *Fundulus heteroclitus* (mummichogs) as an *in situ* bioindicator of the quality of intertidal estuarine environments within the Carolinian Province was explored. Mummichogs are abundant and widely distributed in marshes of the Carolinian Province, where their life history is intimately connected to intertidal estuarine habitats. Adults use the intertidal zone for feeding and spawning and so the mummichog is potentially vulnerable to environmental stresses from both terrestrial and aquatic sources. Intertidal marshes are the principle spawning area and nursery habitat, and numerous distinct cohorts of young are produced with a predictable frequency over a protracted reproductive season (Kneib, 1986 and 1987). Adults forage and spawn in the intertidal zone during flood tides and retreat to intertidal and subtidal creek channels with ebb tides. The relatively large (approximately 2 mm diameter) eggs are typically deposited on emergent

vegetation and empty bivalve eggs high in the intertidal marsh where they are left to incubate primarily in the air and hatch 7-8 days later when inundated by water during spring tides (Able and Castagna, 1975; Taylor et al., 1977; Taylor and DiMichele, 1983). In these studies, newly-spawned eggs of *F. heteroclitus* were deployed in intertidal marshes in Georgia and South Carolina, and the effects on hatching success, embryo survival, and length and weight of hatchlings were evaluated.

The sections regarding benthic communities, fish and shellfish assemblages, and deployed oysters are contained in this chapter. The section regarding *F. heteroclitus* developmental success (prepared by R. Kneib) will be presented as a separate chapter.

METHODS

Fish and Shellfish Assemblages

Fish and shellfish were collected using standard EMAP 16 ft highrise trawl nets (nets were not outfitted with liners or tickler chains). In most cases, two 10 minute trawls were conducted at each station. The contents of the net were sorted and identified, and measured to the nearest cm. Fish and crustaceans were also examined for evidence of gross external pathologies (lumps, tumors, abscesses for fish; shell disease in blue crabs; cotton disease in shrimp).

The trawl data were compiled and indices of diversity (Shannon-Weiner, H-max) were computed. Six functional guilds were defined (opportunistic carnivore, invertebrate carnivore, piscivorous carnivore, detritivore, omnivore, planktivore), and species were categorized. Several fisheries experts on the MRRI staff (J. McGovern, B. Roumillat, G. Steele, and B. Stender) were asked to independently categorize the species. In most cases, the experts agreed, but in those few differences, the majority opinion was accepted. The categorizations as well as the diversity indices were then used in discriminant function analyses to identify those variables that would discriminate between reference and degraded sites.

Deployed Oyster Studies

Hatchery-reared juvenile oysters (approximately 2 cm in height) were deployed at five sites in the Charleston area: Fort Johnson (lower Charleston Harbor), Lighthouse Creek, Brickyard Creek, Shipyard Creek, and Koppers. Dates of deployment began on July 26 for Fort Johnson, Brickyard Creek, and Koppers, and start date for Lighthouse Creek and Shipyard Creek was August 24. Oysters were placed inside rigid Durothene® polyethylene spat bags and secured to poles so that the oysters were suspended approximately 0.5 meters from the bottom. Approximately 800 oysters were initially placed at each site, and sampled periodically over a 3 month period.

The effects on growth were evaluated using both size and weight parameters. For the size measurements, individuals were blotted dry and placed directly on photographic paper, and exposed briefly (1-3 seconds). The photographic paper was developed, resulting in a contact print (Figure 17), from which the lengths (longest distance parallel to the hinge) and heights (distance from hinge to shell margin) were measured with a digitizer. The means and standard deviations of these individual measurements were then computed. For the weight measurements, oysters were shucked, rinsed with cold filtered seawater to remove shell bits and debris, drained and weighed. The wet weights represent weights of composited oysters, and the same samples were then used in contaminant analyses.

The concentrations of metals in oyster tissues were determined by ICP following acid digestion. Samples of dried oyster tissue were digested with nitric acid and hydrogen peroxide in a microwave oven according to National Bureau of Standards methods (Kingston and Jassie, 1986), and filtered. The metal concentrations of the filtrate were then analyzed by ICP. Results are reported as ug/g dry weight. Although standard reference material was not analyzed concurrently with these samples, later analyses with standard oyster tissues (NBS) indicated the following yields: As, 104%; Cd, 70%; Cr, 110%; Cu, 65%; Pb, 125%; Mn, 64%; Ni, 12%; Zn, 75%.

For PAH analyses, composite samples of oysters were dried and extracted with a mixture of methanol, isopropanol, and acetone (2:1:1). A 15% solution of the filtered extract (10-20ml) was then passed through a 500 mg C18 extraction column or Empore disc that had been cleaned by successive rinses with methylene chloride, hexane, ethyl ether, methanol, and 15% extraction solvent. After drying, the C18 column was eluted with methylene chloride and brought up to 1.0ml. The sample was then analyzed on a Tracor model 540 gas chromatograph with a nonpolar (J&W DB-5) capillary column and a flame ionization detector.

Twenty-five oysters from the last sample collection were also processed for evaluation of incidence of Dermo, an oyster disease caused by *Perkinsus marinus*. The incidence of Dermo was determined using the fluid thioglycollate method (Mackin, 1962; Quick and Mackin, 1971; Burrell et al., 1984). Rectal tissue from each oyster was excised, cultured in fluid thioglycollate, and examined after staining with Lugols.

Benthic Assemblages

Bottom samples for the benthic species community indicators were obtained using a 413 cm^2 samples Young-modified Van Veen grab sampler. The sediment and other material in the sample were sieved on a 0.5-mm screen while in the field. The sieving process was conducted so that water was introduced from the bottom of the sieve to avoid damaging soft-bodied organisms. Material collected on the screen was preserved in 10% buffered formalin-rose bengal solution and stored in Nalgene polypropylene containers. Three grabs were collected and processed in the field. All samples were sorted and identified to the lowest taxonomic level by Cove Corporation, Lusby, Maryland.

Figure 17. Copy of a "contact print" used for making size measurements of oysters. Oysters were placed on photographic paper, exposed briefly, and the paper was developed. The dimensions were then measured with a digitizer.

RESULTS AND DISCUSSION

<u>Fish and Shellfish Assemblages</u>

Nektonic communities that are composed of multiple species and feeding types are believed to represent a stable productive food web. The species composition and abundances are affected by the potential food sources of benthic organisms as well as water column characteristics and contaminant inputs. Because fish have relatively long life spans and dominate the upper end of the food web, their responses may integrate multiple environmental perturbations. Fish and shellfish are also important commercial resources, both in terms of harvestable seafood as well as recreational fisheries. Therefore their status is central to public perception of estuarine habitat quality.

There are problems with sampling mobile species, such as patchiness, variations in abundances associated with tidal cycle, catchability, trawl dynamics, etc. During the 1993 pilot studies, successful trawls were conducted at 21 of the 24 stations. At Titusville (TIV), recent tropical storms resulted in large amounts of drift algae, which rapidly filled the net within only a few minutes, so no successful trawls were conducted there. At Ogeechee (OGE), trawl nets were torn by bottom debris. At Whiskey Creek (WKY), the trawl path was too short to allow trawls longer than 3 min. The numbers of individuals from other stations ranged from 4 to 1958, and the number of species ranged from 4 to 21 (Table 20). Penaeid shrimps (primarily *Penaeus aztecus* and *P. setiferus*) were often one of the most abundant components, and blue crabs (*Callinectes sapidus*) were frequently abundant. Fish species that were commonly encountered were *Micropogonius undulatus, Stellifer lanceolatus, Leiostomus xanthurus,* and *Cynoscion regalis.*

Methods for analyzing nektonic community data so that they can be incorporated into assessments of environmental status are not well developed. Generally, the approaches used in the benthic analyses should be applicable to the nektonic communities. However, in many cases, parameters such as trophic guild and pollution sensitivity are unknown, and our ability to predict expected assemblages is very limited at this time. With these limitations in mind and recognizing that the data set from pilot year studies is relatively small, an effort was made to apply the approaches used with the benthic communities to the trawl data. All species captured in the trawls were classified in one of six trophic guilds (Table 21). Discriminant function analyses indicated that three variables, Shannon-Weiner Index (adjusted for salinity), the raw Shannon-Weiner Index, and percent invertebrate carnivores, could be used to discriminate reference from degraded sites (Figure 18). Two sites were misidentified. SPY, a degraded site, was identified as a reference site, and DYT, a reference site was identified as a degraded site.

All fish and shellfish that were collected were examined by the field crews for gross pathologies, such as tumors, lesions, or other abnormalities. There was only one station at which

Table 20. Total numbers of fish and shellfish species and total abundances caught in the Carolinian Province during pilot year studies, summer 1993. * Did not meet criteria for valid trawls. ** Only one trawl was conducted.

| | Trawl 1 | | Trawl 2 | |
Station	Total Species	Total Abundance	Total Species	Total Abundance
MAS	4	7	2	4
INL	7	24	8	22
FTJ **	21	1958		
DYT	9	219	6	276
LTH	11	101	12	95
OGE *	4	12		
SAP	6	28	2	8
NAS	9	111	4	25
TIV *				
WKY*	5	76	8	32
DEY	13	248	14	134
BKY	11	275	12	330
PLM	5	61	5	87
BRU	3	6	7	15
EAU	5	21	10	44
CFT	11	232	11	215
SLO	2	21	5	57
SAM	11	1173	11	491
SAN	11	28	8	104
SPY	11	73	11	242
KOP	14	510	20	938
SED	7	83	9	164
SAV	8	88	8	66
ORT	4	18	6	40

Table 21. Species caught in trawls conducted during pilot year studies in the Carolinian Province, summer 1993. Each species was classified into one of six feeding guilds as shown in the last column.

Scientific Name	Common Name	Family Name	Feeding Guild
Alosa sapidissima	American Shad	Clupeidae	Planktivore
Aluterus schoepfi	Orange Filefish	Balistidae	Opportunistic Carnivore
Anchoa mitchelli	Bay Anchovy	Engraulidae	Planktivore
Archosargus probatocephalus	Sheepshead	Sparidae	Invertebrate Carnivore
Ariopsis felis	Saltwater Catfish	Ariidae	Omnivore
Bagre marinus	Gafftopsail Catfish	Ariidae	Omnivore
Bairdiella chrysoura	Silver Perch	Sciaenidae	Opportunistic Carnivore
Brevoortia tyrannus	Atlantic Menhaden	Clupeidae	Planktivore
Callinectes ornatus	Ornate Blue Crab	Portunidae	Opportunistic Carnivore
Callinectes sapidus	Blue Crab	Portunidae	Opportunistic Carnivore
Callinectes similis	Lesser Blue Crab	Portunidae	Opportunistic Carnivore
Centropristis philidelphica	Bank Sea Bass	Serranidae	Opportunistic Carnivore
Chaetodipterus faber	Spadefish	Ephippidae	Invertebrate Carnivore
Chloroscombrus chrysurus	Atlantic Bumper	Carangidae	Omnivore
Citharichthys spilopterus	Bay Whiff	Bothidae	Opportunistic Carnivore
Cynoscion regalis	Weakfish	Sciaenidae	Piscivorous Carnivore
Dorosoma petenense	Threadfin Shad	Clupeidae	Planktivore
Elops saurus	Ladyfish	Elopidae	Piscivorous Carnivore
Etropus crossotus	Fringed Flounder	Bothidae	Invertebrate Carnivore
Eucinostomus argenteus	Spotfin Mojarra	Gerreidae	Opportunistic Carnivore
Eucinostomus gula	Silver Jenny	Gerreidae	Opportunistic Carnivore
Fundulus heteroclitus	Mummichog	Cyprinodontidae	Omnivore
Hypsoblennius hentzi	Feather blenny	Blenniidae	Opportunistic Carnivore
Ictalurus catus	White Catfish	Ictaluridae	Detritivore
Lagodon rhomboides	Pinfish	Sparidae	Opportunistic Carnivore
Leiostomus xanthurus	Spot	Sciaenidae	Invertebrate Carnivore
Lutjanus sp.	Snapper	Lutjanidae	Piscivorous Carnivore
Menticirrhus americanus	Southern Kingfish	Sciaenidae	Invertebrate Carnivore
Micropogonius undulatus	Atlantic Croaker	Sciaenidae	Opportunistic Carnivore
Ophichthus gomesi	Snake Eel	Ophichthidae	Opportunistic Carnivore
Opsanus tau	Oyster Toadfish	Batrachoididae	Omnivore
Orthopristis chrysoptera	Pigfish	Haemulidae	Opportunistic Carnivore
Paralichthys dentatus	Summer Flounder	Bothidae	Opportunistic Carnivore
Paralichthys lethostigma	Southern Flounder	Bothidae	Opportunistic Carnivore
Penaeus aztecus	Brown Shrimp	Penaeidae	Detritivore
Penaeus duorarum	Pink Shrimp	Penaeidae	Detritivore
Penaeus setiferus	White Shrimp	Penaeidae	Detritivore
Peprilus paru	Harvestfish	Stromateidae	Invertebrate Carnivore
Rhinoptera bonasus	Cow Nose Ray	Myliobatidae	Invertebrate Carnivore
Rhizoprionodon terranovae	Atlantic Sharpnose Shark	Carcharinidae	Opportunistic Carnivore
Selene setapinnis	Atlantic Moonfish	Carangidae	Piscivorous Carnivore
Selene vomer	Lookdown	Carangidae	Piscivorous Carnivore
Sphoeroides maculatus	Northern Puffer	Tetraodontidae	Invertebrate Carnivore
Stellifer lanceolatus	Star Drum	Sciaenidae	Opportunistic Carnivore
Stenotomus aculeatus	Longspine Porgy	Sparidae	Opportunistic Carnivore
Stephanolepis hispidus	Planehead Filefish	Balistidae	Opportunistic Carnivore
Symphurus plagiusa	Blackcheek Tonguefish	Cynoglossidae	Detritivore
Syngnathus fuscus	Northern Pipefish	Syngnathidae	Invertebrate Carnivore
Synodus foetens	Inshore Lizardfish	Synodontidae	Piscivorous Carnivore
Trichiurus lepturus	Cutlassfish	Trichiuridae	Piscivorous Carnivore
Trinectes maculatus	Hogchoker	Soleidae	Detritivore

TRAWL DATA. DISCRIMINANT FUNCTION ANALYSIS

Figure 18. Results of discriminant function analyses of trawl data. Three variables were used: Shannon-Weiner adjusted for salinity, raw Shannon-Weiner, and percent invertebrate carnivores. A reference line is drawn at zero. Stations to the right of the line were identified as degraded, and those to the left were reference.

fish with abnormalities were caught. All specimens of *Micropogonius undulatus* that were caught in the Sampit River had hemorrhagic fins. With shellfish, two types of disease pathologies were noted.

Shrimp were examined for the presence of "cotton" disease, which appears as white fibrous growths subcutaneous to the carapace. The cause of cotton disease (or milk disease as it is sometimes referred to) is believed to be microsporidian parasites (Johnson, 1975 and 1989). Although it tends to be more common in white shrimp (*Penaeus setiferus*), cotton disease has also been observed in other Penaeid species. Shrimp with cotton disease were noted at 4 degraded sites (SPY, SED, SAV, CFT) and one unknown site (DEY) that is metal enriched.

In blue crabs (*Callinectes sapidus*), shell disease is sometimes observed, which can range from rust-like spots on the carapace and appendages to large ulcers and loss of portions of their bodies. The etiology is uncertain, but a number of pathogens (chitinoclastic bacteria belonging to *Vibrio* and *Pseudomonas* genera, and fungi) have been reported from lesions (Johnson, 1983). The occurrence of blue crabs with shell disease is shown in Table 22. No diseased crabs were caught at reference sites. All of the diseased crabs caught in NC were from Clubfoot Creek, and 3 of the 4 diseased crabs caught in FL were from Ortega River. Increased incidences of shell disease have been reported from polluted environments (Young and Pearce, 1975), and there is some evidence that immunological function is compromised (indicated by decreased anti-bacterial activity of blood) in crabs from impacted areas (Noga et al., 1990).

A summary of pathological findings is shown in Table 23. All diseased or abnormal organisms were found at either degraded sites or those unknown sites that are possibly degraded. Although the incidence of pathologies is relatively rare, the presence of diseased organisms can be used as powerful indicators of stress. There is a growing body of evidence that pollutant-associated diseases are increasing in fish and shellfish populations (Sinderman, 1993). EMAP has focused primarily on fish disorders, but the occurrence of shrimp and crab diseases should also be included.

Deployed Oyster Studies

Deployed oysters were used as *in situ* indicators for evaluating the effects of habitat condition on the biota. Growth is a good indicator of environmental effects because it represents an integrated response of physiological and chemical processes (Widdows, 1985; Ringwood, 1992). Because oysters were deployed at Fort Johnson (FTJ), Brickyard (BKY), and Koppers (KOP) at the same time, they will be discussed together. It should be noted that at the FTJ site, crab predation was very intense. At the first sampling day, large quantities of shell debris were found in the bag as well as a large stone crab (*Menippe mercenaria*) and numerous other crabs, which had consumed more than half of the deployed oysters. Therefore most of the comparative

Table 22. Summary of blue crabs (*Callinectes sapidus*) caught during pilot year studies in the Carolinian Province, Summer 1993. The number of trawls, crab abundances, and number of diseased individuals are listed.

STATE	TRAWLS	CRAB ABUNDANCE			DISEASED		
		TOTAL	FEMALE	MALE	TOTAL	FEMALE	MALE
NC	8	67	23	44	3	1	2
SC	23	183	89	94	4	0	4
GA	7	4	1	3	0	0	0
FL	8	16	4	12	4	0	4

Table 23. Summary of diseases and pathologies observed in fish and shellfish caught in trawls conducted during pilot year studies in the Carolinian Province, Summer 1993.

STATION	STATE	CLASS	SHRIMP Cotton Disease	CRAB Shell Disease	FISH Abnormalities
CFT	NC	D	X	X	
SPY	SC	D	X		
SED	SC	D	X	X	
DEY	SC	U	X		
PLM	SC	U		X	
SAM	SC	D		X	X
SAV	GA	D	X		
ORT	FL	D		X	
EAU	FL	U		X	

statements about this deployment set will refer to Brickyard Creek and Koppers. Likewise oysters were deployed at Lighthouse Creek (LTH) and Shipyard Creek (SPY) at the same time so these sites are treated as a set.

Both weight and size parameters are commonly used to evaluate growth, and in bivalves, shell size is frequently measured. Tissue weight (wet weight, g/oyster) was more sensitive than shell size parameters as an indicator of growth effects (Figures 19 and 20). Adverse effects on length or height of oysters deployed at KOP were not detectable before 60 days. In fact, for the first 45 days, oysters at KOP were the same size or larger than those from BKY. However tissue weights of oysters deployed at KOP were only 79% of that of oysters deployed at BKY after only 22 days. By 45 days, oysters from KOP weighed only 66% of those at BKY, and these differences increased over time (Figure 19).

At SPY, adverse effects on shell height but not length were detected by 30 days, but severe adverse effects on weight were observed by this time. Shipyard oysters had shell heights that were 81% of LTH oysters, but tissue weights of SPY oysters were only 60% of those at LTH. In Figure 20, it is apparent that the SPY oysters were so stressed that no increases in tissue weight occurred at this site during deployment. Losses in tissue weight at both sites were observed for the last two sample periods, but different causes are suspected. Weight losses in SPY oysters are believed to be due to stress, whereas weight decreases in LTH oysters are believed to be due to cage fouling effects. During later samplings of oysters deployed at LTH, it was observed that the bags were heavily encrusted with tunicates and bryozoans, both of which would compete with oysters for available food. No fouling was observed on cages deployed at SPY. Also, mortalities at SPY were > 50%, compared to < 10% at LTH.

The growth studies indicate that oysters deployed at degraded sites had significantly slower growth rates; that weight is a better indicator of stress than size; and 3 - 4 weeks is an adequate exposure period for the detection of stress effects. The growth rates of oysters deployed at different stations are listed in Table 24. These values are based on the first sample date (22 or 30 days), since declines in growth and fouling effects reduced the usefulness of later sampling dates. The growth rates of LTH and BKY are remarkably similar. Although both sites support abundant natural populations of oysters, LTH is a sandier, high salinity (32 ppt), more exposed site, whereas BKY is a muddy, less saline (15 ppt), more protected tidal creek. It is intriguing to consider that these rates may represent optimal growth rates in oysters of this age that are under strong endogenous controls. Genetic predisposition for rapid growth at this age may serve to facilitate the use of deployed bivalves in a variety of habitat types. It is clear from this table that virtually no growth was observed in oysters deployed at Shipyard Creek, and growth at the other degraded site (KOP) was < 50%..

Bivalves molluscs have been identified as valuable indicators of pollution because of their pronounced tendencies for bioaccumulation of contaminants (Boyden, 1974; Phillips, 1980; Farrington, 1983). In these studies, integration of tissue contaminant concentrations in conjunction with growth data should provide a powerful means of considering causal factors, or at least determining if adverse effects on growth are more likely the result of natural

DEPLOYED OYSTER GROWTH
Brickyard Creek, Koppers, and Fort Johnson

Figure 19. Growth of oysters deployed at Brickyard Creek, Koppers, and Fort Johnson during pilot year studies in the Carolinian Province, summer 1993. Tissue weight and shell size parameters are shown. ◆ Brickyard Creek, ● Koppers, ■ Fort Johnson.

DEPLOYED OYSTER GROWTH
Lighthouse Creek and Shipyard Creek

Tissue Wet Weight

Shell Height

Shell Length

Figure 20. Growth of oysters deployed at Lighthouse Creek and Shipyard Creek during pilot year studies in the Carolinian Province, summer 1993. Tissue weight and shell size parameters are shown. ● Lighthouse Creek, ■ Shipyard Creek.

environmental variations or due to anthropogenic factors. Bivalves accumulate metals at concentration-dependent rates, bioconcentrating metals several orders of magnitude over exposure concentration (Ritz et al., 1982; Ringwood, 1989). PAHs are sometimes difficult to measure in organisms because they can be readily transformed by mixed function oxidases. The activities of these enzymes are believed to be relatively low in molluscs, so PAH concentrations in bivalve tissues may be useful for identifying PAH exposure.

Table 24. Growth rates (based on tissue wet weight) and disease status of oysters deployed during pilot year studies in the Carolinian Province, summer 1993.

Station	Classification	Growth Rate (mg / day)	Disease (Dermo)
LTH	R	10.3	30%
SPY	D	0.7	68%
KOP	D	4.4	48%
BKY	U	9.1	36%

The concentrations of PAHs from deployed oysters are listed in Table 25. Acenapthene occurred commonly in oysters from all sites. KOP oysters had the highest incidence of PAHs. In general, there were no well-defined patterns or trends. There is the potential that PAH metabolizing systems are operable, so screening for PAH metabolites or cytochrome P450s should be conducted so that tissue data can be interpreted appropriately.

Metals are more readily measured in oysters because they are elements and are not transformed as are PAHs. Measurement of metals in oyster tissues provides an important means of evaluating the bioavailability of environmental contaminants, and is a good general indicator of anthropogenic impacts. The concentrations of metals measured in deployed oysters are listed in Table 26. The initial or control values were the concentrations of metals in the oysters at the time of deployment. Comparisons between sites are most readily made by considering the mean concentrations of the metals over the deployment period for each site. Plots of these mean values for some of the metals are shown in Figure 21. Oysters deployed at SPY had elevated concentrations of Cr and Ni, and slight elevations of Cd, Cu, and Pb as well. Shipyard Creek is known to receive inputs of Cr as well as other metals associated with steel processing. At the other contaminated site, KOP, elevated concentrations of Cu were found. Elevated levels of Zn were found in oysters from BKY but no adverse effects on growth were observed, so it is probable that excess Zn was sequestered by cellular components such as metallothioneins (Engel and Brouwer, 1989).

The incidence of Dermo, a bivalve disease caused by the protistan parasite *Perkinsus marinus*, is increasing all along the Atlantic seaboard, and is believed to be one of the major factors in the decline of the Chesapeake oyster fishery (Sinderman, 1990). It has been hypothesized that the increase in Dermo in bivalves may be related to increased anthropogenic

Table 25. The concentrations of PAH's measured in tissues of oysters deployed during pilot year studies in the Carolinian Provin Province, summer 1993. Concentrations are reported as μg/g dry weight.

	Acenapthene	Anthracene	Pyrene	B(a)Pyrene	B(a)Anthracene	Chrysene	Acenaphthalene	B(b)Flouranthene	B(k)Flouranthene
INITIAL	42.7	14.8		4.6					
KOP									
8-17-93	67.1	26.8	152.7						
9-9-93	61.5	16.5		8.6					
10-1-93	47.8	12.5	29.9		3.2	4.0			
10-21-93									
FTJ									
8-17-93	38.0			4.0					
9-9-93	52.2	15.8	36.6	12.1				12.0	
10-1-93	47.2								
10-21-93				4.9					
BKY									
8-19-93	62.6	22.8	178.7						
9-9-93	58.1	21.8							
10-1-93	50.7								
10-1-93	35.1								
10-21-93									
LTH									
9-23-93	31.3								
10-14-93	43.6						9.9		
11-4-93			3.4						
.									
SPY									
9-22-93	41.3								
10-14-93	23.8	12.2	18.4						
11-4-93								74.3	9.6

Table 26. The concentrations of metals measured in tissues of oysters deployed during pilot year studies in the Carolinian Province, summer, 1993. Concentrations are reported as µg/g dry weight. Deployment date was July 26 for KOP, BKY, and FTJ; deployment date for LTH and SPY was August 24, 1993.

SITE	As	Cr	Zn	Cd	Pb	Cu	Ni	Fe	Mn	Al
Initial	25.30	3.25	833.00	1.33	2.18	77.25	0.65	147.08	11.04	105.15
KOP										
8-17-93	13.80	1.70	1442.22	1.14	1.56	205.09	0.00	267.65	16.05	219.24
9-9-93	28.69	5.69	1319.75	4.31	5.19	185.38	2.87	87.12	8.75	46.87
10-1-93	10.24	0.98	1243.68	1.10	1.53	130.42	0.00	265.81	14.54	320.84
10-21-93	8.90	1.25	951.72	0.96	1.25	107.33	0.17	138.98	8.03	87.10
BKY										
8-19-93	11.50	2.25	1404.75	1.25	3.06	67.15	0.00	332.37	12.51	177.00
9-9-93	8.21	5.40	1404.08	1.02	1.22	72.08	1.32	82.79	7.58	49.11
10-1-93	11.19	1.02	1752.09	1.77	1.27	85.48	0.00	204.00	10.07	221.14
10-21-93	21.28	2.20	2941.12	2.73	3.62	117.73	0.00	350.96	12.55	170.05
FTJ										
8-17-93	15.74	1.42	777.36	1.62	1.05	57.58	0.00	135.96	11.77	113.43
9-9-93	11.76	7.22	999.72	0.94	1.37	68.24	0.54	166.36	13.62	148.90
10-1-93	21.04	3.00	868.48	1.77	2.20	68.69	0.00	384.85	16.34	317.60
10-21-93	27.03	3.43	889.97	1.78	1.48	65.52	0.93	122.50	13.78	75.45
LTH										
9-23-93	22.67	3.19	695.40	1.30	1.00	50.42	0.00	204.67	13.95	277.61
10-14-93	30.02	2.62	678.67	2.72	2.17	49.45	0.00	425.92	24.07	453.95
11-4-93	25.20	2.88	357.09	1.23	2.19	25.74	1.36	244.41	16.31	158.92
SPY										
9-22-93	25.80	17.20	1217.58	1.68	2.52	126.07	8.97	255.80	16.92	158.02
10-14-93	24.05	6.19	858.14	1.80	3.75	95.43	4.60	237.68	16.65	192.77
11-4-93	31.20	5.70	864.45	3.25	3.25	104.05	2.20	254.55	14.05	155.40

Metal Accumulation in Deployed Oysters

Figure 21. Metal concentrations in tissues of oysters deployed in the Carolinian Province during pilot year studies, summer 1993.

Metal Accumulation in Deployed Oysters

Figure 21. (continued).

stress (Sinderman, 1993). The incidence of Dermo in deployed oysters was higher at the degraded sites (Table 24). A group of oysters from the cohort that was deployed was retained in holding tanks at the laboratory. The incidence of Dermo for these laboratory oysters ranged from 8 - 20% during the deployment period.

The potential success of this approach is attributable to the use of hatchery-reared organisms that are pre-reproductive juveniles. These organisms are the same age, with the same exposure histories, and have endogenously high growth rates. Growth is an important physiological indicator that represents the net effects of pollutants, and is more likely to provide a valid estimate of potential chronic toxicity than those based on acute toxicity studies. Bioaccumulation data can provide valuable information that is important to the interpretation of sediment contaminant data. Although contaminants may be detected in sediments, the effects on the organisms and environmental impact will be a function of their bioavailability.

The incidence and prevalence of diseases in marine organisms should be carefully monitored, and may be valuable indicators of stress. Animals may be rendered more susceptible to disease because of adverse effects of pollutants on immune function. Fish, crustacean, and bivalve diseases appear to be increasing, and it has been hypothesized that chronic anthropogenic stressors are one possible or contributing cause. Studies regarding immune responses at the cellular level are needed to provide insight into mechanisms associated with impaired immune function and increases in disease.

Benthic Assemblages

Estuarine benthic assemblages have many attributes that make them reliable and sensitive indicators of ecological condition and pollution stress (Boesch and Rosenberg, 1981; Bilyard, 1987). Benthic species frequently have limited mobility, and cannot avoid exposure to pollutants and adverse conditions. Benthic organisms live in bottom sediments, where chemical contaminants accumulate and low dissolved oxygen stress is most severe (e.g., Holland et al., 1977; Mirza and Gray, 1981; Chapman et al., 1987). Benthic assemblages are taxonomically diverse, and are composed of multiple feeding modes and trophic levels. Different species display a broad range of physiological tolerances and respond to stress in variable ways (Pearson and Rosenberg, 1978; Rhoads et al., 1978; Sanders et al., 1980; Boesch and Rosenberg, 1981; Rygg, 1986; Swartz et al., 1985). Finally, benthic organisms are important links between primary producers and higher trophic levels including economically important species (Virnstein, 1977; Holland et al., 1980; Baird and Ulanowicz, 1989), and their feeding and burrowing activities affect estuarine oxygen, carbon, nutrient, and sediment cycles (Rhoads and Young, 1970; Rhoads, 1974; Cloern, 1982; Blackburn and Henriksen, 1983).

Benthic species composition and abundance have been used to measure the status and trends of estuaries for several decades (Sanders, 1956; Rosenberg, 1976; Boesch, 1973; Pearson and Rosenberg, 1978; Boesch and Rosenberg, 1981; Holland et al., 1987), and many attempts have been made to identify characteristics of benthic assemblages that represent environmental

quality and have broad applicability (Sanders, 1968; Grizzle, 1984; Warwick, 1986). Previously proposed measures include: (1) biodiversity and species richness, (2) species composition, (3) changes in the relative abundance and productivity of functional groups, (4) changes in the relative abundance and productivity of "key" species, (5) changes in biomass, and (6) changes in the relative size of biota. When proposed measures have been evaluated at broad spatial scales, they have generally been shown to have limited applicability at assessing environmental quality (Hawthorne and Dauer, 1983). A major limitation to the development of national and regional scale environmental quality indicators based upon estuarine benthic communities is that natural variations in environmental conditions can have major effects on abundance and distributional patterns (e.g., Carriker, 1967; Rhoads, 1974). Salinity, sediment characteristics, and latitudinal gradients are particularly important environmental factors known to have major effects on benthic species composition and abundance (Carriker, 1967; Gosner, 1971; Boesch, 1977; Holland et al., 1987).

The abundances and numbers of species found in each grab are listed in Table 27. The results when the data are summed over all three grabs are shown in Figure 22. Eight of the nine degraded stations had less than 150 individuals. Although the number of individuals collected at Sampit River was high (760), almost all of the organisms were oligocheates. Only one reference station was characterized by low abundance. The total number of species were also reduced at degraded sites. Eight of the nine degraded stations had less than 10 species, compared to only 1 reference site with less than 10 species.

These simple parameters were somewhat effective at discriminating degraded from reference sites. However categorizing the different species into functional groups (i.e. pollution sensitive species, pollution tolerant species, head down feeders, etc.) and performing discriminant function analyses similar to those conducted with the trawl data should improve the utility of benthic data as an indicator of biotic condition. With this approach a benthic index will be developed using the data from this study as well as data generated during 1994 studies. The larger data set from both years will enable a more valid development of a benthic index that is expected to have a broader application.

Table 27. Summary of benthic data from grabs taken during Pilot Year Studies, summer 1993.

	ABUNDANCE (Number of Individuals per Grab)			SPECIES RICHNESS (Number of Species per Grab)		
	Grab 1	Grab 2	Grab 3	Grab 1	Grab 2	Grab 3
MAS	48	159	217	19	31	36
INL	63	45	98	18	17	19
FTJ	208	117	156	13	8	13
DYT	39	55	15	12	8	5
LTH	56	50	82	5	5	11
OGE	77	50	63	4	5	3
SAP	92	118	131	13	12	16
NAS	75	186	163	8	11	9
TIV	112	136	82	24	35	17
WKY	249	201	196	30	12	15
DEY	49	15	20	10	6	9
BKY	207	293	314	10	11	8
PLM	126	50	55	10	14	8
BRU	608	258	129	14	12	10
EAU	21	8	17	4	3	3
CFT	70	13	39	6	4	4
SLO	24	2	0	2	1	0
SAM	301	320	139	3	5	5
SAN	23	37	47	7	9	6
SPY	59	41	19	2	3	4
KOP	22	31	28	5	5	5
SED	5	6	22	3	3	3
SAV	23	9	3	1	2	1
ORT	12	13	9	3	1	1

Figure 22. Total abundance (A) and species richness (B) of benthic communities sampled during Pilot Year studies, summer 1993. Data are combined over the 3 replicate grabs.

CHAPTER 5. REPRODUCTION SUCCESS OF THE KILLFISH *FUNDULUS HETEROCLITUS* AS A BIOINDICATOR OF NATURAL AND ANTHROPOGENIC STRESSES WITHIN INTERTIDAL MARSHES

INTRODUCTION

The purpose of this research was to evaluate the potential of using reproductive success of the common mummichog *Fundulus heteroclitus* as an *in situ* bioindicator of the quality of intertidal estuarine environments within the Carolinian Province. Mummichogs are abundant and widely distributed in marshes of the Carolinian Province, where their life history is intimately connected to intertidal estuarine habitats. Adults use the intertidal zone for feeding and spawning and so the mummichog is potentially vulnerable to environmental stresses from both terrestrial and aquatic sources. Adult *F. heteroclitus* do not travel large distances within estuaries, and so it may be assumed that individuals captured at a particular site have been exposed to the environmental conditions there for most of their lives. Adults are very hardy and can tolerate wide variation in environmental conditions, but chronic exposure to environmental stress could be expected to show first in the reproductive success of a population.

The intertidal marsh is the principal spawning area and nursery habitat for *Fundulus heteroclitus*, which produces numerous distinct cohorts of young with a predictable frequency over a protracted reproductive season (Kneib, 1986, 1987). Adults forage and spawn in the intertidal zone during flood tides and retreat to intertidal and subtidal creek channels with the ebbing tide. In Georgia marshes, spawning occurs primarily on the high amplitude spring tides that are associated with the full and new moon stages of the lunar cycle from March until September, resulting in the annual production of 11-12 distinct cohorts of eggs and young (Kneib, 1986). There is a cessation in spawning for a 6-wk period beginning about mid-May, during which the adult population replenishes energy reserves that were depleted in the spring spawning effort; spawning then resumes from July through September (Kneib, 1986).

The relatively large (~2 mm diam.) eggs usually are deposited on emergent vegetation and in empty bivalve shells high in the intertidal marsh (Able and Castagna, 1975, Taylor and DiMichele, 1983) where they are left to incubate primarily in the air (Taylor *et al.,* 1977). Embryological development requires as little as 7-8 days at 25°C (Taylor *et al,* 1977), but hatching is delayed until the embryos are immersed in water (DiMichele & Taylor 1980). In the field, hatching may be delayed for several days until embryos are inundated by the series of spring tides following those on which they were spawned, ~2 wk earlier (Taylor *et al.,* 1977).

After hatching the young remain in puddles of residual tidal water that form in low-lying areas between clumps of intertidal vegetation as the tide recedes. Here they have a refuge from high densities of adult mummichogs and other predators that become concentrated in the adjacent creek channels at low tide (Kneib, 1987). Aquatic microhabitats (i.e. tidal puddles) on the marsh surface are more abundant during the spring than neap portion of the tidal cycle, which has a profound influence on the survival of mummichog cohorts. Adult *F. heteroclitus*

can tolerate a wide range of environmental conditions (Dunson *et al.*, 1993), but survival through the early life history stages is dependent on maintaining a delicate coordination between the reproductive biology of this species and the tidal cycle. If development is delayed and the young hatch when tidal amplitude is declining toward neap tide, the chances of survival are greatly reduced (Kneib, 1993). At the foundation of the present research, which explored the potential for using the early life history stages of this species as a bioindicator of environmental stress, was the premise that factors causing hatching to occur too early or too late will result in the loss of a cohort's contribution to the annual recruitment of the population.

MATERIALS AND METHODS

Study Area

The research was conducted at 10 intertidal marsh locations during March - May 1994. Eight of the sampling locations were in Georgia: 6 'clean' estuarine sites on Sapelo Island and 2 'polluted' (high mercury levels) sites on the Turtle River near the city of Brunswick (Figure 23). Also included were two sites near Charleston, SC (Koppers Creek and Long Creek). Salinity (using optical refractometer) and oxygen (YSI Model 58 meter) measurements were taken during visits to the sampling locations in Georgia and the same variables were monitored by Hydrolab Datasonde recorders located near the SC locations.

Newly-spawned eggs of *Fundulus heteroclitus* were exposed to the field conditions at each of the sampling locations for 6 days, then returned to the laboratory at the Marine Institute at Sapelo Island to complete embryological development. There were 5 separate runs of the experiment, coinciding with peaks in the spawning activity of the *F. heteroclitus* population. All 10 of the sampling locations were not included in each experimental run. The SC locations were included only in Run 5 and the locations near Brunswick, GA were included only in Runs 2-4 (Table 28). The response variables measured in this experiment included: (1) proportion of embryos surviving 6-d exposure to field conditions, (2) proportion of embryos hatching between 12 and 14 days post-spawn, (3) proportion of embryos hatching between 12 and 20 days post-spawn, (4) standard (tip of snout to end of vertebral column) and total length (tip of snout to end of caudal fin) of hatchlings, and (5) average dry weight of hatchlings.

Stocks of Eggs

Eggs of *Fundulus heteroclitus* were obtained by allowing adult fish to spawn without artificial inducement using a system developed in a previous series of experiments with this species (Kneib and Parker 1991, Kneib, 1993). Every 2 wks beginning 6-7 d before expected peaks in spawning activity (associated with new and full moons), adult fish were collected in baited minnow traps on incoming tides from intertidal creeks on Sapelo Island, Georgia. Approximately 90 adult fish (50-80 mm TL) were held at a sex ratio of 3♀:2♂ in each of six 80-liter fiberglass aquaria, containing spawning boards (wooden blocks with a series of 4 mm wide slots, held on the bottom of the tank by a pavement brick) that provided a suitable substrate into

Sapelo Island, GA Sites:

UD = Upper Duplin
TD = Top of Duplin
LL = Lumber Landing
KF = Kenan Field
ML = Marsh Landing
OC = Oakdale Creek

Brunswick, GA Sites:

GP = Georgia Pacific
BI = Blythe Island

Figure 23. Sampling locations in Georgia -- Sapelo Island and Brunswick (Turtle River).

Table 28. Sampling locations and physical measurements. Sampling locations involved in each run are indicated (✓).

Sampling location	Salinity range ppt (n)	O$_2$ range mg/l (n)	Run number 1	2	3	4	5
Sapelo Island, GA sites							
Upper Duplin	16-21 (22)	1.04-3.47 (4)	✓	✓	✓	✓	✓
Top of Duplin	0-15 (21)	2.75-4.06 (3)	✓	✓	✓	✓	
Lumber Landing	0-18 (22)	4.98-5.51 (4)	✓	✓	✓	✓	✓
Kenan Field	14-20 (21)	3.53-3.98 (2)	✓	✓	✓	✓	
Oakdale Creek	2-16 (21)	3.24-5.26 (3)	✓	✓	✓	✓	
Marsh Landing	13-23 (22)	4.69-7.33 (4)	✓	✓	✓	✓	✓
Brunswick, GA sites							
Georgia Pacific	15-18 (6)	4.68 (1)		✓	✓	✓	
Blythe Island	16-20 (6)	5.02 (1)		✓	✓	✓	
Charleston, SC sites							
Koppers Creek	19-24 (268)	2.24-7.76 (268)					✓
Long Creek	20-28 (326)	3.80-7.55 (326)					✓

which the fish deposited their eggs. Fish were fed an ad libitum diet of grass shrimp (*Palaemonetes pugio*). Eggs were harvested twice daily (once in the morning and again in the evening) and placed on moist paper towels in plastic petri dishes. Fertilized eggs were placed at the field locations within 36 hr of being spawned. After the spawning peak, the adult fish were released and replaced with new individuals for the next experimental run.

Experimental protocol for bioassay

Fundulus heteroclitus eggs are normally deposited in protected intertidal sites (e.g. empty bivalve shells or within leaf sheaths of cordgrass *Spartina alterniflora*). An egg chamber (Figure 24) was constructed by modifying a 7 ml high-density polyethylene vial to provide exposure conditions similar to those normally experienced in the field and to allow the easy deployment and retrieval of embryos. A 1 cm hole was drilled in the screw cap of each vial and covered with a circular piece of polyester. A 0.5 cm hole was drilled in the bottom of the vial, which was then filled 1/3 full of polyester filter fiber material; the filter fiber blocked the opening at the bottom of the vial and retained water to provide a moist environment for the eggs when the chambers were not immersed by the tide. A total of 4 chambers, each containing 25 fish embryos (<36 hr old), were attached by plastic cable ties to a 1.5 cm diameter wooden dowel (60 cm in length); a pair of chambers was attached at each of two elevations above the marsh surface (5 cm and 25 cm). The dowels were attached to wooden stakes (Figure 24) that were permanently located at sites within each sampling location. This arrangement essentially mimicked the natural placement of eggs on the stems of emergent marsh vegetation.

Three stakes (~10 m apart) were placed along an intertidal creekbank (near the edge of the emergent marsh vegetation) at each sampling location. An additional set of 3 stakes (parallel to the first) was placed at a higher intertidal elevation within the vegetated marsh at 3 locations on Sapelo Island (Upper Duplin, Kenan Field and Marsh Landing), 1 location in Brunswick (Georgia Pacific) and both locations in the Charleston area. Finally, a third set of 3 stakes was placed even higher (and further from the creekbank) in the intertidal zone at the Upper Duplin and Kenan Field locations on Sapelo Island.

Rarely were enough eggs spawned on a single day to deploy all egg chambers simultaneously. Consequently, the egg chambers were usually deployed over a 2-3 day period, with each day providing eggs for one replicate (stake) at each intertidal site within a sampling location. For example, the first eggs produced by the captive adult fish during a given spawning episode (run) were placed in the field on one stake at each intertidal site, eggs produced on the following day were placed on a second stake at each intertidal site, and those produced on the third day of a spawn were placed on the third stake at each site. Embryos were retrieved from the field on the same staggered schedule after they had been exposed to conditions at each field location for 6 days. Sufficient eggs were available to support 3 replicates at each intertidal site in Runs 1, 2, and 4 and two replicates in Run 2 (Table 29). In Run 5, all replicates were composed of eggs spawned on a single day.

Figure 24. Drawing of egg chamber and stakes used to expose Fundulus heteroclitus embryos to intertidal conditions at each field location. Egg chambers were attached by plastic cable ties to wooden dowels that could be removed from wooden stakes permanently installed at each field site.

Three groups of ~100 control eggs were retained each day and maintained on moist paper towels in plastic petri dishes at ambient outdoor temperatures. These were rinsed daily with artificial (Instant Ocean) seawater of either 5, 15 or 30 ppt.

After 6 days, the exposed embryos were returned to the laboratory where, like the controls, they were held at ambient outdoor temperatures in plastic petri dishes lined with moist paper towels. Beginning at 12 days post-spawn, all embryos were immersed in water (15 ppt) for 30 min each day to simulate tidal inundation, which normally triggers hatching. The number of larvae hatching each day was recorded and the newly-hatched larvae were immediately preserved in 10% formalin. The preserved specimens were later measured for standard length (tip of snout to end of vertebral column) and total length (tip of snout to end of caudal fin) to the nearest 0.1 mm. Groups of hatchlings from both egg chambers at each height on a replicate stake were dried to constant weight at 60°C and weighed to the nearest 0.01 mg. Dividing the group weight by the number of larvae in the group yielded an average individual larval weight for each replicate stake (and height above the marsh).

Table 29. Spawning dates of eggs used in each run.

Run	Spawning dates of *Fundulus*	Initial no. of eggs placed in:	
		Controls	Field sites
1	14-16 March 1994	750	2750
2	27-29 March 1994	800	4500
3	10-11 April 1994	600	2900
4	25-27 April 1994	900	4400
5	9 May 1994	300	240

RESULTS

Environmental Variables

Salinity and oxygen measurements taken at each field location are summarized in Table 28. Across all sites, salinities ranged from 0 to 28 ppt and dissolved oxygen values were between 1.04 and 7.76 mg/l; a high variance was associated with both variables within a given location.

Responses of Embryos

There was considerable variation in responses of embryos among runs, but there were no consistent differences that seemed to be associated with either intertidal elevation, height above the marsh surface or location within the runs. The proportion of embryos surviving 6 days of exposure to conditions at each field location varied from a low of 0.46 to a high of 0.88; in

general survival was best in Runs 2 and 3 (Table 30). Survival of embryos in the laboratory controls ranged from 0.64 to 0.92 (Table 33), slightly higher than in the field.

The proportion of embryos that hatched between 12 and 14 days post-spawn was greatest during Run 3 in both the field group (Table 31) and controls (Table 33). Again, there was relatively little variation among locations within any run. The same pattern was maintained when the measure of hatching success included embryos that were up to 20 days post-spawn (Tables 32 and 33).

The standard lengths and average individual dry weights of larvae in each run are shown in Tables 34 through 38. Length at hatching varied very little, averaging between 5.5 and 5.9 mm across all runs. Average weights of hatchlings varied from 0.42 to 0.60 mg, but the greatest variation occurred among the lab controls. The most robust larvae were recorded from the 5 ppt control group in Run 1 (Table 34) and the least robust from the 5 ppt control group in Run 5 (Table 38).

Given that there were no consistent patterns in the embryo responses associated with either intertidal site or the height of egg chambers above the marsh surface, the data were pooled across these variables to facilitate comparisons among sampling locations and runs. The information is presented graphically in Figures 25 through 29. Visual examination of these figures provides little evidence for any differences among the suspected 'clean' and 'polluted' locations examined in this research. The natural variation in responses of embryos was most pronounced among runs. The proportion of larvae that hatched successfully at the appropriate time (12-14 d post-spawn) was greatest in Run 3 (Fig. 26), the middle of the spring reproductive period. This suggests that the quality of the eggs and survival of embryos produced by the natural population of *Fundulus heteroclitus* varies temporally. These results are consistent with other recent findings on natural variation in egg size and quality in this species (Parker, 1993) and survival of larval cohorts in the field (Kneib, 1993).

CONCLUSIONS AND RECOMMENDATIONS

Although this system provided the opportunity to address issues concerning the reproductive success of a widely-distributed estuarine species at an experimentally manageable spatial scale, eggs and embryos of *Fundulus heteroclitus* from the pristine marshes of Sapelo Island seemed too robust to be useful as a bioassay of estuarine quality. However, the early life history stages of *F. heteroclitus*, as well as other estuarine fish species (Lasker, 1987), may still hold considerable potential for use as bioindicators of estuarine habitat quality. In reconsidering the design of a bioassay involving this species, I would suggest considering the collection of adult fish from 'clean' and 'polluted' locations, spawning them in the laboratory and examining the same response variables for their offspring as were measured in the present research. This approach would have the advantage of being able to measure sublethal, but chronic effects of exposure to different quality environments that may be passed from adults to their offspring.

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Table 30. Proportion of *Fundulus heteroclitus* embryos surviving after 6-d exposure to field sites. Table values are means ± SD; (n)=number of replicates at each site. Data from eggs placed at 5 cm and 25 cm above the substrate are given separately. Each replicate usually started with 50 eggs (25 in each of two vials at each elevation on each stake).

Field site	Run 1		Run 2		Run 3		Run 4		Run 5	
	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	0.65±0.181 (9)	0.60±0.166 (9)	0.88±0.037 (9)	0.85±0.110 (9)	0.73±0.065 (6)	0.72±0.094 (6)	0.79±0.113 (9)	0.79±0.073 (9)	0.61±0.119 (6)	0.58±0.089 (6)
Top of Duplin	0.69±0.503 (3)	0.55±0.050 (3)	0.77±0.378 (3)	0.85±0.194 (3)	0.82±0.028 (2)	0.80±0.028 (2)	0.69±0.012 (3)	0.68±0.251 (3)	--	--
Lumber Landing	0.56±0.106 (3)	0.75±0.116 (3)	0.87±0.090 (3)	0.89±0.070 (3)	0.75±0.042 (2)	0.77±0.127 (2)	0.79±0.012 (3)	0.79±0.031 (3)	0.46±0.057 (2)	0.58±0.057 (2)
Kenan Field	0.54±0.140 (9)	0.58±0.154 (9)	0.78±0.162 (9)	0.84±0.140 (9)	0.75±0.095 (6)	0.80±0.067 (6)	0.73±0.151 (9)	0.74±0.086 (9)	--	--
Oakdale Creek	0.54±0.140 (3)	0.63±0.070 (3)	0.87±0.064 (3)	0.80±0.072 (3)	0.76±0.085 (2)	0.78±0.028 (2)	0.69±0.122 (3)	0.80±0.087 (3)	--	--
Marsh Landing	0.58±0.124 (6)	0.58±0.140 (6)	0.83±0.107 (6)	0.87±0.064 (6)	0.76±0.100 (4)	0.78±0.082 (4)	0.76±0.103 (6)	0.75±0.099 (6)	0.47±0.101 (4)	0.58±0.041 (4)
Georgia Pacific (Brunswick)	--	--	0.66±0.164 (6)	0.72±0.191 (6)	0.81±0.064 (6)	0.70±0.169 (6)	0.82±0.037 (5)	0.73±0.140 (5)	--	--
Blythe Island (Brunswick)	--	--	0.79±0.126 (6)	0.80±0.480 (6)	0.79±0.068 (6)	0.83±0.079 (6)	0.80±0.078 (6)	0.84±0.065 (6)	--	--
Koppers Creek (Charleston)	--	--	--	--	--	--	--	--	0.56±0.083 (6)	0.57±0.098 (6)
Long Creek (Charleston)	--	--	--	--	--	--	--	--	0.58±0.133 (6)	0.56±0.102 (6)

Table 31. Proportion of *Fundulus heteroclitus* embryos hatching up to Age 14-d. Table values are means ± SD; (n)=number of replicates at each site. Data from eggs placed at 5 cm and 25 cm above the substrate are given separately.

Field site	Run 1		Run 2		Run 3		Run 4		Run 5	
	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	0.06±0.130 (9)	0.08±0.156 (9)	0.10±0.133 (9)	0.11±0.147 (9)	0.45±0.141 (6)	0.45±0.149 (6)	0.20±0.179 (9)	0.31±0.229 (9)	0.18±0.129 (6)	0.23±0.154 (6)
Top of Duplin	0.13±0.140 (3)	0.17±0.180 (3)	0.00±0.000 (3)	0.01±0.012 (3)	0.68±0.028 (2)	0.63±0.042 (2)	0.23±0.257 (3)	0.27±0.232 (3)	--	--
Lumber Landing	0.00±0.000 (3)	0.00±0.000 (3)	0.01±0.012 (3)	0.06±0.087 (3)	0.56±0.000 (2)	0.36±0.226 (2)	0.05±0.042 (3)	0.03±0.058 (3)	0.04±0.028 (2)	0.03±0.042 (2)
Kenan Field	0.09±0.147 (9)	0.05±0.118 (9)	0.10±0.124 (9)	0.04±0.056 (9)	0.43±0.320 (6)	0.48±0.244 (6)	0.15±0.164 (9)	0.18±0.161 (9)	--	--
Oakdale Creek	0.00±0.000 (3)	0.00±0.000 (3)	0.00±0.000 (3)	0.01±0.012 (3)	0.32±0.057 (2)	0.41±0.042 (2)	0.05±0.061 (3)	0.05±0.050 (3)	--	--
Marsh Landing	0.01±0.024 (6)	0.01±0.017 (6)	0.05±0.086 (6)	0.09±0.130 (6)	0.40±0.248 (4)	0.52±0.102 (4)	0.12±0.216 (6)	0.08±0.178 (6)	0.12±0.118 (4)	0.20±0.161 (4)
Georgia Pacific (Brunswick)	--	--	0.05±0.114 (6)	0.01±0.010 (6)	0.35±0.206 (6)	0.36±0.294 (6)	0.10±0.093 (5)	0.16±0.094 (5)	--	--
Blythe Island (Brunswick)	--	--	0.05±0.083 (6)	0.07±0.113 (6)	0.26±0.286 (6)	0.23±0.236 (6)	0.20±0.263 (6)	0.28±0.294 (6)	--	--
Koppers Creek (Charleston)	--	--	--	--	--	--	--	--	0.03±0.028 (6)	0.10±0.114 (6)
Long Creek (Charleston)	--	--	--	--	--	--	--	--	0.04±0.053 (6)	0.14±0.154 (6)

Table 32. Proportion of *Fundulus heteroclitus* embryos hatching up to Age 20-d. Table values are means ± SD; (n)=number of replicates at each site. Data from eggs placed at 5 cm and 25 cm above the substrate are given separately.

Field site	Run 1		Run 2		Run 3		Run 4		Run 5	
	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	0.30±0.166 (9)	0.26±0.175 (9)	0.56±0.171 (9)	0.57±0.161 (9)	0.56±0.143 (6)	0.53±0.092 (6)	0.53±0.132 (9)	0.56±0.164 (9)	0.49±0.116 (6)	0.50±0.085 (6)
Top of Duplin	0.35±0.042 (3)	0.48±0.131 (3)	0.49±0.356 (3)	0.56±0.290 (3)	0.71±0.071 (2)	0.72±0.028 (2)	0.48±0.231 (3)	0.42±0.278 (3)	--	--
Lumber Landing	0.21±0.031 (3)	0.33±0.050 (3)	0.55±0.147 (3)	0.53±0.232 (3)	0.64±0.028 (2)	0.51±0.099 (2)	0.43±0.117 (3)	0.41±0.182 (3)	0.30±0.113 (2)	0.32±0.255 (2)
Kenan Field	0.23±0.148 (9)	0.24±0.140 (9)	0.48±0.204 (9)	0.50±0.168 (9)	0.60±0.152 (6)	0.65±0.173 (6)	0.48±0.109 (9)	0.45±0.188 (9)	--	--
Oakdale Creek	0.15±0.147 (3)	0.29±0.042 (3)	0.47±0.153 (3)	0.47±0.150 (3)	0.47±0.071 (2)	0.51±0.014 (2)	0.39±0.070 (3)	0.29±0.133 (3)	--	--
Marsh Landing	0.20±0.149 (6)	0.28±0.170 (6)	0.38±0.134 (6)	0.59±0.159 (6)	0.58±0.101 (4)	0.61±0.093 (4)	0.42±0.245 (6)	0.41±0.176 (6)	0.30±0.053 (4)	0.46±0.077 (4)
Georgia Pacific (Brunswick)	--	--	0.37±0.079 (6)	0.40±0.195 (6)	0.63±0.090 (6)	0.52±0.281 (6)	0.43±0.093 (5)	0.38±0.117 (5)	--	--
Blythe Island (Brunswick)	--	--	0.33±0.195 (6)	0.31±0.115 (6)	0.57±0.121 (6)	0.61±0.122 (6)	0.45±0.174 (6)	0.48±0.159 (6)	--	--
Koppers Creek (Charleston)	--	--	--	--	--	--	--	--	0.40±0.098 (6)	0.45±0.122 (6)
Long Creek (Charleston)	--	--	--	--	--	--	--	--	0.39±0.152 (6)	0.43±0.097 (6)

Table 33. Data from lab controls for each run and 3 salinities (5, 15, 30 ppt). Each replicate started with 100 *Fundulus heteroclitus* eggs.

Salinity	Run 1	Run 2	Run 3	Run 4	Run 5
Proportion of embryos surviving for 6 days					
5	0.69±0.050 (3)	0.92±0.066 (3)	0.74±0.028 (2)	0.82±0.025 (3)	0.62±0.000 (1)
15	0.74±0.163 (3)	0.81±0.116 (3)	0.76±0.028 (2)	0.85±0.083 (3)	0.74±0.000 (1)
30	0.65±0.121 (3)	0.88±0.078 (2)	0.87±0.035 (2)	0.86±0.078 (3)	0.64±0.000 (1)
Proportion of embryos hatching up to Age 14 d postspawn					
5	0.18±0.236 (3)	0.15±0.130 (3)	0.44±0.064 (2)	0.20±0.164 (3)	0.13±0.000 (1)
15	0.21±0.291 (3)	0.34±0.250 (3)	0.54±0.042 (2)	0.43±0.138 (3)	0.30±0.000 (1)
30	0.13±0.179 (3)	0.12±0.163 (2)	0.74±0.078 (2)	0.40±0.171 (3)	0.39±0.000 (1)
Proportion of embryos hatching up to Age 20 d postspawn					
5	0.23±0.193 (3)	0.36±0.165 (3)	0.54±0.092 (2)	0.35±0.040 (3)	0.39±0.000 (1)
15	0.32±0.191 (3)	0.58±0.096 (3)	0.60±0.021 (2)	0.57±0.093 (3)	0.48±0.000 (1)
30	0.28±0.112 (3)	0.61±0.382 (3)	0.79±0.042 (2)	0.56±0.158 (3)	0.55±0.000 (1)

Table 34. Mean standard length (SL, mm) and average individual dry weight of hatchling *Fundulus heteroclitus* exposed for 6 days to conditions at each field site in Run 1. Means are shown ± SD; (n) = replicates (number of stakes per site).

Field sites	n	No. of individuals		Standard length (mm)		Avg. individual dry wgt (mg)	
Control 5 ppt	3	66		5.7±0.08		0.60±0.025	
Control 15 ppt	3	94		5.7±0.04		0.56±0.036	
Control 30 ppt	3	76		5.5±0.06		0.53±0.033	
		5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	9	118	113	5.8±0.07	5.8±0.07	0.52±0.067	0.51±0.044
Top of Duplin	3	44	55	5.8±0.12	5.8±0.02	0.48±0.015	0.50±0.042
Lumber Landing	3	31	40	5.8±0.07	5.8±0.08	0.52±0.065	0.58±0.066
Kenan Field	8/9	83	89	5.8±0.10	5.8±0.11	0.54±0.042	0.52±0.056
Oakdale Creek	3	25	34	5.7±0.07	5.7±0.23	0.52±0.069	0.57±0.068
Marsh Landing	6	59	67	5.7±0.18	5.8±0.18	0.56±0.059	0.56±0.076

Table 35. Mean standard length (SL, mm) and average individual dry weight of hatchling *Fundulus heteroclitus* exposed for 6 days to conditions at each field site in Run 2. Means are shown ± SD; (n) = replicates (number of stakes per site).

Field sites	n	No. of individuals		Standard length (mm)		Avg. individual dry wgt (mg)	
Control 5 ppt	3	113		5.8±0.09		0.50±0.035	
Control 15 ppt	3	170		5.8±0.07		0.51±0.030	
Control 30 ppt	2	122		5.8±0.03		0.54±0.031	
		5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	9	262	258	5.8±0.11	5.8±0.10	0.51±0.027	0.49±0.044
Top of Duplin	3	74	88	5.8±0.11	5.8±0.12	0.55±0.016	0.54±0.056
Lumber Landing	3	84	77	5.8±0.03	5.8±0.01	0.55±0.028	0.52±0.008
Kenan Field	9	219	218	5.8±0.05	5.8±0.04	0.54±0.036	0.47±0.164
Oakdale Creek	3	76	69	5.9±0.12	5.7±0.09	0.52±0.019	0.50±0.034
Marsh Landing	6	125	174	5.8±0.12	5.8±0.06	0.52±0.023	0.53±0.022
Georgia Pacific (Brunswick)	6	104	118	5.7±0.04	5.7±0.09	0.53±0.033	0.53±0.031
Blythe Island (Brunswick)	6	130	104	5.8±0.08	5.8±0.08	0.53±0.024	0.55±0.045

Table 36. Mean standard length (SL, mm) and average individual dry weight of hatchling *Fundulus heteroclitus* exposed for 6 days to conditions at each field site in Run 3. Means are shown ± SD; (n) = replicates (number of stakes per site).

Field sites	n	No. of individuals		Standard length (mm)		Avg. individual dry wgt (mg)	
Control 5 ppt	2	103		5.8±0.02		0.45±0.006	
Control 15 ppt	2	119		5.8±0.03		0.48±0.014	
Control 30 ppt	2	151		5.7±0.04		0.49±0.025	
		5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	6	167	159	5.8±0.07	5.8±0.06	0.46±0.018	0.46±0.020
Top of Duplin	2	63	69	5.8±0.08	5.7±0.04	0.50±0.032	0.48±0.014
Lumber Landing	2	67	50	5.7±0.04	5.8±0.21	0.51±0.047	0.55±0.020
Kenan Field	6	174	187	5.8±0.06	5.7±0.09	0.47±0.019	0.46±0.024
Oakdale Creek	2	49	48	5.7±0.06	5.6±0.17	0.49±0.046	0.47±0.022
Marsh Landing	4	114	119	5.7±0.08	5.7±0.06	0.45±0.015	0.45±0.009
Georgia Pacific (Brunswick)	6	182	150	5.7±0.11	5.7±0.13	0.49±0.036	0.47±0.078
Blythe Island (Brunswick)	6	160	171	5.8±0.07	5.7±0.09	0.44±0.022	0.43±0.020

Table 37. Mean standard length (SL, mm) and average individual dry weight of hatchling *Fundulus heteroclitus* exposed for 6 days to conditions at each field site in Run 4. Means are shown ± SD; (n) = replicates (number of stakes per site).

Field sites	n	No. of individuals		Standard length (mm)		Avg. individual dry wgt (mg)	
Control 5 ppt	3	106		5.8±0.04		0.47±0.024	
Control 15 ppt	3	161		5.9±0.05		0.48±0.017	
Control 30 ppt	3	156		5.8±0.09		0.49±0.017	
		5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	7	193	202	5.9±0.08	5.9±0.09	0.51±0.031	0.52±0.030
Top of Duplin	2	62	48	5.8±0.03	5.8±0.14	0.52±0.048	0.51±0.019
Lumber Landing	3	69	62	5.8±0.01	5.9±0.09	0.51±0.027	0.48±0.018
Kenan Field	7	174	173	5.8±0.07	5.8±0.08	0.49±0.031	0.51±0.027
Oakdale Creek	3	63	45	5.8±0.03	5.8±0.07	0.49±0.059	0.48±0.026
Marsh Landing	6	130	122	5.8±0.12	5.8±0.11	0.49±0.030	0.51±0.029
Georgia Pacific (Brunswick)	5	107	92	5.8±0.10	5.8±0.09	0.48±0.019	0.51±0.022
Blythe Island (Brunswick)	1	33	36	5.8±0.00	5.9±0.00	0.52±0.000	0.49±0.000

Table 38. Mean standard length (SL, mm) and average individual dry weight of hatchling *Fundulus heteroclitus* exposed for 6 days to conditions at each field site in Run 5. Means are shown ± SD; (n) = replicates (number of stakes per site).

Field sites	n	No. of individuals		Standard length (mm)		Avg. individual dry wgt (mg)	
Control 5 ppt	1	37		5.7±0.00		0.42±0.000	
Control 15 ppt	1	47		5.7±0.00		0.44±0.000	
Control 30 ppt	1	55		5.8±0.00		0.45±0.000	
		5 cm	25 cm	5 cm	25 cm	5 cm	25 cm
Upper Duplin	6	139	150	5.8±0.06	5.8±0.06	0.48±0.016	0.45±0.033
Lumber Landing	2	30	31	5.8±0.13	5.8±0.10	0.53±0.011	0.44±0.128
Marsh Landing	4	60	88	5.7±0.17	5.7±0.09	0.52±0.047	0.46±0.035
Koppers Creek (Charleston)	6	119	123	5.7±0.11	5.8±0.04	0.48±0.056	0.50±0.021
Long Creek (Charleston)	6	115	124	5.8±0.03	5.8±0.04	0.49±0.032	0.46±0.038

Figure 25. Comparison of embryo survivorship at 6 days post-spawn in laboratory controls and across field locations by experimental run. Dates include the time from when the first eggs were spawned to when the last replicates were retrieved from the field in each of the 5 runs. Eggs were not deployed at the Brunswick sites in Runs 1 and 5, nor at the Charleston sites in Runs 1 through 4.

Figure 26. Comparison of hatching success between 12 and 14 days post-spawn in laboratory controls and across field locations by experimental run. Dates include the time from when the first eggs were spawned to when the last replicates were retrieved from the field in each of the 5 runs. Eggs were not deployed at the Brunswick sites in Runs 1 and 5, nor at the Charleston sites in Runs 1 through 4.

Figure 27. Comparison of hatching success between 12 and 20 days post-spawn in laboratory controls and across field locations by experimental run. Dates include the time from when the first eggs were spawned to when the last replicates were retrieved from the field in each of the 5 runs. Eggs were not deployed at the Brunswick sites in Runs 1 and 5, nor at the Charleston sites in Runs 1 through 4.

Figure 28. Comparison of average standard length of larvae in laboratory controls and across field locations by experimental run. Dates include the time from when the first eggs were spawned to when the last replicates were retrieved from the field in each of the 5 runs. Eggs were not deployed at the Brunswick sites in Runs 1 and 5, nor at the Charleston sites in Runs 1 through 4.

Figure 29. Comparison of average dry weight of larvae in laboratory controls and across field locations by experimental run. Dates include the time from when the first eggs were spawned to when the last replicates were retrieved from the field in each of the 5 runs. Eggs were not deployed at the Brunswick sites in Runs 1 and 5, nor at the Charleston sites in Runs 1 through 4.

CHAPTER 6. CONCLUSIONS AND COMMENTS

SUMMARY OF INDICATORS AND PROPOSED CRITERIA OF STRESS

A variety of indicators have been described in detail in the preceding chapters. Some were validated as valuable indicators of habitat condition and others were questioned. Some candidate indicators were introduced and some developmental indicators were tested more rigorously. An effort was also made to stimulate some thinking regarding interpretation of data, particularly with regards to the presence of multiple stressors and the potential for chronic toxicity.

The results across stations are summarized in Table 39. Conditions identified as stressful are marked with an X. The following major categories are listed: dissolved oxygen, pH, sediment PAHs, sediment metals, sediment toxicity, benthic communities, disease status, and oyster growth. Conditions were designated as stressful based on the following criteria:

- Dissolved Oxygen. Sites were classified as stressed if minimal criteria or range/rate criteria were met.
  Minima: < 0.3 mg/L at any time, < 1.0 mg/L 10% of the time, < 2.0 mg/L 20% of the time, < 5.0 mg/L all of the time.
  Range/Rate: DO range deviation > 3.5 mg/L and DO change rate > 0.5 mg/L/hr.

- pH. Sites were classified as stressed if minimal or range criteria were met.
  Minima: < 7.0 at any time.
  Range: pH range deviation > 0.5.

- Sediment PAHs. Sites were classified based on ER-L and ER-M criteria.
  ER-L: Sites with ≥ 1 PAH contaminant exceeding ER-L values.
  ER-M: Sites with ≥ 1 PAH contaminant exceeding ER-M values.

- Sediment Metals. Sites were classified as stressful based on ER-L and ER-M criteria as well as the computed criteria of summed proportional metal concentrations.
  ER-L: Sites with ≥ 1 metal contaminant exceeding ER-L values.
  ER-M: Sites with ≥ 1 metal contaminant exceeding ER-M values.
  PMC:ER-L: Sites with summed proportional metal concentrations (metal concentration divided by ER-L value) > 1.
  PMC:ER-M: Sites with summed proportional metal concentrations (metal concentration divided by ER-M value) > 1.

Table 39. Summary of indicator studies for stations sampled during pilot year studies in the Carolinian Province, summer 1993. Shaded boxes signify indicators not evaluated for certain stations. X indicates stressful condition; blanks indicate no apparent stress.

Station	Dissolved Oxygen		pH		Sediment PAHs		Sediment Metals				Sediment Toxicity				Benthos	Disease	Oyster Growth
	Min	Range/Rate	Min	Range	≥1 ER-L	≥1 ER-M	≥1 ER-L	≥1 ER-M	PMC:ER-L	PMC:ER-M	Amphipod	Mysid	Microtox	Seed Clam			
MAS														░			░
INL																	░
FTJ				X							░	░	X	░			░
DYT														░		X	░
LTH											X			░			
OGE			X		X						X			░		X	░
SAP														░			░
NAS			X														░
TIV					X									░			░
WKY				X				X					X	░			░
DEY								X	X				X	░	X	X	░
BKY	X		X	X	X			X					X	░			
PLM		X						X					X	X		X	░
BRU	X				X			X					X	░			░
EAU	X							X	X				X		X	X	░
CFT		X			X		X	X						░	X	X	░
SLO		X		X		X	X	X					X	░	X		░
SAM	X		X	X	X			X	X				X	X	X	X	░
SAN		X	X	X	X	X							X	X	X		░
SPY		X		X	X		X		X				X	X	X	X	X
KOP	X			X	X	X	X	X	X				X	░	X		X
SED	X	X	X	X	X								X	X	X	X	░
SAV	X	X	X		X		X		X				X	░	X	X	░
ORT		X		X	X	X	X	X	X	X			X	X	X	X	░

- Sediment Toxicity. Sites were classified as stressful if significant toxicity was observed with any of the four toxicity tests:

  Amphipod: Significant toxicity to *Ampelisca abdita*.

  Mysid: Significant toxicity to *Mysidopsis bahia*.

  Microtox: EC_{50} of $\leq 1.0\%$ (using water-corrected values).

  Clam: Significant toxicity if seed clam growth was $\leq 80\%$ of controls.

- Benthic Communities. Sites were classified as stressed if the number of species was less than 10 or the number of individuals was less than 150.

- Disease. Sites were classified as stressed based on the presence of diseased fish or shellfish collected in the trawls.

- Oyster Growth. Sites were classified as stressed if deployed oysters had growth rates that were $\leq 75\%$ of controls.

The data summarized in Table 39 can be interpreted in a number of ways. If the number of times that there is evidence of stress is summed over the first seven categories (excluding oyster growth), the final tally is shown in Table 40. From this table, stressful conditions are apparent at the degraded sites. The most degraded sites were Sampit River, Savannah River, and Ortega River. This is consistent with our a prior assumptions and our knowledge of inputs. This summation approach is very simplistic and certainly more sophisticated metrics, etc. would be appropriate.

INDICATOR EFFICACY

Some categories, such as dissolved oxygen, sediment contaminants, and benthic index have functioned as valuable core indicators in the Virginian and Louisianian Provinces. Studies in the Carolinian Province support their continued use. Strategies such as range/rate criteria, summed proportional metal criteria, and discriminant function analyses may serve to extend the utility of the data and facilitate identification of chronically stressed habitats. Some means of incorporating the significance of multiple contaminants must be developed.

A number of issues were raised concerning the potentially stressful conditions associated with cyclical patterns of parameters such as dissolved oxygen and pH. Although water quality parameters based on minima or mean values may help to identify some stressful conditions, they may underestimate the potential impacts of fluctuations. It is clear that many estuarine environments experience fluctuations in dissolved oxygen, pH, and salinity. The biota must channel some energy into compensating for normal environmental variations, and their ability to tolerate other challenges associated with anthropogenic activities may be reduced. Likewise the allocation of energetic and metabolic resources for detoxification mechanisms may reduce the

Table 40. The number of categories in which stressful conditions were observed for each station. The categories were: Dissolved oxygen, pH, Sediment PAHs, Sediment metals, Sediment toxicity, Benthic index, Disease. Therefore maximum number of stressful categories = 7.

STATION	Number of Stress
MAS	0
INL	0
FTJ	2
DYT	0
LTH	1
OGE	3
SAP	1
NAS	1
TIV	2
WKY	3
DEY	3
BKY	5
PLM	4
BRU	4
EAU	5
CFT	6
SLO	5
SAM	7
SAN	5
SPY	6
KOP	5
SED	6
SAV	7
ORT	7

ability of biota to respond to fluctuations in habitat parameters. Dissolved oxygen cycles that are driven in part by tidal and diurnal processes were frequently observed. This presents a high potential for reperfusion damage. The importance of pH in driving the outcome of both physical and biological parameters was discussed. Mean pH values do not sufficiently characterize the potential stress associated with what may seem like minor variations. The use of relative criteria (such as range or rate functions) may be more biologically relevant, and would rely less on absolute values. Absolute criteria are subject to errors associated with regional differences and limitations of monitoring machinery (for example, DO concentrations in mg/L are salinity dependent such that DO values increase as salinity decreases).

The abundances of fish and shellfish in the trawls did not provide an acceptable approach for discriminating reference from degraded sites. In fact, the results suggest that abundances and diversity are often high in even the most degraded sites. It is difficult to correlate the conditions at a site with mobile populations. Trawls are best conducted to collect specimens for contaminant analysis or disease status.

The incidence of diseases in organisms caught in trawls is relatively rare, but still appears to be a valuable indicator. It is recommended that the disease status of shrimps and crabs should be tallied as well as fish. In the southeast, the invertebrate species are common components of trawls, and both diseases (shell disease in blue crabs and cotton disease in shrimp) are relatively easy to identify by field crews. Moreover they are important commercial species.

The amphipod and mysid toxicity tests did not demonstrate sufficiently high powers of discrimination to justify their continuation. The amphipod toxicity test was used in both the Virginian and Louisianian Provinces, so there is an understandable caution about discarding it. However, there are sufficient concerns about its robustness that development of additional toxicity tests is essential. Seed clam toxicity was introduced as a candidate indicator. The seed clam assay appears to hold promise as an indicator of chronic toxicity, so further testing is warranted.

Microtox® emerged as a promising tool for identifying potential toxicity of sediments. Some concerns, such as sensitivity to sediment characteristics and aluminum, as well as the sample size used for the analyses, need to be addressed. From Table 39, it appears that there is a relatively good association between sediment contaminants and toxicity. Notice that Microtox tended to agree with the PMC:ER-L parameter. Therefore Microtox appears valuable as an indicator of potential chronic toxicity.

The oyster growth studies were conducted on a very limited basis, but indicate the potential utility of deployed organisms. The use of young organisms from a common stock is absolutely essential to the success of these types of studies. A deployment time of 3 - 4 weeks is adequate for detecting adverse effects, and weight rather than size is the most important growth parameter to measure. Laboratory assays simply cannot mimic the combinations or variations in environmental conditions that organisms encounter. Evaluating resident populations can be problematic because the data are often characterized by high background noise associated with

comparing diverse assemblages of organisms that are different ages, sizes, etc. *In situ* deployments enable us to control some of these factors so that biotic responses to actual conditions can be evaluated. Deployed bivalves are also valuable for addressing bioavailability issues, since some contaminants may be so tightly conjugated that they are virtually inconsequential.

Studies with *Fundulus heteroclitus* indicated that the approach tested was not effective at discriminating between reference and degraded sites. Perhaps this is not too surprising since fish embryos tend to be encased in elaborate chorionic membranes which provide a protective barrier to most pollutants. The recommendations regarding the use of adult organisms, which would evaluate the viability of gametes based on adult history, deserves consideration.

The pilot year studies have served to identify indicators that can discriminate reference from degraded sites in southeastern estuaries. Many of the methods and approaches used in the Virginian and Louisianian Provinces were used successfully in the Carolinian Province. The utility of some indicators (DO and pH) was extended by considering range criteria as well as absolute minima. Sediment toxicity assays continue to be the weakest aspect. No single assay is likely to ever be ideal, so at least two types should be employed to eliminate potential errors due to false positives and false negatives. It is uncertain if the failure of the *Ampelisca abdita* assays was due to less toxic nature of southeastern sediments or some species-specific effect. It is difficult to understand why sites such as Ortega River or Sampit River would not cause toxic effects. An effort was made to use some criteria for the identification of sites that suffer from chronic stress as well as acute conditions. Microtox, seed clam assays, multiple metals, and range/rate criteria were discussed toward this aim, and there are certainly others that warrant development. Although EMAP uses ecological indicators, physiological indices would provide significant information for ecological assessments. Cellular and molecular tools represent emerging technologies that should be developed and exploited. They provide the greatest potential for identifying chronic stress in organisms.

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