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## Biological Criteria, Environmental Health and Estuarine Macrobenthic Community Structure

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**Biological criteria for defining water quality and the presence of acceptable levels of benthic resources are evaluated for estuarine macrobenthic communities of the lower Chesapeake Bay, USA. Models of expected community values as a function of salinity are presented for community biomass, numbers of individuals, species richness, percent biomass of deep-dwelling species, percent biomass of equilibrium species, and percent biomass of opportunistic species. The models presented may serve as, or be used to develop, biological criteria for estuaries. Tidal freshwater and oligohaline regions had the highest variability in model parameters due to patchily distributed, large-sized bivalve species. In the absence of data from pristine habitats, the models were developed from a 5 year data set (1985-1989) for stations considered to be minimally impacted.**

The models produced were used to evaluate benthic communities of two regions of the Chesapeake Bay—one exposed to summer low dissolved oxygen events (hypoxia/anoxia) and the other characterized by sediments contaminated with heavy metals and polynuclear aromatic hydrocarbons. Stations exposed to stress from either low dissolved oxygen events or contaminated sediments were characterized by *1. reduced community biomass, 2. reduced species richness, 3. less biomass consisting of deep-dwelling species and equilibrium species and 4. more biomass consisting of opportunistic species.* Some unstressed habitats can be highly dominated by shallow-dwelling long-lived species, thus dominance of deep-dwelling species in biomass must be used with caution as a biological criterion. The number of individuals per m<sup>2</sup> was highly variable for some stressed stations and this parameter is probably of limited value as a biological criterion characterizing the quality of estuarine habitats. No single method or analysis is likely to produce stress classifications without unacceptable misclassifications. Ecological stress, from any source, is best measured by multiple methods or analyses with different assumptions. The consistency of classification between different approaches would provide the robustness necessary to judge the reliability of a stress classification.

The evaluation, restoration, and maintenance of water quality and associated living resources are major goals of environmental management. Recently the U.S. Environmental Protection Agency (1990) has directed the development of biological water quality criteria as part of water quality standards for each state in the USA. Biological criteria are considered important components of water quality standards programmes because *1. they are direct measures of the condition of the biota, 2. they may uncover problems undetected or underestimated by other methods, and 3. such criteria provide measurements of progress of restoration efforts.* Biological criteria are intended to supplement toxicity and chemical assessment methods and serve as independent evaluations of the quality of marine and estuarine ecosystems.

Implementation of biological criteria to assess environmental health is dependent upon the definition of reference conditions that define attainable or desirable biological or habitat conditions. Habitats that are unaffected by anthropogenic stresses may not exist in many estuaries; therefore, habitats characterized as least impacted are used to define reference conditions.

Estimates of the benthic macrofaunal community (here defined as organisms retained on a 0.5 mm screen) are often used to indicate environmental health because benthic animals *1. are relatively sedentary (cannot avoid deteriorating water/sediment quality conditions), 2. have relatively long life spans (indicate and integrate water/sediment quality conditions), 3. consist of different species that exhibit different tolerances to stress (can be classified into functional groups), 4. are commercially important or are important food sources for economically or recreationally important species, and 5. have an important role in cycling nutrients and other chemicals between the sediments and the water column* (Berner, 1976; Virnstein, 1979; Aller, 1978, 1982; Holland *et al.* 1980; Swartz and Lee, 1980; Boesch and Rosenberg, 1981; Dauer *et al.* 1982a,b; Hartley, 1982; Hargrave and Theil, 1983; Philips and Segar, 1986; Bilyard, 1987; Gray *et al.* 1988; Warwick *et al.* 1990; Weston, 1990). This study presents graphical models of expected values

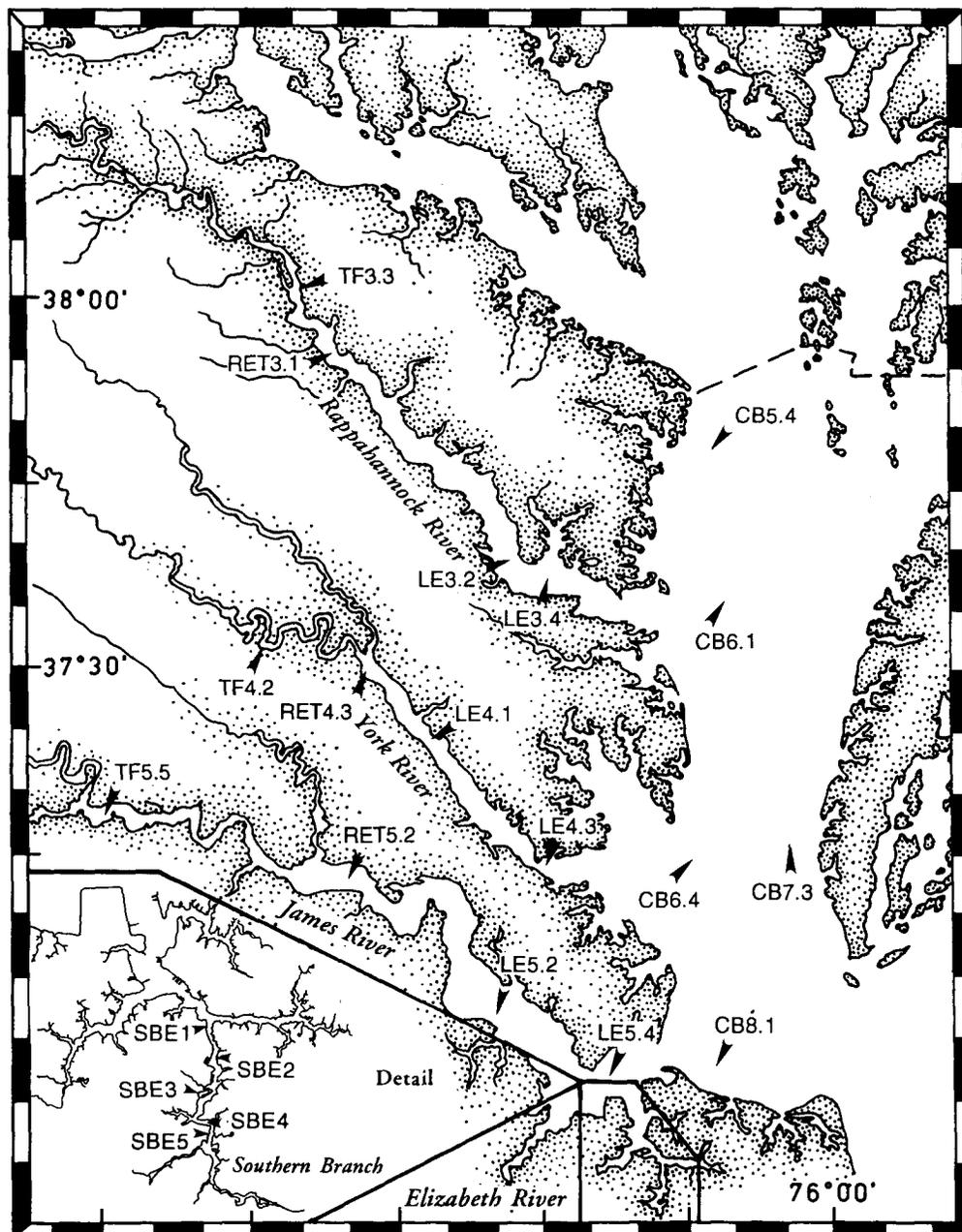


Fig. 1 Map of lower Chesapeake Bay showing station locations.

of macrobenthic community structure as a function of salinity for the lower Chesapeake Bay. Six benthic community parameters are modeled: total community biomass, number of individuals, species richness, percent biomass of deep-dwelling species, percent biomass of equilibrium species, and percent biomass of opportunistic species. The models produced are used to evaluate benthic communities of two regions of the Chesapeake Bay—one identified as exposed to summer low dissolved oxygen events (hypoxia/anoxia) and the other exposed to sediments contaminated with heavy metals and polynuclear aromatic hydrocarbons. These models may serve as biological criteria to evaluate the environmental health of the estuary as indicated by macrobenthic community structure.

### Lower Chesapeake Bay Benthic Monitoring Program

All benthic data reported here were collected as part

of the Virginia Benthic Biological Monitoring Program and the same methodology was used at all stations on all collection dates. Macrobenthic community structure was estimated from quarterly collections during the months of March, June, September, and December at 16 stations in the lower Chesapeake Bay from March 1985 through December 1989. Stations were located within the mainstem of the Bay and three major tributaries—the James, York, and Rappahannock rivers (Fig. 1). In the tributaries, stations were located within the tidal freshwater zone (TF5.5, TF4.2, TF3.3), the turbidity maximum (transitional) zone (RET5.2, RET4.3, RET3.1), the lower estuarine mesohaline muds (LE5.2, LE4.1, LE3.2), and the lower estuarine polyhaline silty-sands (LE5.4, LE4.3). The tidal freshwater station within the York River estuary was located in the Pamunkey River. In the mainstem of the Bay, three stations were located off the mouths of the major tributaries (CB8.1, CB6.4, CB6.1) and two stations in the deeper channels—one near the bay mouth (CB7.3E)

TABLE 1

Station parameters. Data are means for data collected from March 1985 through December 1989 (n=60). Values for SBE2 and SBE5 are for 1989 only (n=12). Salinity is in parts per thousand, water depth in meters, grain size in phi units and silt-clay is a percentage by weight.

Station	Coordinates	Salinity	Depth	Grain Size	Silt-clay Content
<b>Reference Stations</b>					
TF4.2	37°32'49" N, 76°58'29" W	0.33	9.65	5.48	55.05
TF5.5	37°18'46" N, 77°13'59" W	0.35	9.70	4.61	61.24
TF3.3	38°01'08" N, 76°54'37" W	2.48	6.15	6.53	72.03
RET5.2	37°12'38" N, 76°47'35" W	2.68	8.20	6.43	80.70
RET3.1	37°55'12" N, 76°49'18" W	8.18	5.65	7.56	87.59
RET4.3	37°30'49" N, 76°47'20" W	12.03	6.70	6.69	55.05
LE5.2	37°03'30" N, 76°35'36" W	13.40	7.70	5.32	57.91
LE4.1	37°25'06" N, 76°41'36" W	17.03	8.55	4.68	50.62
LE5.4	36°57'05" N, 76°23'19" W	20.93	7.90	2.44	10.50
LE4.3	37°14'27" N, 76°29'06" W	21.35	7.75	3.99	37.99
CB6.4	37°14'11" N, 76°12'18" W	23.69	11.90	4.51	53.45
CB8.1	36°59'07" N, 76°10'07" W	26.58	9.25	4.09	38.97
CB7.3E	37°13'29" N, 76°03'19" W	27.69	21.35	3.17	19.73
<b>Hypoxic/Anoxic Stations</b>					
LE3.2	37°40'13" N, 76°33'16" W	17.20	12.30	7.39	90.94
CB5.4	37°47'28" N, 76°10'33" W	20.59	33.00	6.85	89.17
CB6.1	37°35'18" N, 76°09'45" W	21.38	12.65	5.79	84.67
<b>Contaminated Sediment Stations</b>					
SBE2	36°48'45" N, 76°17'27" W	19.85	12.50	7.29	90.71
SBE5	36°45'54" N, 76°18'00" W	17.38	8.50	7.37	96.55

and the other above the Rappahannock River near the Virginia-Maryland border (CB5.4). To assess further the impacts of contaminated sediments, two stations were added in March 1989 in the Southern Branch of the Elizabeth River (SBE2, SBE5).

On each quarterly collection cruise, four box core samples were collected at each station. Each sample had a surface area of 184 cm<sup>2</sup> and penetrated to at least 25 cm within the sediment. One sample at each station was partitioned into depth intervals of 0–2, 2–5, 5–10, 10–15, 15–20, and 20–25 cm. The sediment of each depth interval and each unpartitioned sample was sieved separately on a 0.5 mm screen, the residue relaxed in dilute isopropyl alcohol, and preserved with a buffered formalin-rose bengal solution. In the laboratory, the organisms of each depth interval and each unpartitioned sample were sorted, and individuals identified to the lowest possible taxonomic level and enumerated. From 1985 to 1989 a total of 358 taxa were collected and 277 (77.4%) identified to the species level. Biomass, in each sample and depth interval, was measured for each taxon, as ash-free dry weight (AFDW) by drying to constant weight at 60°C and ashing at 550°C for four hours.

On each collection date a subsample of surface sediment (approximately 50 g) was collected at all stations for sediment analysis. The sand fraction of the sediment (>63 µm) was dry sieved and the silt-clay fraction was quantified by pipette analysis using the techniques of Folk (1974). Total volatile solids contained in the sediment were determined as the AFDW of the sediment divided by the dry weight of the sediment, expressed as a percentage.

Bottom salinity and temperature were measured on each cruise at all stations with a conductive salinometer (Beckman RS5-3). Additional data to characterize the bottom waters were obtained from the Virginia Water Quality Monitoring Program.

Table 1 lists sedimentary, salinity, and water depth measurements for the stations presented in this study.

From previous studies, two sets of stations were identified as potentially stressed by either low dissolved oxygen events or contaminated sediments (Dauer *et al.* 1989a, 1992, 1993). Stations with more than 29% of the June–September readings below 2 ppm were classified as anoxic/hypoxic affected. From 1985–1989, semi-monthly dissolved oxygen measurements of bottom water showed that low dissolved oxygen events (<2 ppm oxygen) occurred in 43% of the readings at station CB5.4 (northern mainstem, total measurements=40), 46% of the readings at LE3.2 (lower Rappahannock River, total measurements=37), 31% of the readings at CB6.1 (off Rappahannock River, total measurements=49), 5% of the readings at LE4.3 (lower York River, total measurements=40); no low oxygen readings were recorded at 11 remaining stations (Dauer *et al.* 1989a). The spatial distribution of impacted macrobenthic communities in lower Chesapeake Bay primarily reflects regions where the water column is highly stratified and gravitational circulation is unable to replenish bottom oxygen (Kuo and Neilson, 1987).

Two stations in the Southern Branch of the Elizabeth River (SBE2, SBE5) were identified as having heavily contaminated sediments (Dauer *et al.* 1989b; Alden *et al.* 1991; Dauer *et al.* 1993). Sediments were considered contaminated if one or more of the selected metals or semi-volatile organic compounds exceeded the Long and Morgan (1990) median value (ER-M), which represents that concentration above which biological effects were frequently or always observed or predicted among species tested; the Long and Morgan ER-M values (shown in parentheses) are in ppm for metals (mg kg<sup>-1</sup> dry weight of sediment) and in ppb for semi-volatile organic compounds (µg kg<sup>-1</sup> dry weight of sediment). Copper (390), lead (110), mercury (1.3), and

zinc (270) as well as benzo(a)anthracene (1600), benzo(a)pyrene (2500), chrysene (2800), fluorene (640), phenanthrene (1380), pyrene (2200) are metals and organic contaminants of concern. Values from Alden *et al.* 1991 showed that SBE2 exceeded the Long and Morgan ER-M concentration for: lead (358), mercury (2.4), zinc (1029), and pyrene (5480). SBE5 exceeded the Long and Mercury ER-M concentration for: zinc (276), benzo(a)anthracene (1630), and pyrene (2560). No other stations in this study exceeded the Long and Morgan ER-M concentrations.

### Biological Criteria—Macrobenthic Community Structure Parameters

The major assumption in developing the present models of expected values is that healthy benthic communities can be characterized by high biomass estimates dominated by long-lived, often deep-dwelling, species and high species richness. The presence of long-lived (high biomass) species indicates a past history of good water/sediment quality conditions (Rhoads and Boyer, 1982; Warwick, 1986). Many long-lived species (e.g. bivalve molluscs, malvanid polychaetes, burrowing shrimp, and burrowing anemones) are also relatively deep-dwelling species (Pearson and Rosenberg, 1978; Rhoads and Boyer, 1982; Dauer *et al.* 1987). Estimates of species richness have been used to characterize community structure showing a pattern of low values for habitats *a priori* classified as stressed, and community composition changes from dominance by long-lived equilibrium species in relatively unstressed situations to dominance by short-lived opportunistic species in relatively stressed situations.

Models of expected values of macrobenthic community structure are presented for six parameters—three commonly used to characterize macrobenthic community structure (community biomass, numbers of individuals, species richness) and three less often used (percent biomass of deep-dwelling species, percent biomass of equilibrium species, percent biomass of opportunistic species). Community biomass was calculated as the total ash-free dry weight per m<sup>2</sup> for all species collected. The number of individuals was calculated as individuals per m<sup>2</sup>. Species richness was calculated as the mean number of species per replicate. In contrast to other estimates of diversity, this estimate is directly interpretable; it is also highly correlated with informational indices that are often used in benthic monitoring programmes (see Dauer *et al.* 1989a). The percent biomass of deep-dwelling species was calculated as the percent of the total community biomass found below 5 cm in the sediment. Species used for percent biomass of opportunistic species were relatively short-lived, eurytopic species often characterized as dominating disturbed or stressed habitats (Boesch, 1977; Pearson and Rosenberg, 1978). Species used for percent biomass of equilibrium species were relatively long-lived species that dominate the community biomass in undisturbed or unstressed habitats (Warwick, 1986). This classification approach is similar to several schemes previously applied to macrobenthic communities, for

TABLE 2

Species groups used in community composition comparisons

A. Opportunistic Species Group	
Annelida : Polychaeta	
	<i>Asabellides oculata</i> (Webster)
	<i>Eteone heteropoda</i> Hartman
	<i>Glycinde solitaria</i> (Webster)
	<i>Leitoscoloplos fragilis</i> (Verrill)
	<i>Mediomastus ambiseta</i> (Hartman)
	<i>Nereis succinea</i> (Frey and Leuckart)
	<i>Paraprionospio pinnata</i> (Ehlers)
	<i>Polydora ligni</i> Webster
	<i>Streblospio benedicti</i> Webster
Annelida : Oligochaeta	
	<i>Limnodrilus</i> spp.
Mollusca : Bivalvia	
	<i>Mulina lateralis</i> (Say)
B. Equilibrium Species Group	
Cnidaria : Anthozoa	
	<i>Cerianthus americanus</i> (Verrill)
Annelida : Polychaeta	
	<i>Asychis elongata</i> (Verrill)
	<i>Clymenella torquata</i> (Leidy)
	<i>Diopatra cuprea</i> (Bosc)
	<i>Macroclymene zonalis</i> (Verrill)
Mollusca : Bivalvia	
	<i>Anadara ovalis</i> (Brugiere)
	<i>Anadara transversa</i> (Say)
	<i>Cyrtopleura costata</i> Linnaeus
	<i>Macoma balthica</i> Linnaeus
	<i>Mercenaria mercenaria</i> (Linnaeus)
	<i>Mya arenaria</i> Linnaeus
	<i>Rangia cuneata</i> Sowerby
	<i>Tagelus divistus</i> (Spengler)
Echinodermata : Ophiuroidea	
	<i>Microphiopholis atra</i> (Stimpson)

example, 1. the Group I (opportunistic), II, and III (equilibrium) species of McCall (1977), 2. the adaptive strategies groups *r* (*r*-selected), *K* (*K*-selected), and *T* (stress tolerant) of Gray (1979), 3. the successional stage approach (pioneering versus equilibrium stages) of Rhoads and Boyer (1982), and 4. the ecological basis for the Abundance Biomass Curve (ABC) method of Warwick (1986). Table 2 lists the species included in the two groups. No attempt was made to classify all 358 taxa identified from 1985–1989. Species listed in Table 2 account for 89.6% of the biomass recorded at all stations.

### Models of Macrobenthic Community Structure

In the absence of reliable historical data on macrobenthic communities, these models serve as indicators of expected values. Models were developed using data from all the benthic monitoring stations except those stations considered impacted by either low dissolved oxygen events or the presence of contaminated sediments. Models represent plots of the mean value of each parameter from 1985 through 1989 with 95% confidence intervals. Means and confidence intervals for depth distribution and indicator groups (based on percentage data) were calculated after angular transformation and are presented as back transformed values. Salinity was chosen as the major factor affecting benthic community structure along estuarine gradients. The significance of salinity in determining spatial distributional patterns in estuaries has long been recognized (Remane and Schleiper, 1971). Analysis of the 1985 to

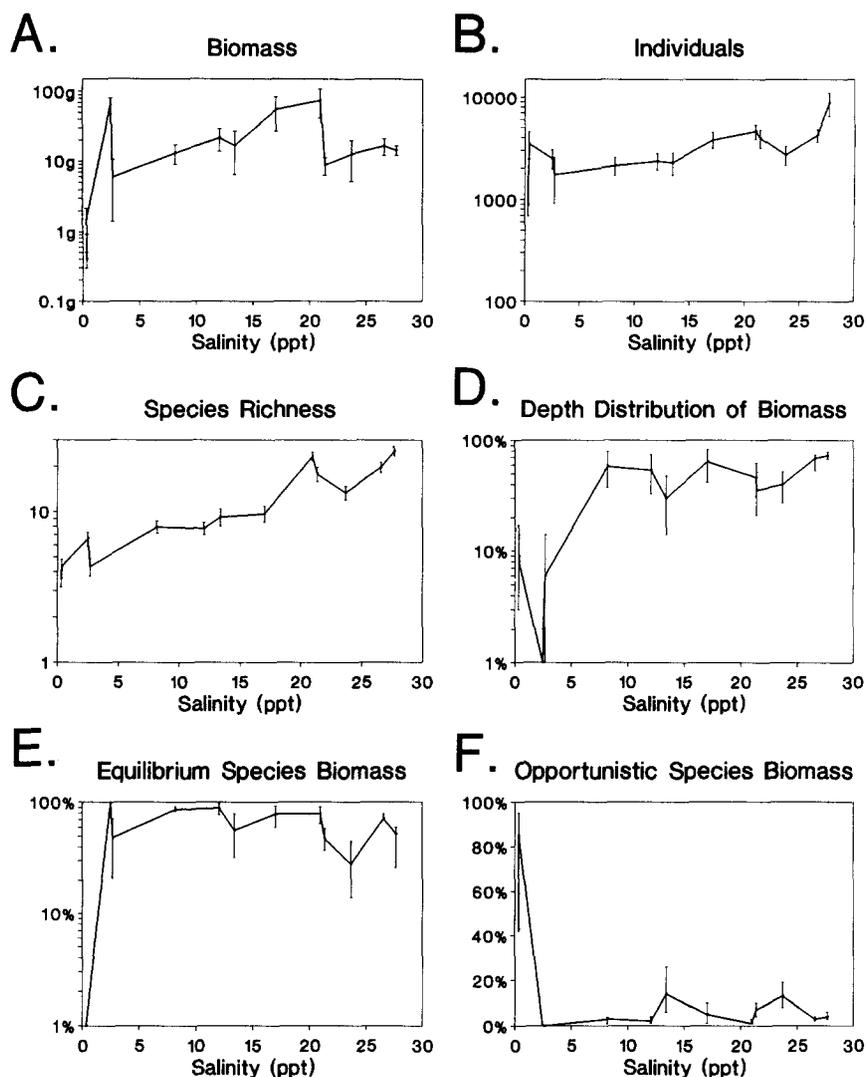


Fig. 2 Community parameters as a function of salinity. Salinity values are five year means (1985–1989) and 95% confidence intervals ( $n=60$ ). A. Community biomass in  $\text{g m}^{-2}$ , B. Number of individuals  $\text{m}^{-2}$ , C. Species richness in species/replicate, D. Deep-dwelling biomass as percentage of community biomass below 5 cm, E. Percentage of community biomass composed of equilibrium species, F. Percentage of community biomass composed of opportunistic species.

1989 biomass data set, using the techniques of Williams and Stephenson (1973), showed that 95% of the variance in the data set was accounted for by spatial patterns, 4% by temporal patterns and 1% by spatial-temporal interactions (Dauer *et al.* 1989a). Plots of stations in canonical discriminant or principal components space consistently were highly correlated with salinity on the first axis. Sedimentary characteristics are also well documented to affect benthic community structure (Gray, 1974; Rhoads, 1974) and the present models are intended to represent patterns of macrobenthic community structure for the dominant sediment type for subtidal habitats in each of the major salinity regions (tidal freshwater, transitional/oligohaline, mesohaline and polyhaline). Figure 2 shows the models of expected benthic community parameters for community biomass (Fig. 2A), numbers of individuals (Fig. 2B), species richness (Fig. 2C), percent of deep-dwelling biomass (Fig. 2D), percent of community biomass composed of equilibrium species (Fig. 2E) and the percent of community biomass composed of opportunistic species (Fig. 2F).

## Results and Discussion

Most macrobenthic communities of Chesapeake Bay had mean total community biomass values greater than  $5 \text{ g per m}^2$  and more than 2000 individuals per  $\text{m}^2$  except for tidal freshwater region (Fig. 2A, 2B). Species richness generally increased from low values in the tidal freshwater region to high values in the polyhaline region (Fig. 2C). The amount of community biomass composed of deep-dwelling species generally increased as a function of deep salinity, with most mesohaline and polyhaline values greater than 40% (Fig. 2D). Station LE5.4 in the James River was an exception to this pattern and was greatly dominated by the shallow-dwelling bivalve *Mercenaria mercenaria*. The percentage of community biomass composed of equilibrium species was generally greater than 40% (Fig. 2E) and the percentage of community biomass composed of opportunistic species was generally less than 10% (Fig. 2F), except for tidal freshwater and oligohaline regions of the estuary. The development and application of biocriteria will be most difficult in the tidal freshwater and oligohaline regions

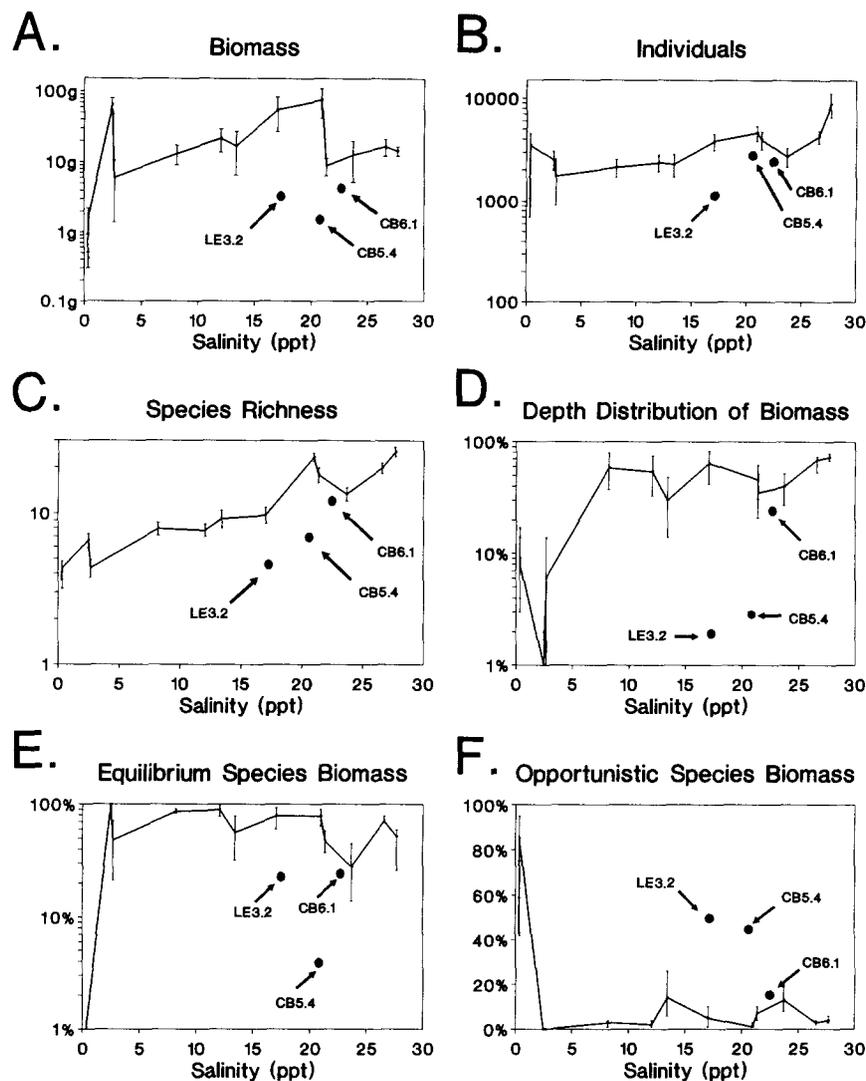


Fig. 3 Comparison of expected community parameters with stations exposed to low dissolved oxygen events in the Mainstem of the Bay (CB5.4, CB6.1) and the lower Rappahannock River (LE3.2). Values for CB5.4, CB6.1 and LE3.2 are mean values for data from 1989 ( $n=12$ ). A. Community biomass in  $g\ m^{-2}$ , B. Number of individuals  $m^{-2}$ , C. Species richness in species/replicate, D. Deep-dwelling biomass as percentage of community biomass below 5 cm, E. Percentage of community biomass composed of equilibrium species, F. Percentage of community biomass composed of opportunistic species.

due to the high statistical variation of the community parameters. This variation is due to very patchy distribution in space (both between replicates on a collection date and between stations within the salinity range of tidal freshwater and oligohaline regions) and in time (between collection dates) of large sized bivalve species such as *Rangia cuneata*.

Macrobenthic communities from regions of lower Chesapeake Bay exposed to low dissolved oxygen events (Fig. 3) and contaminated sediments (Fig. 4) were characterized by lower values for community biomass (Figs. 3A, 4A), number of individuals (Figs. 3B, 4B), species richness (Figs. 3C, 4C), and the amount of biomass consisting of deep-dwelling species (Figs. 3D, 4D). In addition, the communities at stations exposed to low dissolved oxygen events and contaminated sediments had lower dominance by equilibrium species (Figs. 3E, 4E) and greater dominance by opportunistic species (Figs. 3F, 4F). The models also were able to separate values for macrobenthic communities at

stations exposed to intermediate levels of stress as indicated by the values for stations CB6.1, where summer low dissolved oxygen events occurred at a lower frequency than for CB5.4 and LE3.2.

Highly stressed macrobenthic communities have been described as dominated by species that are shallow-dwelling, short lived, and primarily annelids (Pearson and Rosenberg, 1978; Rhoads *et al.* 1978; Rhoads and Boyer, 1982). High mortalities of benthic communities associated with periodic hypoxic or anoxic events have several predictable effects (Tenore 1972; Holland *et al.* 1977; Rosenberg, 1977; Pearson and Rosenberg, 1978; Santos and Simon 1980; Officer *et al.* 1984; Gaston, 1985; Dauer *et al.* 1992); longer-lived benthos, generally also deeper dwelling within the sediment, cannot survive long enough to become either biomass dominants or established in the deeper depth intervals. Therefore such a community becomes dominated by short-lived shallow-dwelling species. Estimates of benthic communities are generally lower due to higher mortality

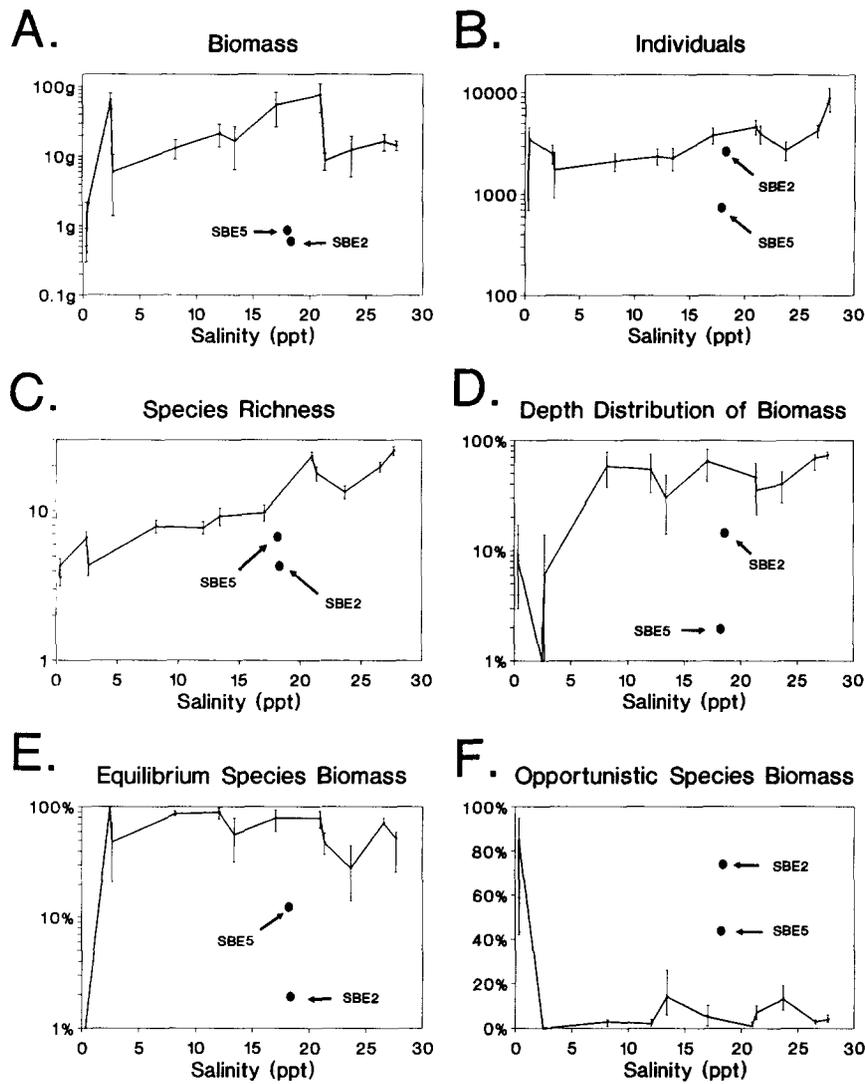


Fig. 4 Comparison of expected community parameters with stations exposed to contaminated sediments of the Southern Branch of the Elizabeth River (SBE2 and SBE5). Values for SBE2 and SBE5 are mean values for data from 1989 (n=12). A. Community biomass in  $g\ m^{-2}$ , B. Number of individuals  $m^{-2}$ , C. Species richness in species/replicate, D. Deep-dwelling biomass as percentage of community biomass below 5 cm, E. Percentage of community biomass composed of equilibrium species, F. Percentage of community biomass composed of opportunistic species.

rates; however, the density of individuals may actually be higher due to adaptive strategies that allow rapid local recruitment by opportunistic species in disturbed or stressed habitats. Species richness, as measured by the average number of species per replicate, is lower due to the smaller number of species with the physiological adaptations, behavioural characteristics or spatio-temporal recruitment patterns necessary to overcome the effects of periodic hypoxic or anoxic stress.

Contaminated sediments present a diversity of stresses for benthic communities and result in patterns of lower biomass, lower species richness and shifts in community composition to a dominance by shallow-dwelling, opportunistic species. Contaminants are diverse chemical compositions and have cumulative effects on the macrobenthos as well as upon consumers of the macrobenthos. Contamination of marine and estuarine sediments therefore is a serious threat to both living resources and human health (Baker, 1980a, 1980b; National Research Council, 1989).

## Conclusions

The graphical models presented in this study may serve as biological criteria to evaluate relative environmental health of Chesapeake Bay as indicated by macrobenthic community structure. These models are relatively simple to apply and interpret when attempting to determine the status of estuarine habitats in regard to exposure to stress. Limitations of the present models include: 1. data from habitats of greatly different sediment type or water depth but of similar salinity may not conform to the model, 2. the expected values only apply to subtidal habitats, and 3. the potential necessity to update models due to long-term trends at the reference stations. In regard to the first limitation, habitats in the same salinity region with substantially different sediment types, for example on shallow flanking shoals found in the tributaries, may require different biological criteria. Stations in the present monitoring program of the lower Chesapeake Bay are intended to

represent the predominant sediment type in the major salinity regions of each tributary. Future use of expected values from these models would be optimized by comparisons with data collected from similar sediment types. In regard to the third limitation, Dauer (1991) reported significant trends in community biomass, species richness and proportion of opportunistic and equilibrium species biomass for nine of the stations used to develop the expected values models. Most trends were not of sufficient magnitude to alter the conclusions of this study; however, updating the present models may be necessary depending upon the results of future trend analyses.

A variety of data reduction/simplification methods or approaches have been applied to benthic communities in determining unacceptable stress as indicated by community structure (e.g. indicator species: Pearson and Rosenberg 1978; Gray 1979; nematode-copepod ratios: Raffaelli and Mason, 1981; lognormal species distribution: Gray, 1979, 1981; ABC method: Warwick, 1986; Warwick and Ruswahyani, 1987; Warwick *et al.* 1987, 1990; Gray *et al.* 1988; Warwick, 1988a, 1988b; Austen *et al.* 1989) but none has been widely accepted. No single method, analysis, or variable is likely to produce stress classifications without unacceptable misclassifications. Tests for significant effects, environmental impacts, or violations of biocriteria should be powerful (minimize declaring no significant effect when one exists), conservative (minimize declaring a significant effect when none exist), and robust (decisions about effects are not greatly affected by violations of assumptions of the test). Methods of analysis, such as in the study, are designed to be powerful, but may not be conservative. Conservative decisions are made using scientific interpretation of the ecological significance of test results, based upon ecological knowledge of the biotic community. Consistency of classification between different approaches will provide the robustness necessary to judge the reliability of a stress classification (Green, 1979); therefore, ecological stress, from any source, is best measured by using multiple variables, methods, or analyses with different assumptions.

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