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**Long-Term Monitoring of Ecological Conditions  
In Gray's Reef National Marine Sanctuary:  
Comparison of Soft-Bottom Benthic Assemblages  
and Contaminant Levels in Sediments and Biota  
in Spring 2000 and 2005**



**NOAA Technical Memorandum NOS NCCOS 68**

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# Long-Term Monitoring of Ecological Conditions In Gray's Reef National Marine Sanctuary: Comparison of Soft-Bottom Benthic Assemblages and Contaminant Levels in Sediments and Biota in Spring 2000 and 2005

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## Abstract

As part of an ongoing program of benthic sampling and related assessments of sediment quality at Gray's Reef National Marine Sanctuary (GRNMS) off the coast of Georgia, a survey of soft-bottom benthic habitats was conducted in spring 2005 to characterize condition of macroinfaunal assemblages and levels of chemical contaminants in sediments and biota relative to a baseline survey carried out in spring 2000. Distribution and abundance of macrobenthos were related foremost to sediment type (median particle size, % gravel), which in turn varied according to bottom-habitat mesoscale features (e.g., association with live bottom versus flat or rippled sand areas). Overall abundance and diversity of soft-bottom benthic communities were similar between the two years, though dominance patterns and relative abundances of component species were less repeatable. Seasonal summer pulses of a few taxa (e.g., the bivalve *Ervilia* sp. A) observed in 2000 were not observed in 2005. Concentrations of chemical contaminants in sediments and biota, though detectable in both years, were consistently at low, background levels and no exceedances of sediment probable bioeffect levels or FDA action levels for edible fish or shellfish were observed. Near-bottom dissolved oxygen levels and organic-matter content of sediments also have remained within normal ranges. Highly diverse benthic assemblages were found in both years, supporting the premise that GRNMS serves as an important reservoir of marine biodiversity. A total of 353 taxa (219 identified to species) were collected during the spring 2005 survey. Cumulatively, 588 taxa (371 identified to species) have been recorded in the sanctuary from surveys in 2000, 2001, 2002, and 2005. Species Accumulation Curves indicate that the theoretical maximum should be in excess of 600 species. Results of this study will be of value in advancing strategic science and management goals for GRNMS, including characterization and long-term monitoring of sanctuary resources and processes, as well as supporting evolving interests in ecosystem-based management of the surrounding South Atlantic Bight (SAB) ecosystem.





## 1.0 Introduction

In April 2000, the National Centers for Coastal Ocean Science (NCCOS) and the Office of National Marine Sanctuaries (ONMS) began a partnership with the purpose of augmenting the management of National Marine Sanctuaries (NMS) through increased scientific understanding of sanctuary sites (ONMS/NCCOS 2004). An ongoing program of benthic sampling and related assessments of sediment quality was initiated at Gray's Reef National Marine Sanctuary (GRNMS) to help address strategic science and management goals, including long-term monitoring of environmental conditions and characterization of basic oceanographic processes. Such activities are important to fulfilling key research and monitoring goals for GRNMS to “support, promote and coordinate scientific research and long-term monitoring to enhance the understanding of the Sanctuary environment and to improve management decision-making” (NOAA 2006).

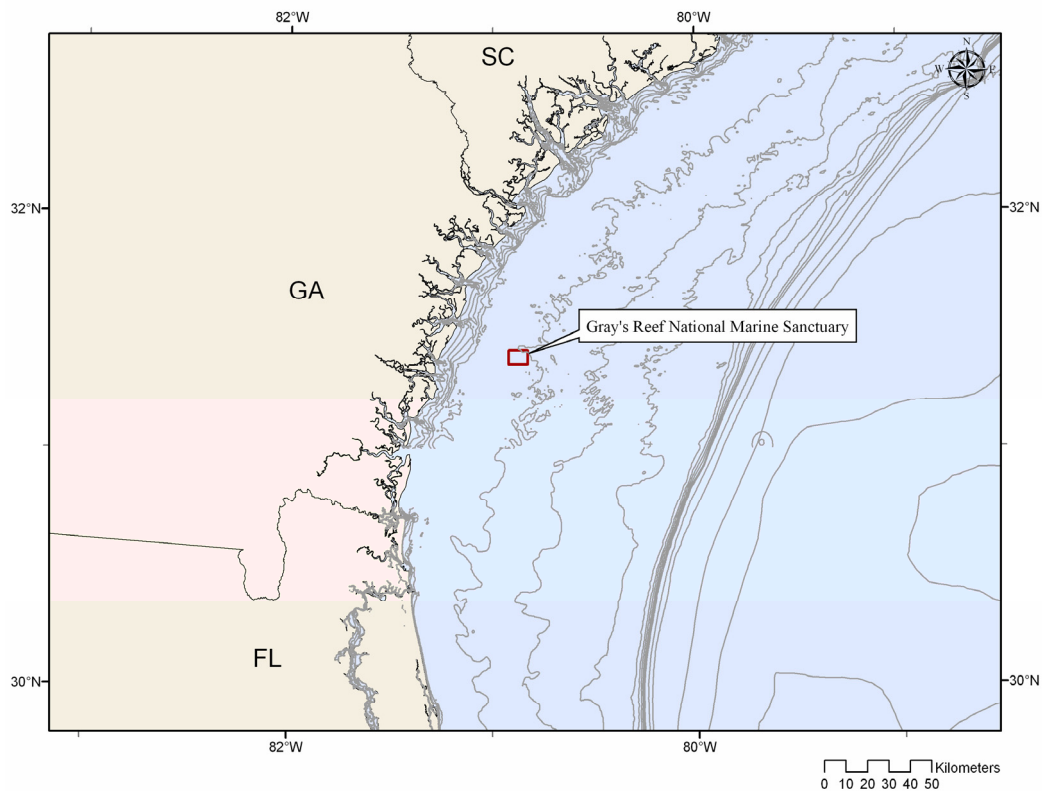


Figure 1.1. Location of Gray's Reef National Marine Sanctuary.

GRNMS is a marine protected area located 32 km offshore of Sapelo Island, Georgia (Fig. 1.1), encompassing 58 km<sup>2</sup> of “live bottom” habitat and associated sandy substrate. Named in recognition of Milton B. Gray, a taxonomist and curator who studied the area in the 1960s to obtain collections of reef-associated fauna for the University of Georgia Marine Institute (Hunt 1974), Gray's Reef was designated a National Marine Sanctuary in January, 1981. It is one of the largest near-shore rocky reefs off the southeastern United States, and lies in a transition zone between temperate and tropical waters. Located at the boundary between the inner and mid-continental shelf portion of the South Atlantic Bight (SAB), which extends from Cape Hatteras,

North Carolina to Cape Canaveral, Florida, Gray's Reef is influenced by freshwater inflow, winds, and tides, with some influence from the Gulf Stream, which flows along the shelf edge (NOAA 2006). Water depths throughout the sanctuary average approximately 20 m. The bottom consists of extensive but discontinuous rock outcroppings of moderate height (2-3 m) interspersed with unconsolidated sediments of sand and shell hash (ONMS/NCCOS 2004).

An initial characterization of GRNMS was conducted in spring 2000 (3-6 April) to evaluate the condition and distribution of benthic macroinfauna, including sediment-associated stressors, to provide a quantitative benchmark for tracking any future changes due to natural or human disturbances. The results of the spring 2000 survey suggest that soft-bottom habitats associated with GRNMS support highly diverse infaunal assemblages, a finding that contradicts a commonly-held assumption that these relatively featureless expanses of substrate surrounding "live bottom" habitat are biological voids (Cooksey et al 2004, Hyland et al. 2006). Levels of man-made, chemical contaminants were found at low, but detectable, concentrations in sediments and edible tissues of target species (black sea bass, zebra ark), suggesting background conditions while highlighting the need for future monitoring to track potential changes in levels of these substances.

This report presents the results of a follow-up survey, conducted in spring 2005 (3-7 May), to assess ecological conditions associated with soft-bottom benthic habitat in GRNMS relative to the initial spring 2000 baseline study. The objectives of this study were to characterize benthic macroinfaunal assemblages and concentrations of chemical contaminants in sediments and biota (black sea bass, *Centropristis striata*, and zebra ark, *Arca zebra*) comparing 2005 survey data to the baseline study results to see how conditions may have changed. Results are intended to address sanctuary goals as noted above. Additionally, in assessing the linkages of ecological condition across multiple species in relation to a variety of environmental controlling factors and processes, this study supports evolving interests within NOAA and other institutions to move toward ecosystem-based approaches to coastal resource management (Murawski 2007, Marine Ecosystems and Management 2007).

## 2.0 Methods

Sampling sites (Fig. 2.1) were selected as randomly generated latitude/longitude coordinate pairs from each of 20 sampling cells (2.9 km<sup>2</sup> each) using the grid design developed for the spring 2000 baseline study (see Hyland et al 2006). At each site, surface-to-bottom profiles of physical properties of water (depth, temperature, salinity, conductivity, pH, and dissolved oxygen) were taken using a Sea-Bird SBE 19 SEACAT CTD profiler (Sea-Bird 2006).

Three replicate sediment samples for macroinfaunal analysis were collected at each site using a 0.04 m<sup>2</sup> Young grab sampler. Each replicate was sieved in the field through a 0.5-mm mesh screen and preserved in 10% buffered formalin. Sediment samples were shipped to the laboratory, where they were rinsed through a 0.5-mm mesh sieve to remove preservatives and sediment, stained with Rose Bengal, and stored in 70%-isopropanol until processing. Infauna were sorted from sample debris under a Wild M-5A dissecting microscope and identified to the lowest practical identification level, usually species.

The upper 2-3 cm of sediment from additional multiple grabs taken at each station were combined into a single composite sample, then sub-sampled for analysis of metals, organics (PAHs, pesticides, PCBs), total organic carbon (TOC), and grain size. Sediment texture was determined at half-phi intervals (Krumbein  $\Phi$  scale; Krumbein and Sloss 1963) using the

hydrometer technique for fractions smaller than 44 $\mu$ m and nested sieves for larger particle fractions. TOC was measured as ash-free dry weight expressed as a percentage (Vittor 2006).

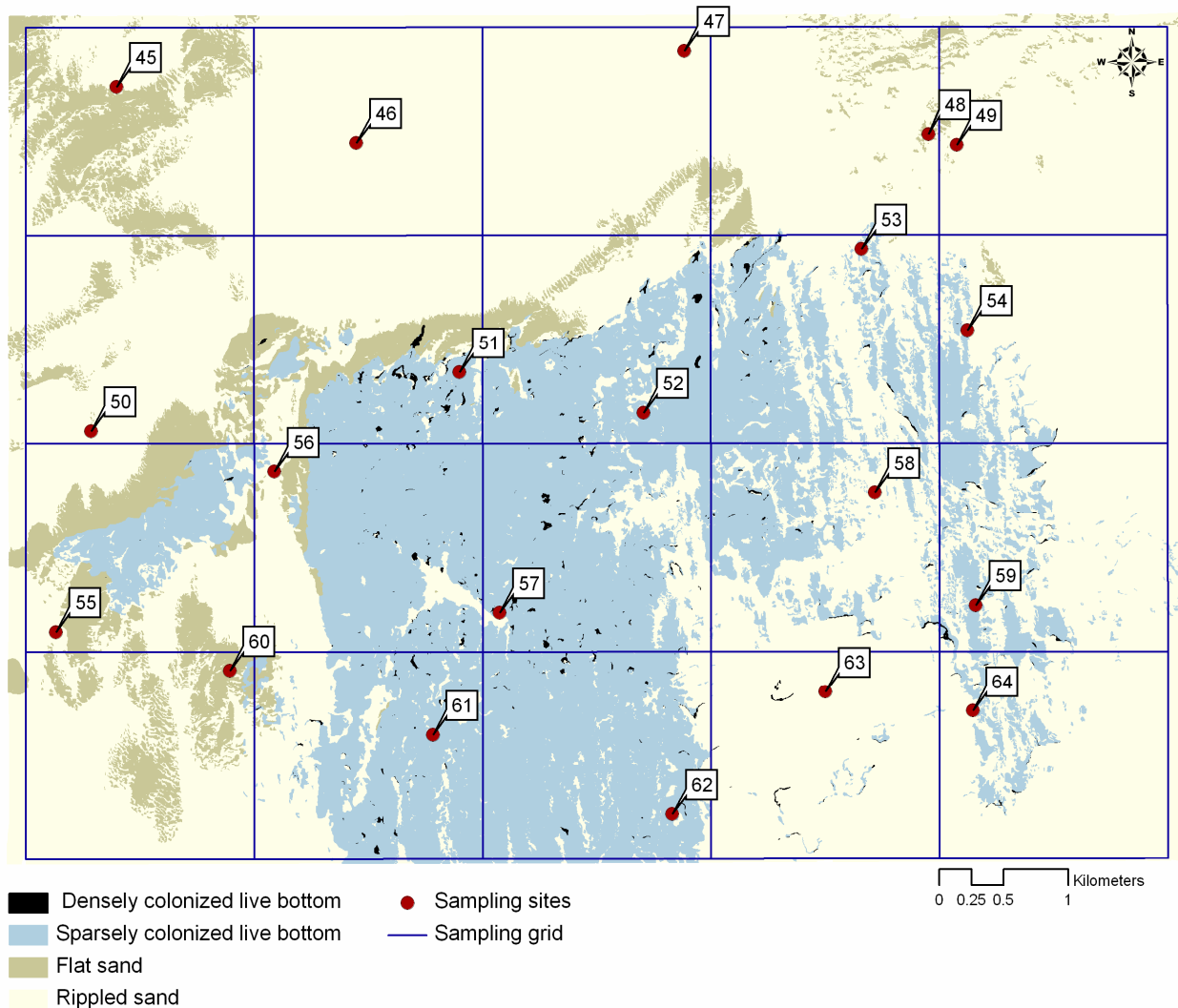


Figure 2.1. Location of sites sampled in spring 2005 at GRNMS. Also shown are the sampling grid (Hyland et al 2006) and map of benthic habitat types (Kendall et al 2005).

Black sea bass (*Centropristis striata*) and zebra ark (*Arca zebra*) were collected by hook-and-line fishing and scuba diving, respectively, at targeted stations for analysis of chemical contaminants in tissues. Fishing was conducted at targeted sites until a total of 15 black sea bass were collected. Each specimen was wrapped individually in heavy aluminum foil, placed in two nested plastic bags, sealed, and frozen at -20° C until transferred on ice to the laboratory for analysis. A total of 22 zebra arks were collected by divers, rinsed with ambient seawater, wrapped in heavy aluminum foil, placed in double plastic bags, and frozen (-20° C) until analysis. The 22 specimens yielded a total of eight composite samples (composed of individuals collected from each of the eight dive sites where found), each consisting of one to four individuals.

Methods for analysis of chemical contaminants followed those of Sanders (1995), Fortner et al. (1996), Kucklick et al. (1997), and Clum et al. (2002). While matrix-specific extraction methods were required for some chemicals (e.g., all metals except Hg), subsequent instrumental analyses were the same for both sediments and tissues. Trace metal analyses were performed using inductively coupled plasma mass spectrometry (ICP/MS) for the following suite of metals: Al, Cr, Cu, Fe, Mn, Ni, Sn, and Zn. Additional trace metals (Ag, As, Cd, Pb, Se) were analyzed using graphite furnace atomic absorption (GFAA). Cold vapor atomic absorption (CVAA) was used for analysis of total Hg. Two classes of organic compounds (PCBs and pesticides) were analyzed by dual-column gas chromatography with electron capture detection (GC-ECD). An ion-trap mass spectrometer equipped with a gas chromatograph (GC/MS-IT) was used for analysis of PAHs.

The biological significance of observed chemical contaminant concentrations in sediments was evaluated in relation to Effects Range-Low (ERL) and Effects Range-Median (ERM) sediment quality guidelines (SQG) values from Long et al. (1995), where available. ERL values are lower-threshold bioeffect limits, below which adverse effects on sediment-dwelling organisms are not likely to occur. ERM values, in contrast, represent mid-range concentrations of chemicals above which adverse effects are likely to occur. Overall sediment contamination from multiple chemicals was expressed using the mean ERM quotient (Long et al. 1998, 2000; Long and MacDonald 1998; Hyland et al. 1999), which is calculated as the mean of the ratios of individual chemical concentrations in a sample relative to corresponding published ERM values.

A variety of population and community-level indices were used to characterize the benthic infaunal assemblages. These included numbers of species, H' diversity (Shannon and Weaver 1949) derived with base-2 logarithms, density ( $m^{-2}$ ) of total fauna (all species combined), and density of numerically dominant fauna.

Patterns in the distribution of benthic infauna were examined using normal (Q-mode) cluster analysis (Boesch 1977). An unweighted pair-group method using arithmetic averages (UPGMA; Sneath & Sokal 1973) was used as the clustering method and Bray-Curtis similarity (BCS; Bray & Curtis 1957) was used as the resemblance measure. Analyses were run on double-square-root transformed abundances, combined over replicates within a station, using the PRIMER software package (Clarke and Gorley 2001). Rare taxa (i.e., those representing <1% of the total abundance of a sample) were excluded from the analysis to improve clustering interpretability and comparability with methods used in conjunction with the previously published, spring 2000 study (Hyland et al 2006; Cooksey et al 2004).

Canonical discriminant analysis was used to evaluate whether the group separation derived from cluster analysis of the macroinfaunal species data could be explained by other measured abiotic environmental factors (Green and Vascotto 1978; Hyland et al 1991). The analysis produced a reduced set of discriminant (canonical) functions that best described the separation of the pre-declared site groups based on data represented by the different environmental variables. Total structure coefficients, which are the correlations between the original variables and the discriminant scores on each function, provided a measure of the relative contribution of each variable to group separation. The analysis was performed using the CANDISC procedure in SAS (2004). Non-metric Multidimensional Scaling (NMDS) also was used to confirm the site groupings obtained through cluster analysis.

Species accumulation curves (SACs) were generated using R statistical software (R Development Core Team 2006) and the “vegan” community ecology extension to R (Oksanen et al 2007). Additional data collected in 2001 and 2002 at a subset of six stations (see Hyland et al.

2006, Cooksey et al. 2004) were included in this analysis. SACs were used to illustrate the cumulative number of taxa encountered with increasing sample size, beginning in spring 2000 and continuing with each successive sampling event in 2001, 2002, and 2005. SACs were generated as the mean of random permutations of the data (sites added in random order) or subsampling without replacement (Gotelli and Colwell 2001). The purpose of this particular exercise was to help further our understanding and predictions of marine biodiversity within the sanctuary.

### 3.0 Results and Discussion

#### 3.1 Spring 2005 Sampling

Station depths varied between 15.9 and 21.9 m. Near-bottom (lower 3 m) salinities were characteristic of an open-ocean environment (typically between 33.2 and 34.4PSU) and dissolved oxygen (DO) levels were within a narrow range of 7.5 – 7.7 mg L<sup>-1</sup> (Fig. 3.1) well above a benthic hypoxic-effect threshold of about 1.4 mg L<sup>-1</sup> (Diaz and Rosenberg 1995). Sediments were composed mainly of sand and gravel ( $\geq 90\%$ ), with all but two sites (stations 50 and 51) having  $< 2\%$  silt-clay content (Fig. 3.2). Median sediment particle size ( $\Phi$ ) values ranged between 1.93 (medium sand) and 0.30 (coarse sand). The sorting coefficient, which is a measure of the distribution and diversity of grain sizes, ranged from 0.5 to 1.6. Percent total organic carbon (TOC) was low at all stations, varying between 0 (Station 50) and 1.9% (Fig. 3.3) which is well below a reported range ( $> 36$  mg g<sup>-1</sup>, or 3.6%) associated with a high risk of disturbance from organic over-enrichment (Hyland et al. 2005). The station with the highest sediment TOC and silt-clay content (Station 51) also had the most diverse (poorly sorted, with highest sorting coefficient) and finest (largest median particle size) sediment. A summary of the values of each of these abiotic environmental variables by station is given in Appendix A.

Concentrations of most chemical contaminants in sediments appeared to be at low background levels at all sites

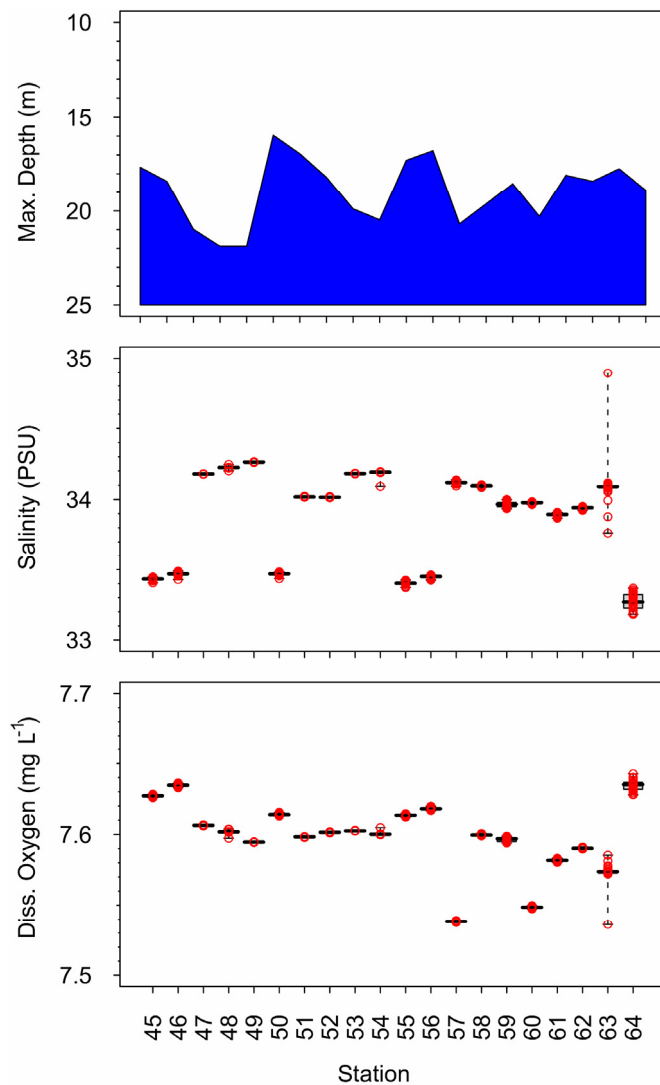


Figure 3.1. Depth, salinity, and dissolved oxygen measured at 20 sites sampled in spring 2005 at GRNMS. Observations represent conditions in the lower 3 m of the water column.

sampled (Appendix B). There were no exceedances of published sediment quality guidelines (SQG) for the higher ERM or lower ERL bioeffect levels for any individual contaminant at any of the sites. Sediment concentrations of pesticides and PCBs were below the limit of detection at all sites. While a number of PAHs and metals were found at detectable levels in sediments throughout the sanctuary, these concentrations all were well below published ERM/ERL values.

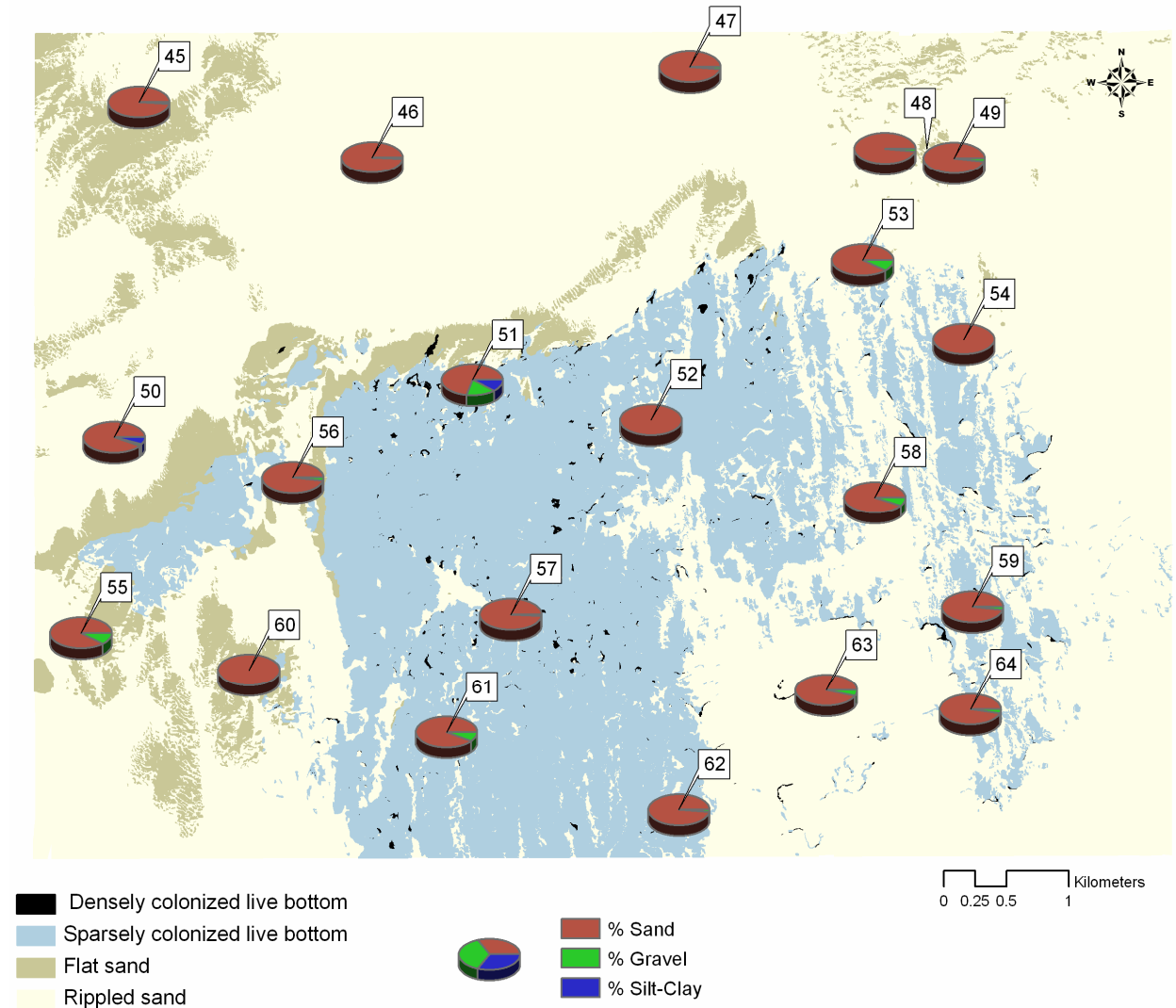


Figure 3.2. Sediment sand, gravel, and silt-clay fractions (%) at sites sampled in spring 2005 at GRNMS. Map of benthic habitat types is from Kendall et al (2005).



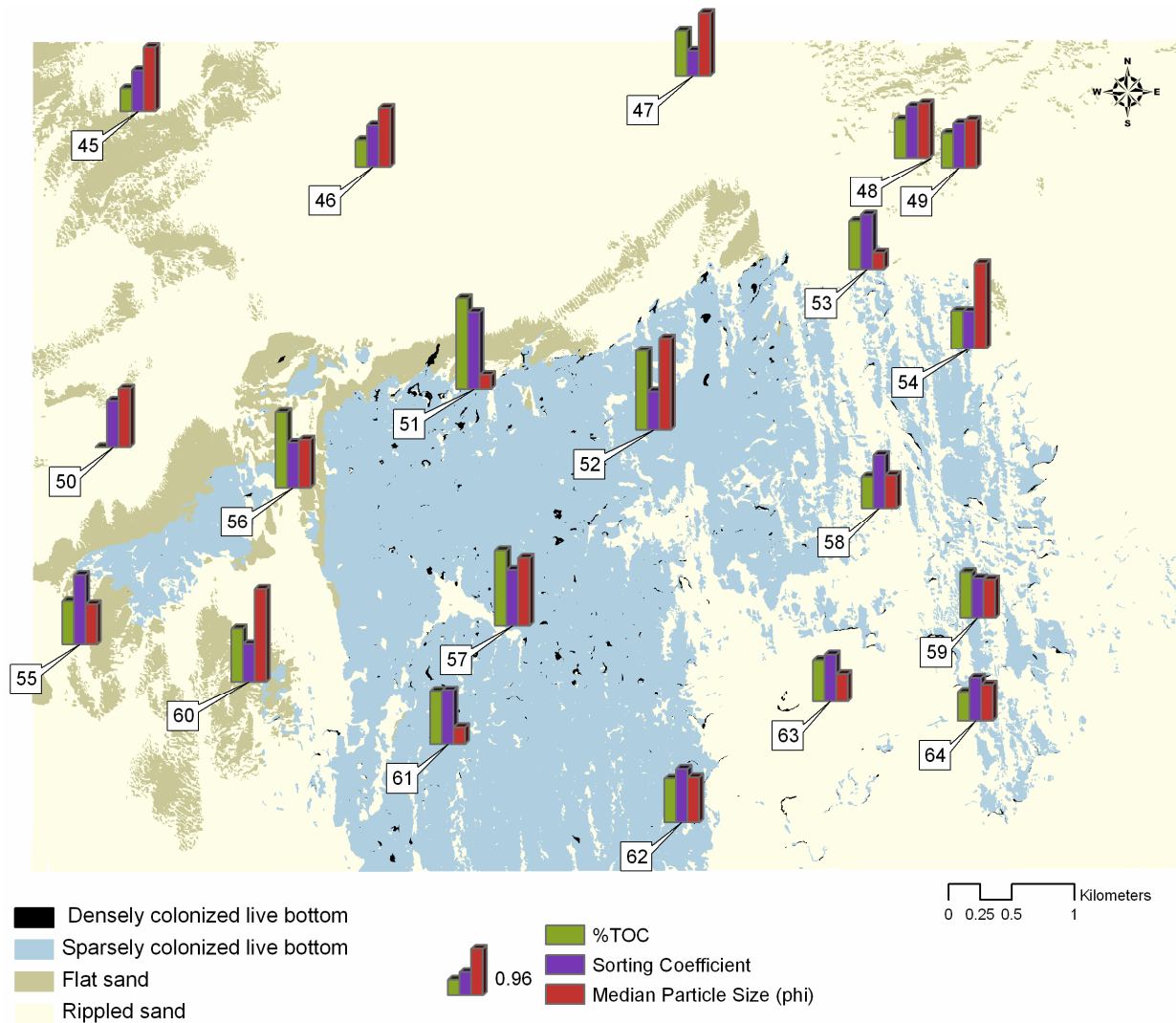


Figure 3.3. Percent TOC, sediment sorting coefficient, and median particle size ( $\Phi$ ) measured at 20 sites sampled in spring 2005 at GRNMS. The height of the tallest bar in the legend represents a median particle size of 0.96 mm. Note that because phi is an inverse scale, bar heights are inversely related to median particle diameter. Map of benthic habitat types is from Kendall et al (2005).

There were no stations with mean ERM quotients in the range (i.e., above a critical point of 0.06, Hyland et al. 1999) found to be associated with a high incidence of impaired benthic condition (Fig. 3.4a). Mean ERM quotients all were in the lower portion of the range previously found to be associated with a low incidence of benthic effects in southeastern estuaries (i.e., below a critical point of 0.02; Hyland et al. 1999, Hyland et al. 2003). Of those contaminants found above detection limits (PAHs and metals), metals were the main contributor to total (summed) ERM quotients (Figs. 3.4b and c). Note that the mean ERM quotients in Fig. 3.4a are obtained by dividing the summed ERM quotient at each site (Fig. 3.4b) by the number of contaminants for which ERM values are available ( $n=24$ ; see Appendix B). The dominant metals were As, Cd, Cr, Pb, and Zn (Fig. 3.4c). PAHs above detection limits included biphenyl, dibenz[a,h]anthracene, 2,6-dimethylnaphthalene, fluoranthene, fluorene, indeno[1,2,3-c,d]pyrene, 1- and 2-methylnaphthalene, naphthalene, phenanthrene, and pyrene.

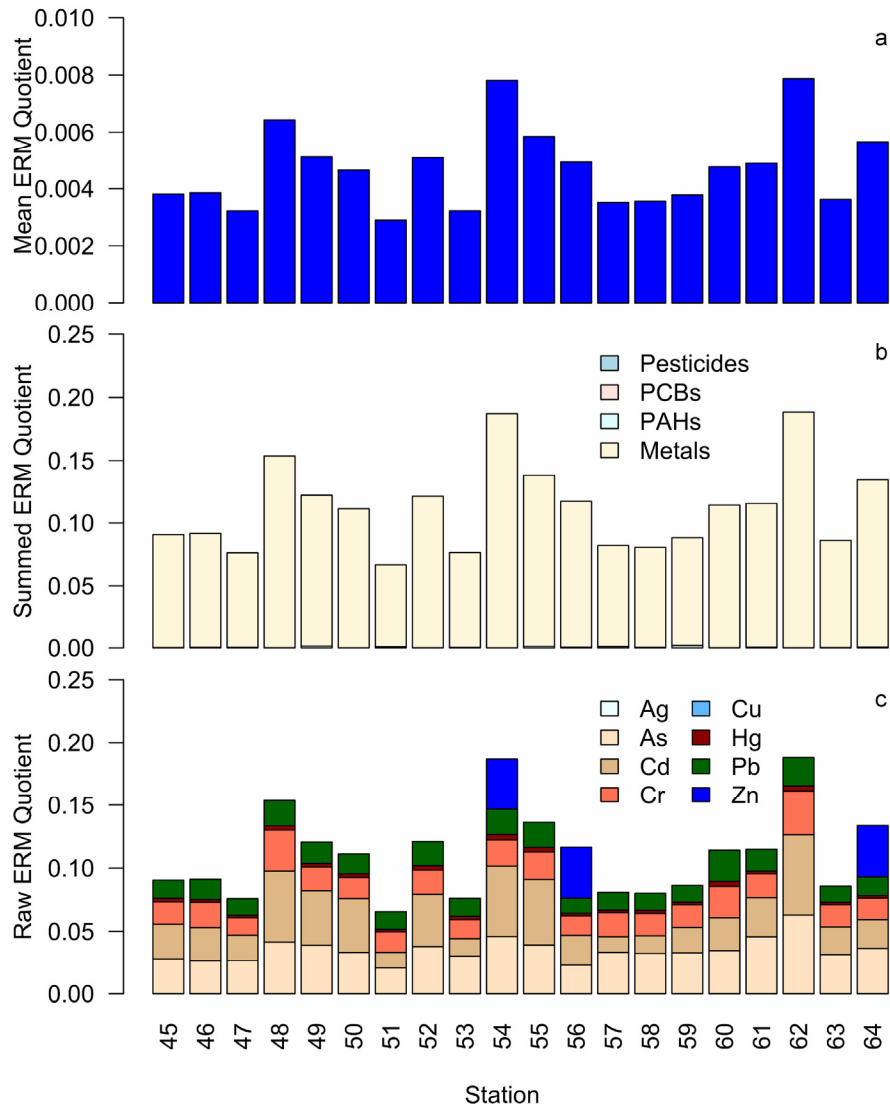


Figure 3.4. Distribution of a) mean ERM quotients (calculated for 24 contaminants), b) summed ERM quotients (n=24 contaminants, calculated separately by contaminant class), and c) raw ERM quotients (metals only) among 20 sites in Gray's Reef National Marine Sanctuary.

Levels of chemical contaminants (mean and ranges) in tissues of black sea bass (*Centropristis striata*) and zebra ark (*Arca zebra*) are given in Appendix C. FDA human-health guidelines (Action Levels or Levels of Concern) are included for comparison, where available (FDA 1994; FDA 1993a-e; FDA 1984). Similar to concentrations observed in sediments, levels of pesticides, PCBs, and most PAHs were below method detection limits, and no exceedances of FDA guideline values were observed in either species. As, Cr, Fe, Zn, and Al were the dominant metals in both species. PAHs in tissues included acenaphthene (both species), acenaphthylene (both species), benz[a]anthracene (zebra ark), benzo[b]fluoranthene and benzo[j+k]fluoranthene (zebra ark), benzo[g,h,i]perylene (zebra ark), biphenyl (both species), chysene+triphenylene (zebra ark), dibenz[a,h]anthracene (zebra ark), 2,6-dimethynaphthalene (both species), fluoranthene (both species), 1- and 2-methylnaphthalene (both species), naphthalene (both



species), phenanthrene (both species), pyrene (zebra ark), and 1,6,7-trimethylnaphthalene (zebra ark).

A total of 353 taxa (219 identified to species level) were enumerated from 60 replicate grab samples (3 per site, each 0.04 m<sup>2</sup>) collected at the twenty GRNMS sites. A diverse macroinfaunal assemblage, with an average of 47 taxa and 154 individuals per 0.04 m<sup>2</sup> grab (3,850 m<sup>-2</sup>), and a mean H' diversity of 4.7, was observed at the 20 stations (Table 3.1).

Table 3.1. Number of taxa, abundance, density, and H' diversity (base-2 logarithms) of benthic macroinfauna at GRNMS sites sampled in spring 2005.

Station	Mean No. Taxa per grab	Total No. Taxa (pooled replicates)	Mean Abundance (0.04 m <sup>-2</sup> )	Mean Density (m <sup>-2</sup> )	Mean H' Diversity
45	39	71	107	2667	4.7
46	51	104	178	4458	4.8
47	51	95	191	4783	4.6
48	45	81	187	4667	4.4
49	53	97	148	3692	5.0
50	34	62	95	2383	3.9
51	62	119	246	6158	4.9
52	53	99	145	3625	4.9
53	64	123	245	6125	5.3
54	27	55	57	1425	4.2
55	61	112	224	5600	5.1
56	29	56	78	1942	4.3
57	48	89	151	3783	4.9
58	42	91	99	2475	4.8
59	44	84	178	4442	4.7
60	33	69	88	2192	4.1
61	33	63	99	2483	4.1
62	54	106	170	4242	5.0
63	51	97	148	3692	4.8
64	63	114	247	6167	5.1
Mean:	47	89	154	3850	4.7

Dominant taxa (10 most abundant) are shown in Table 3.2 and included the spionid polychaetes *Spiophanes bombyx* and *Spio pettibonae* as the two most abundant taxa. Sub-dominants included the minor jackknife clam *Ensis minor*, tubificid Oligochaetes, the gastropod *Caecum johnsoni*, the lancelet *Branchiostoma* spp., the dorvilleid polychaete *Protodorvillea kefersteini*, the chrysopetalid polychaete *Bhawania goodei*, unidentified members of the genus *Spio*, and the pilargid polychaete *Synelmis ewingi*. Collectively these 10 dominants made up 37% of the total abundance of all infaunal taxa and, of this amount, polychaetes represented 22%. The distribution of major phyla is shown in Fig. 3.5. Stations with the highest percentages of crustaceans (e.g., 52 and 54) and echinoderms (e.g., 57 and 62) were in areas of live bottom. Stations with the highest percentages of mollusks (e.g., 50 and 56) tended to be in sandy areas.

Table 3.2. Ten dominant (most abundant) taxa found at GRNMS stations sampled in spring 2005.

Taxon	Group	Mean Density (m <sup>-2</sup> )	% of Total Abundance	Cumulative % Abundance	% Station Occurrence
<i>Spiophanes bombyx</i>	Polychaeta	248.8	6.5	6.5	100
<i>Spio pettiboneae</i>	Polychaeta	201.7	5.2	11.7	85
<i>Ensis minor</i>	Bivalvia	156.7	4.1	15.8	85
Tubificidae	Oligochaeta	149.6	3.9	19.7	95
<i>Caecum johnsoni</i>	Gastropoda	134.2	3.5	23.1	85
<i>Branchiostoma</i> spp.	Cephalochordata	130.4	3.4	26.5	90
<i>Protodorvillea kefersteini</i>	Polychaeta	125.8	3.3	29.8	95
<i>Bhawania goodei</i>	Polychaeta	107.9	2.8	32.6	90
<i>Spio</i> spp.	Polychaeta	82.1	2.1	34.7	70
<i>Synelmis ewingi</i>	Polychaeta	80.0	2.1	36.8	60

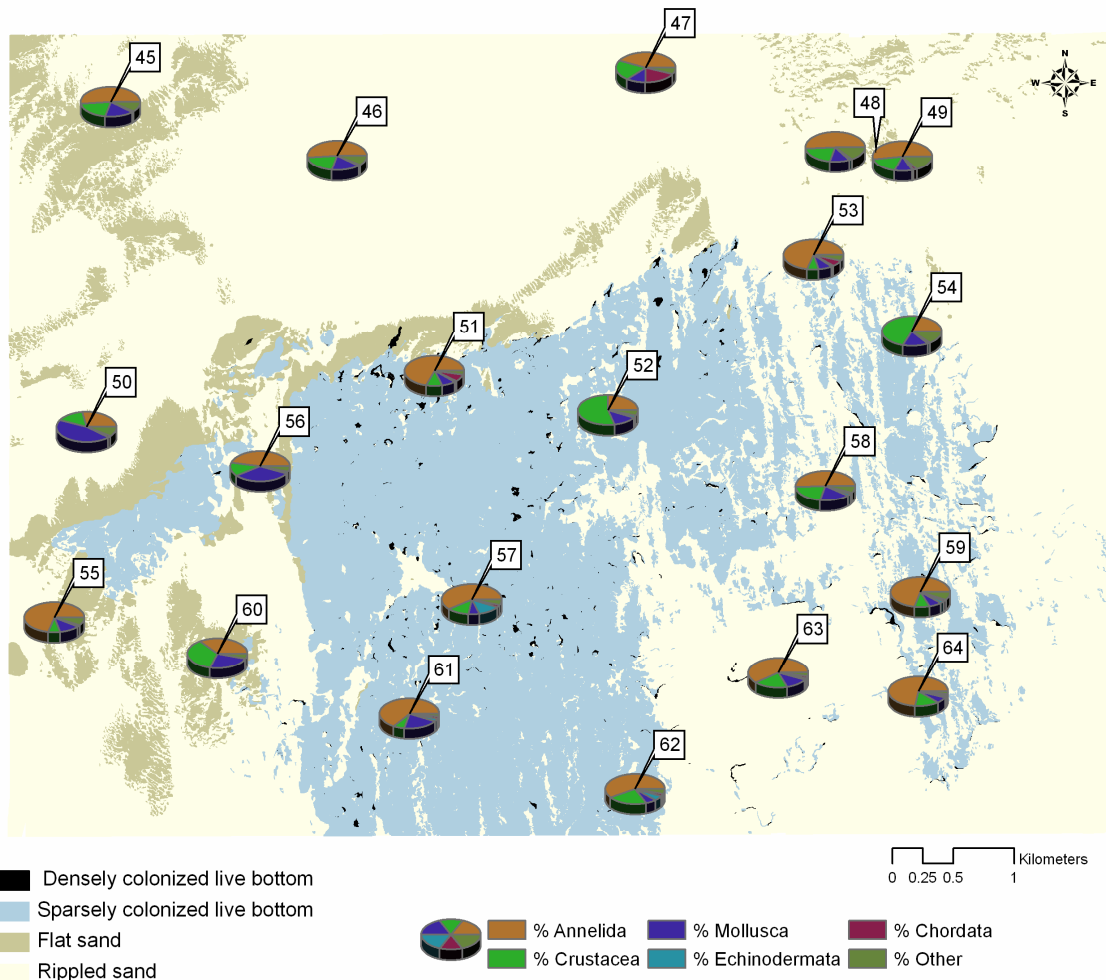


Figure 3.5. Distribution of major phyla at sites sampled in spring 2005 at GRNMS. Map of benthic habitat types is from Kendall et al (2005).

Hierarchical clustering of macroinfaunal abundances, in which stations were ordered into groups of increasingly greater similarity based on resemblances of component-taxon abundances, yielded three site groups, as shown in Fig. 3.6. Site groups were defined based on a Bray-Curtis similarity value of 0.55 as a separation criterion.

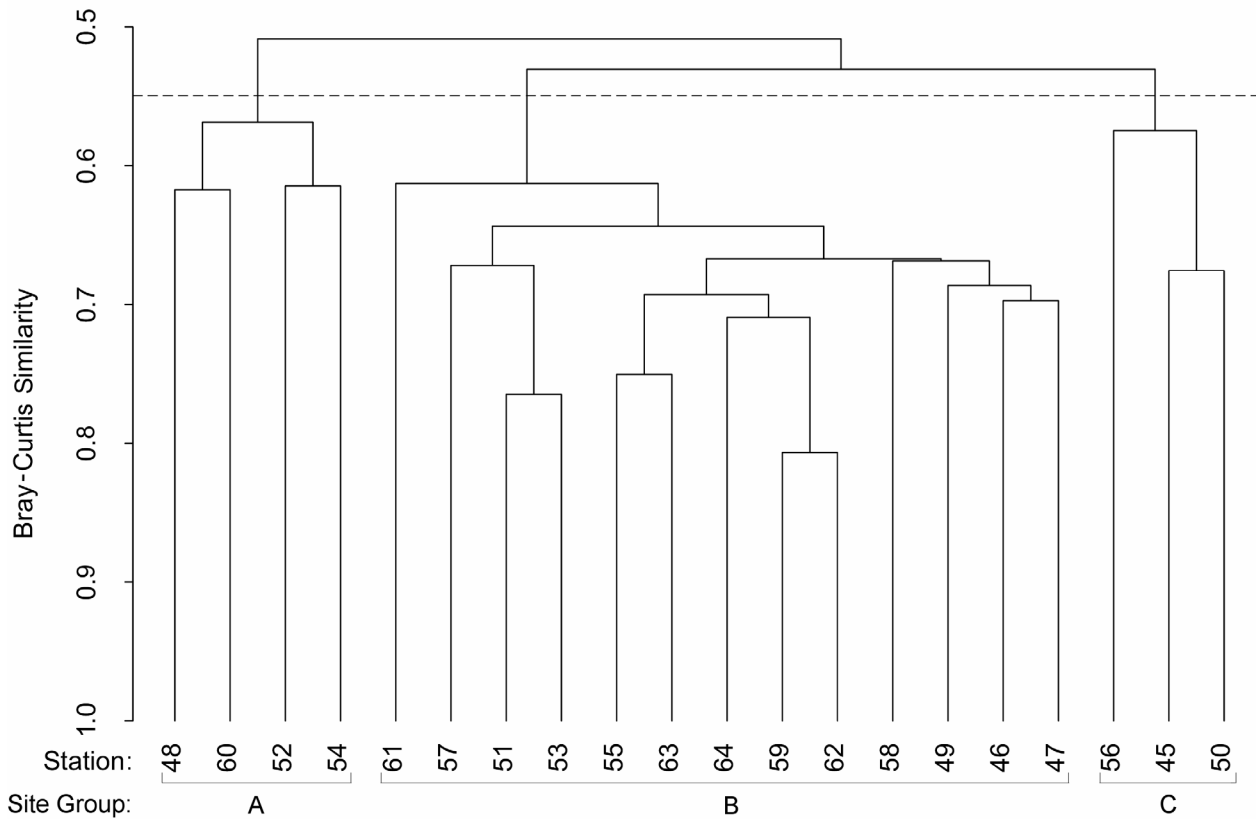


Figure 3.6. Dendrogram resulting from hierarchical clustering of macroinfaunal abundances at sites sampled in spring 2005, using Bray-Curtis (BC) similarity and group-average sorting. Samples within each station were combined over all 3 replicates. A BC similarity value of 0.55 was used to define site groups.

Dominant taxa within each site group (SG) are shown in Table 3.3, along with measures of diversity and abundance by site group. Of the three site groups, SG B had the highest infaunal density,  $H'$  diversity, and number of taxa. Differences in these parameters were significant only for the lowest (SG C) and highest (SG B) values; no significant differences were observed between the intermediate values (SG A) and either of the other two site groups. SG C, which had the lowest density, diversity, and numbers of taxa, consisted of stations (45, 50, 56) located in the northwest sector of the sanctuary.

Table 3.3. Dominant taxa in each of the site groups obtained from hierarchical clustering of sites.

Site Group	Taxon	Dominant fauna			Mean density (m <sup>-2</sup> ) <sup>a</sup>	Mean # taxa per grab	Mean H' per grab	Mean # taxa per station <sup>b</sup>
		Mean density (m <sup>-2</sup> )	Cum. %	% Station Occurrence				
A	<i>Synelmis ewingi</i> (P)	296	10	75	2977	40	4.41	75
	<i>Ensis minor</i> (B)	248	18	100				
	<i>Acanthohaustorius millsii</i> (C)	194	25	75				
	<i>Oxyurostylis smithi</i> (C)	194	31	100				
	<i>Metharpinia floridana</i> (C)	169	37	100				
	<i>Spiophanes bombyx</i> (P)	131	41	100				
	Turbellaria (Pl)	106	45	100				
	<i>Spio</i> spp. (P)	75	47	50				
	<i>Acanthohaustorius</i> spp. (C)	65	50	75				
	Nemertea	54	51	100				
B	<i>Spiophanes bombyx</i> (P)	317	7	100	4469	52	4.86	99
	<i>Spio pettiboneae</i> (P)	303	14	92				
	Tubificidae (O)	203	18	100				
	<i>Caecum johnsoni</i> (G)	187	23	100				
	<i>Branchiostoma</i> spp. (Ce)	183	27	100				
	<i>Protodorvillea kefersteini</i> (P)	167	30	100				
	<i>Bhawania goodei</i> (P)	155	34	92				
	<i>Sphaerosyllis piriferopsis</i> (P)	102	36	92				
	<i>Spio</i> spp. (P)	85	38	69				
	<i>Goniadides carolinae</i> (P)	79	40	100				
C	<i>Ensis minor</i> (B)	383	16	67	2330	34	4.30	63
	<i>Ensis directus</i> (B)	122	22	33				
	<i>Tanaissus psammophilus</i> (C)	117	27	100				
	<i>Spiophanes bombyx</i> (P)	108	31	100				
	<i>Parapionosyllis longicirrata</i> (P)	83	35	100				
	<i>Caecum johnsoni</i> (G)	83	38	100				
	<i>Spio</i> spp. (P)	81	42	100				
	Tubificidae (O)	75	45	100				
	<i>Protodorvillea kefersteini</i> (P)	67	48	100				
	<i>Crassinella lunulata</i> (B)	64	51	100				

P = Polychaeta, G = Gastropoda, B = Bivalvia, C = Crustacea, O = Oligochaeta, Pl = Platyhelminthes, Ce = Cephalochordata.

<sup>a</sup> All taxa combined.

<sup>b</sup> Total number of taxa at a station (3 replicate, 0.04-m<sup>2</sup> grabs combined) averaged over all stations within the same site group.

Dominant phyla within each site group included Annelida (primarily Polychaeta), Crustacea (Malacostraca), and Mollusca (Bivalvia and Gastropoda). These 3 phyla accounted for approximately 90% of all taxa in each site group. Site group A was composed of Annelida (35.7% Polychaeta, 1.1% Oligochaeta), Crustacea (35.8% Malacostraca), and Mollusca (14.1% Bivalvia, 2.5% Gastropoda, 0.2% Polyplacophora). Site group B included Annelida (56% Polychaeta, 5.9% Oligochaeta), Crustacea (15% Malacostraca), and Mollusca (5.8% Gastropoda, 5.3% Bivalvia). Site group C was characterized by Annelida (35.8% Polychaeta, 3.8% Oligochaeta), Mollusca (27.4% Bivalvia, 7.3% Gastropoda), and Crustacea (15.3% Malacostraca). The distribution of phyla among Site Groups is given in Table 3.4.

Table 3.4. Dominant phyla contributing more than 10% abundance of all taxa in each of three site groups identified from hierarchical clustering.

	Taxon	Mean density (m <sup>-2</sup> )	% of Total Site Group Abundance	Cumulative % Abundance	% Station Occurrence
SG A (n=4)	Annelida	1096	37	37	100
	Crustacea	1067	36	73	100
	Mollusca	502	17	90	100
SG B (n=13)	Annelida	2768	62	62	100
	Crustacea	674	15	77	100
	Mollusca	508	11	88	100
SG C (n=3)	Annelida	922	40	40	100
	Mollusca	811	35	75	100
	Crustacea	356	15	90	100

Mean values of measured abiotic variables for the three site groups are displayed in Table 3.5. Significant differences in mean depth, salinity, and median particle size ( $\Phi$ ) exist among site groups, though salinity differences likely are not biologically meaningful, due to the narrow range of salinities (mean salinity between 33.4 and 34.1). These results suggest that the site groupings are related mainly to sediment characteristics and secondarily to depth (not corrected to Mean Lower Low Water datum). SG C consisted of the shallowest stations. Coarsest sediments with lowest  $\Phi$  values and highest percentages of gravel were observed at SG B stations. SG A had the finest sediments with highest  $\Phi$  values and lowest percentages of gravel.

Table 3.5. Mean values of abiotic environmental variables by site group and results of univariate tests for significant differences (ANOVA) among site groups for each variable.

Variable	Site Group			ANOVA Results	
	A	B	C	F-value	Pr > F
Depth (m)	20.2	19.0	16.8	4.90	0.021
Temperature (°C)	18.8	18.8	18.8	0.01	0.990
Salinity (PSU)	34.1	33.9	33.4	5.27	0.017
DO (mg L <sup>-1</sup> )	7.6	7.6	7.6	1.65	0.222
pH	8.2	8.2	8.2	0.59	0.564
Median particle size ( $\Phi$ )	1.7	0.8	1.2	9.99	0.001
% Sand	98.8	93.5	96.2	1.11	0.351
% Gravel	0.9	5.4	1.8	2.02	0.163
% Silt-clay	0.4	1.1	2.0	0.37	0.695
% TOC	1.1	1.0	1.0	0.10	0.902
Mean ERM quotient	0.006	0.004	0.004	2.32	0.128

Canonical discriminant analysis reveals that these differences are related foremost to sediment grain size. Table 3.6 lists the total structure coefficients (TSCs) for canonical discriminant analysis that includes depth, median particle size ( $\Phi$ ), % sand, % gravel, and mean ERM quotient. The first canonical variable is significant ( $p = 0.0038$ ,  $df=10,26$ ) and accounts for 71% of the among-group variation in abiotic variables. The second canonical variable was marginally significant ( $p=0.054$ ,  $df=4,14$ ) and accounts for the remaining 29% of the variation in abiotic variables among site groups. The TSCs suggest that the first canonical variable is most highly correlated with median particle size ( $\Phi$ ). A plot of the first and second canonical variables (Fig. 3.7) shows fairly good separation of site groups, with SG A (highest  $\Phi$  values indicative of finer sediments) well-separated from the other two site groups on the first canonical axis. Some separation between SGs B and C on the second canonical axis (most highly correlated with station depth) is also apparent, with SG C having the shallowest mean depth.

Table 3.6. Total structure coefficients (TSCs) from canonical discriminant analysis of abiotic environmental variables using the site groups obtained from hierarchical clustering of the macroinfaunal data.

Variable	Total Structure Coefficient	
	Can1	Can2
Depth	0.4970	0.6534
Median particle size ( $\Phi$ )	<u>0.8093</u>	-0.4563
% Sand	0.3593	-0.2475
% Gravel	-0.3945	0.4326
Mean ERM quotient	0.5602	-0.0643

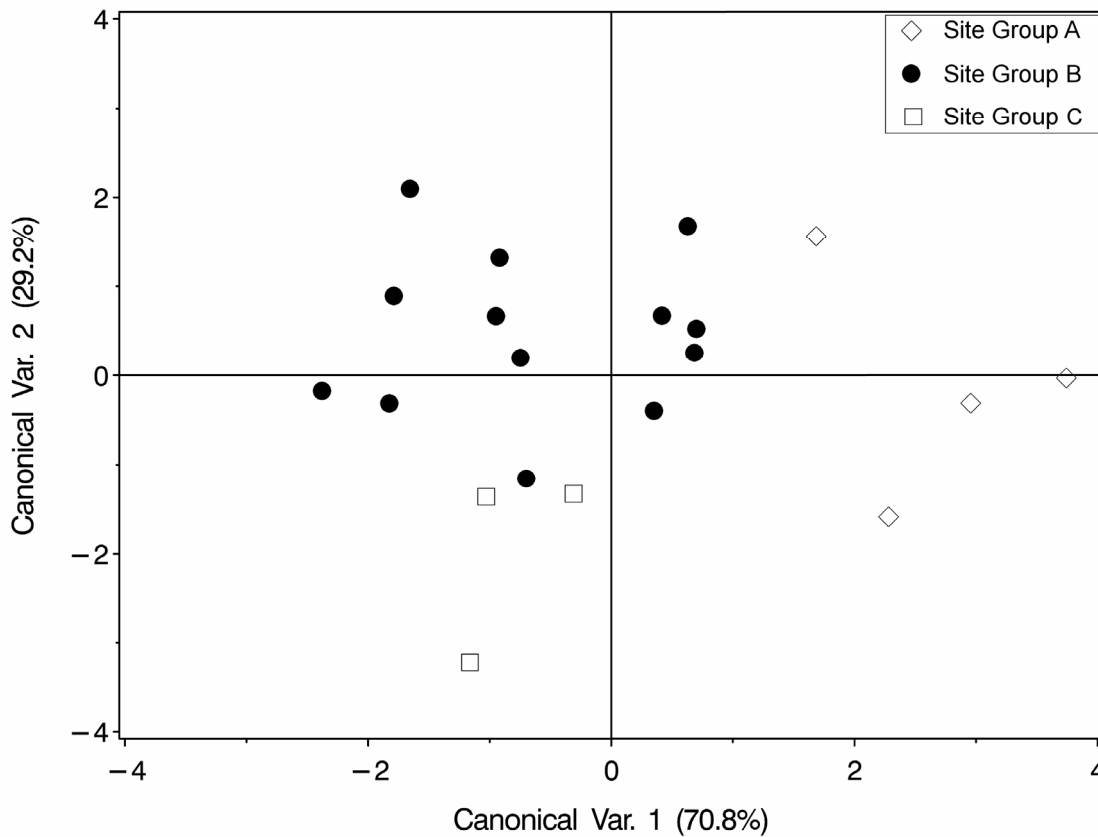


Figure 3.7. Plot of the first and second canonical variables obtained from canonical discriminant analysis of abiotic environmental variables based on site groups obtained from hierarchical clustering.

Non-metric multidimensional scaling (NMDS) of the benthic infauna data (Fig. 3.8) provides confirmation of the site groupings obtained from hierarchical clustering. Fitting vectors of environmental variables onto the ordination shows the directions in the ordination space towards which the environmental variables change most rapidly and to which they have maximal correlations with the ordination configuration. Similar to the results obtained from canonical discriminant analysis, SG A is separated based on particle size (fine-grained sands), with SG B having a range of varying particle sizes, but typically larger particle diameters (calculated from median particle size,  $\Phi$ , as  $D = D_0 \cdot 2^{-\Phi}$ , where  $D_0$  is a reference diameter, equal to 1 mm to make the equation dimensionally consistent). The red circles in the figure are proportional to particle diameter in mm.

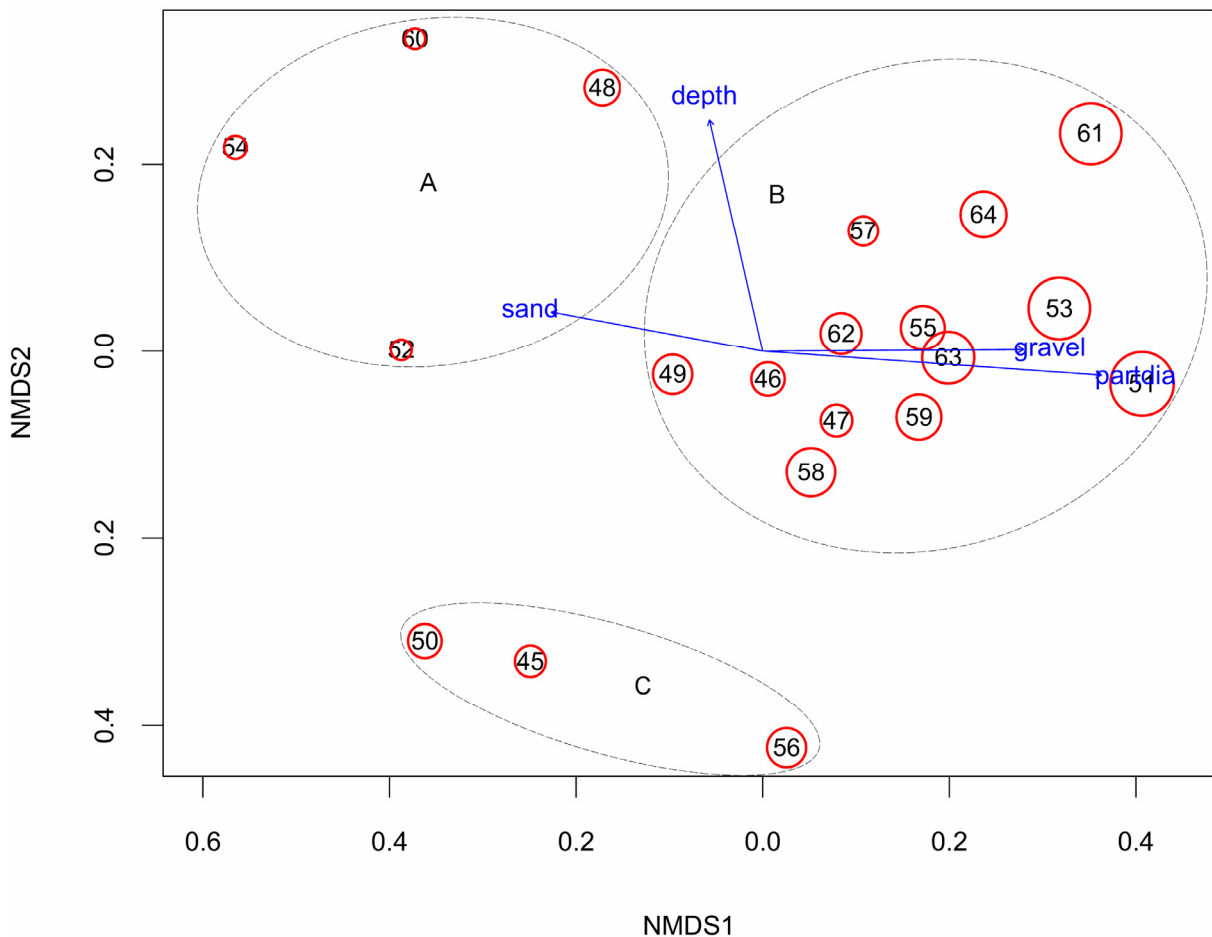


Figure 3.8. Plot of results of non-metric multidimensional scaling (NMDS) of the macroinfaunal abundance data. Site groups obtained from hierarchical clustering are shown as ellipses, with corresponding site group labels. Vectors show the directions in the ordination space towards which the environmental variables change most rapidly and to which they have maximal correlations with the ordination configuration. Red circles are proportional to median sediment particle diameter.

While sediment texture and particle size appear to influence the distribution of benthic taxa at GRNMS, these parameters are also related to bottom habitat type. Kendall et al (2005) developed a map of benthic habitat types at GRNMS based on combined data from sonar imagery, towed-camera video transects, and diver observations collected in 2001. Four distinct classes of bottom habitat (or benthoscape features) were defined as follows:

Flat sand plain (FS): stable sand deposits; no sudden changes in relief; grain size appears to be smaller than in areas with rippled sand.

Rippled sand (RS): sediment with regular ridges or ripples; troughs often dominated by coarser material such as shell fragments; crests composed primarily of sand.

Sparsely colonized hard bottom (SC): very low relief areas of partially exposed limestone substrate colonized with a sparse assemblage of sessile benthic organisms; thin veneer of sand 1-2 cm thick covers much of the bottom but is thin or ephemeral enough to allow sessile benthic organisms to attach to the limestone.

Densely colonized hard bottom (DC): exposed limestone that is colonized with a nearly continuous coverage of sessile benthic organisms such as soft corals, sponges, and tunicates; characterized by ledges and other areas of high relief (0.5 – 2 m).

Locations of stations in the present 2005 study coincide spatially with three of the four habitat types (FS, RS, and SC) included in the map by Kendall et al. (2005). Two of the 20 total sites (48 and 60) correspond to the FS bottom type, seven (51 – 54, 57, 59, 61) correspond to the SC bottom type, and the remaining eleven are in RS habitat. No densely colonized (DC) habitats were represented in this study.

With respect to median particle size ( $\Phi$ ), samples collected from sites in the FS bottom type were characterized by smaller particle sizes (large  $\Phi$  values); sites in the RS bottom habitat typically had larger particle sizes; and those in the SC bottom type had the highest median and widest range of particle sizes (Fig. 3.9). While particle-size distributions among the three benthic site groups showed similar patterns (Fig. 3.9), the site

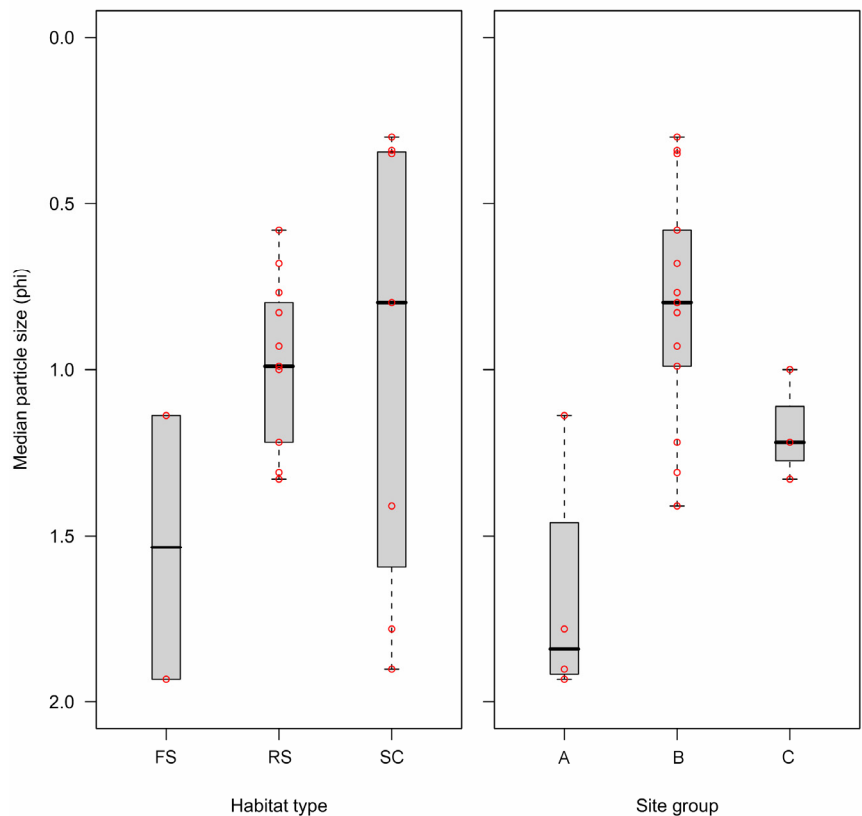


Figure 3.9. Median particle size ( $\Phi$ ) versus habitat type and site group. The scale of the y-axis is reversed to reflect that larger values of  $\Phi$  correspond to smaller particle diameters. Habitat types follow Kendall et al (2005). FS=Flat sand; RS=Rippled sand; SC=Sparsely colonized live bottom.



groups did not appear to be strongly correlated with these larger mesoscale features of benthoscape relief. For example, SG A included sites from both the FS and SC habitat types, SG B stations included both the RS and SC habitat types, whereas SG C included sites in the RS habitat type only. The site group designations and their locations in relation to bottom habitat types are displayed in Fig. 3.10. The 2000 data showed a much stronger match between spatial patterns of benthic site groups and the Kendall et al. (2005) mosaic of benthoscape types (Hyland et al. 2000).

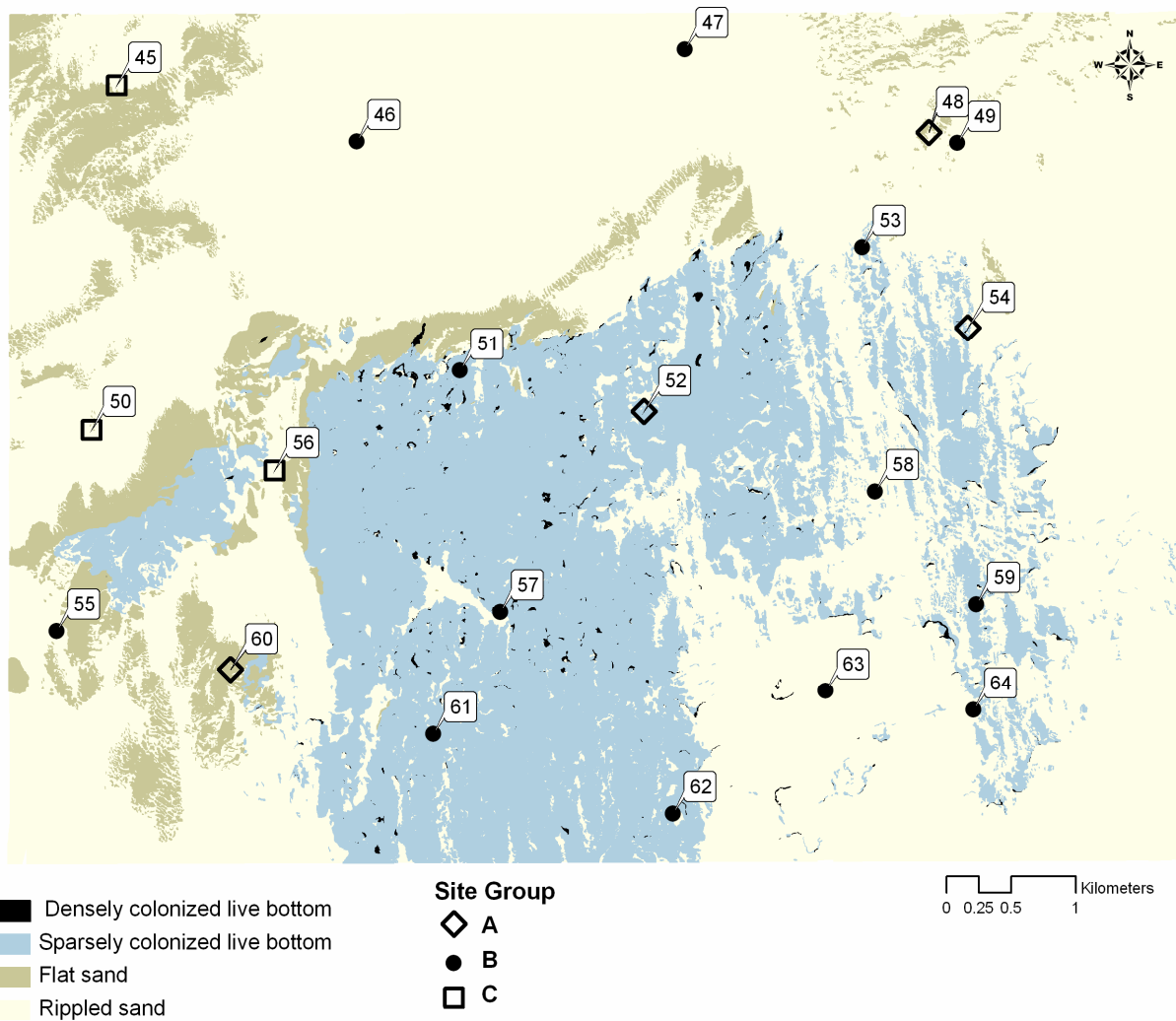


Figure 3.10. Relationship between the site groups obtained from hierarchical clustering of macroinfaunal abundance and site locations with respect to bottom habitat type in GRNMS. Benthic habitat map is from Kendall et al (2005).

### 3.2 Comparison with results of the spring 2000 survey

Ranges of abiotic environmental factors (depth, salinity, dissolved oxygen, sediment characteristics) measured in the spring 2005 sampling effort were similar to those observed in the spring 2000 baseline survey. In both years, DO and sediment organic matter (TOC) concentrations were at normal levels outside reported bioeffect ranges. Also, as in the baseline survey, 2005 levels of chemical contaminants in sediments and tissues of black sea bass (*C. striata*) and zebra ark (*A. zebra*) were below sediment-quality and human-health guideline values and were often below the instrumental detection limits. In both years, mean ERM quotients (mERMq) for sediments were well below levels found to be harmful to benthic infaunal organisms (mERMq=0.06; Hyland et al 2003), and were in the lower portion of the range associated with a low incidence of benthic effects (mERMq=0.02; Hyland et al 2003). The average mERMq across sites were 0.0051 and 0.0047 in 2000 and 2005, respectively. Thus, any shifts in the characteristics and condition of benthic communities are not likely due to adverse effects of environmental stressors.

Abundance and number of infaunal taxa did not differ significantly between the two sampling periods (at  $\alpha = 0.05$ ), though Shannon diversity ( $H'$ ) was significantly different (mean of 4.7 in 2005 vs. 3.9 in 2000,  $p = 0.016$ ). A likely explanation for this difference can be found in Hyland et al. (2006), which reported peaks in population densities of several taxa at some sites, most notably *Ervilia* sp. A. Observed densities of *Ervilia* sp. A (Mollusca) exceeded 46,000 m<sup>-2</sup>, and accounted for over 90% of all taxa found at some sites in spring 2000. Such high relative abundances of one or two taxa affect the evenness component of the diversity index, resulting in much lower values of  $H'$  at those sites. This seems to be a plausible explanation, which is confirmed by removing *Ervilia* sp. A from the 2000 data set prior to calculating mean density and diversity. When this species is excluded, there is no longer a significant difference in  $H'$  diversity between the two sampling periods (Table 3.7). Also, the difference in total density between years is reduced dramatically.

Table 3.7. Comparison of mean density (m<sup>-2</sup>), number of taxa, and  $H'$  diversity at GRNMS in spring 2000 and 2005.

Variable	Survey		Statistical Test Results		
	Spring 2000	Spring 2005	df	t-value	Pr >  t
Mean density (m <sup>-2</sup> )	8831	3850	19.5 <sup>b</sup>	1.73	0.099
Mean # taxa	45	47	38	-0.58	0.565
Total # taxa <sup>a</sup>	87	89	38	-0.36	0.721
Mean $H'$	3.9	4.7	22.7 <sup>b</sup>	-2.59	0.016
Mean density (m <sup>-2</sup> ) <sup>c</sup>	3893	3850	38	0.09	0.929
Mean # taxa <sup>c</sup>	44	47	38	-0.67	0.508
Total # taxa <sup>a,c</sup>	86	89	38	-0.48	0.634
Mean $H'$ <sup>c</sup>	4.5	4.7	38	-1.51	0.138

<sup>a</sup> Pooled replicates.

<sup>b</sup> Corrected for unequal variances using Satterthwaite's approximation.

<sup>c</sup> After excluding *Ervilia* sp. A from the 2000 data.

Dominant taxa found in 2000 and 2005 (10 most abundant in each year) are displayed in Table 3.8. As discussed above, densities of *Ervilia* sp. A were extremely high in comparison

with other taxa, accounting for over 50% of the total abundance of all taxa found in spring 2000, while all other taxa contributed < 5% individually. Benthic taxa found in 2005 were much more evenly distributed, with each taxon contributing  $\leq 5\%$  to the total infaunal abundance. Comparison of the dominance rankings between years for the combined set of 16 taxa indicates a low degree of correlation in dominance hierarchies (Kendall's  $\tau = -0.10$ ,  $p = 0.59$ ). Note that the rankings are independent of the absolute densities of the respective taxa. Removing *Ervilia* sp. A from the 2000 data set and repeating the analysis using the next 10 dominants from 2000 yields a similar result ( $\tau = 0.03$ ,  $p = 0.88$ ), revealing a low degree of concordance between rankings for the two years, and demonstrating the dynamic nature of infaunal assemblages in GRNMS.

Table 3.8. Dominant (10 most abundant) taxa identified in spring 2000 and 2005 at GRNMS.

	Taxon	Mean Density	Cumul. % Abundance	Frequency (% of grabs)
Spring 2000	<i>Ervilia</i> sp. A	4937.5	55.9	60.0
	<i>Caecum johnsoni</i>	300.8	59.3	83.3
	<i>Crassinella lunulata</i>	267.5	62.3	90.0
	<i>Branchiostoma</i> spp.	250.8	65.2	76.7
	<i>Aspidosiphon muelleri</i>	217.5	67.7	91.7
	<i>Spiophanes bombyx</i>	163.8	69.5	98.3
	<i>Spio pettiboneae</i>	158.3	71.3	75.0
	<i>Oxyurostylis smithi</i>	155.0	73.1	98.3
	Ophiuroidea	125.4	74.5	68.3
	Actiniaria	102.1	75.6	46.7
Spring 2005	<i>Spiophanes bombyx</i>	248.8	6.5	98.3
	<i>Spio pettiboneae</i>	201.7	11.7	60.0
	<i>Ensis minor</i>	156.7	15.8	70.0
	Tubificidae	149.6	19.7	85.0
	<i>Caecum johnsoni</i>	134.2	23.1	71.7
	<i>Branchiostoma</i> spp.	130.4	26.5	73.3
	<i>Protodorvillea kefersteini</i>	125.8	29.8	85.0
	<i>Bhawania goodei</i>	107.9	32.6	56.7
	<i>Spio</i> spp.	82.1	34.7	51.7
	<i>Synelmis ewingi</i>	80.0	36.8	35.0

Hierarchical cluster analysis of the combined 2000 and 2005 benthic data yielded the dendrogram displayed in Fig. 3.11, which exhibits station groupings that correspond closely to the site groups obtained independently for the 2000 (Cooksey et al 2004) and 2005 data (this report). From the 2000 results, Cooksey et al (2004) identified two main site groups, A and B, while a subsequent publication (Hyland et al 2006) further divided SG B into two sub-groups B1 and B2, based on bottom substrate type. SG A in 2000 was characterized by stations concentrated in the northwest portion of the sanctuary having somewhat lower abundances and diversity compared to other sanctuary sites. Similarly, in the present study, the lowest mean values of abundance, numbers of species, and diversity were associated with SG C, which comprises three sites in the same northwest quadrant of the sanctuary (Table 3.3; Fig. 3.10). Another result that is demonstrated by this overall dendrogram is the clear between-year separation of the two large B-00 and B-05 site groups, which reflects differences in the relative abundance of species between the two sampling periods (and thus the dynamic nature of these offshore shelf assemblages).

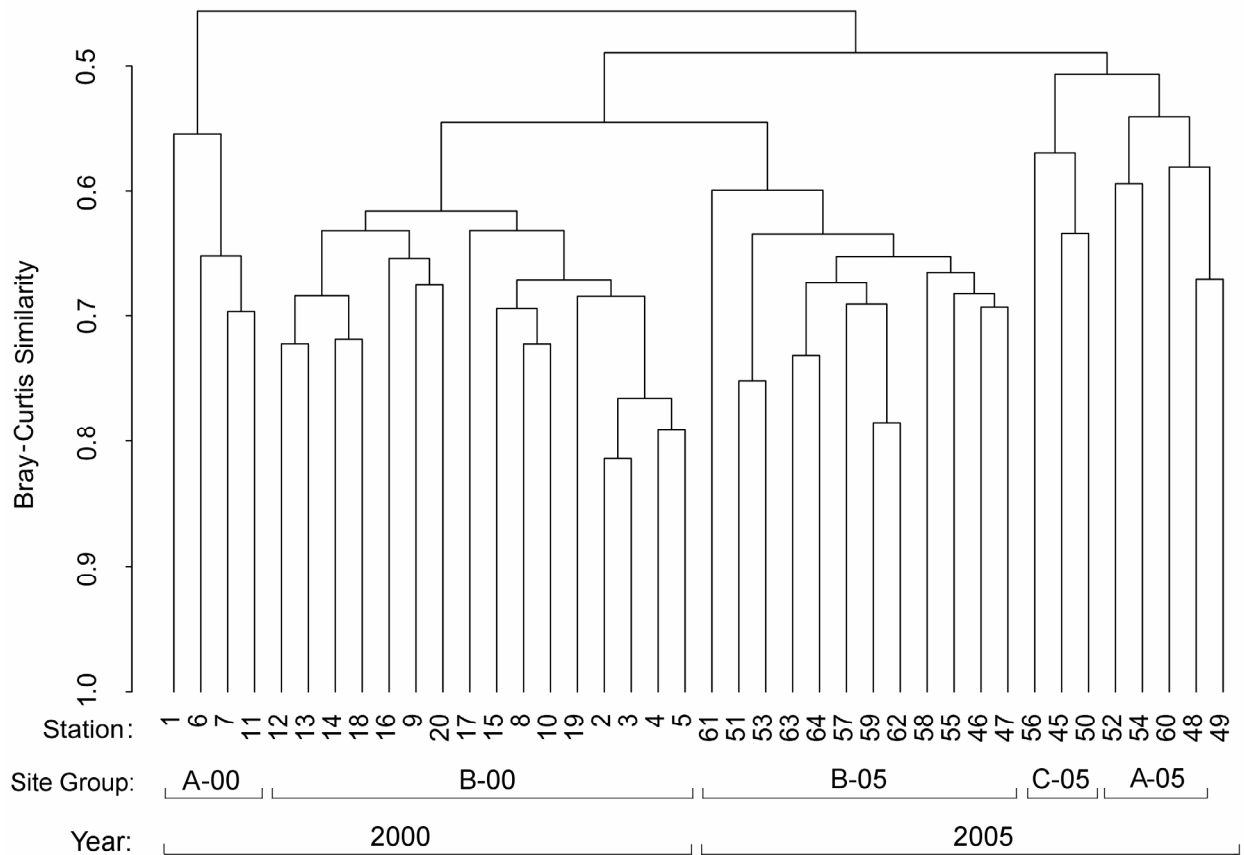


Figure 3.11. Dendrogram resulting from hierarchical clustering of macroinfaunal abundance from the combined 2000 and 2005 GRNMS benthic data.

In both the spring 2000 and 2005 surveys, one of the most strikingly apparent features of GRNMS is the highly diverse nature of macroinfaunal assemblages. A total of 348 taxa (223 identified to species) were collected during the spring 2000 baseline survey and 353 taxa (219 identified to species) were identified from the 2005 survey. In the spring 2000 survey, mean number of taxa per grab ranged from 27 to 64 (median=45) and mean  $H'$  diversity ranged from 0.8 to 5.1 (median=4.3); similarly, mean number of taxa per grab ranged from 27 to 64 (median=49) and  $H'$  diversity ranged from 3.9 to 5.3 (median=4.8) in the 2005 survey. In both studies, mean numbers of taxa per grab were nearly identical, and as noted previously,  $H'$  diversity, which is sensitive to the evenness component of biological assemblages, was lower in 2000 due to disproportionately high densities of a few dominant taxa (Table 3.7).

The combined 2000 and 2005 benthic dataset includes 483 taxa, which means that an additional 135 taxa were identified in 2005 that were not found in the 2000 survey. Six GRNMS sites also were sampled in 2001 and 2002 (three replicate, 0.04 m<sup>2</sup> grabs at each site) and these sampling occasions also added to the total number of infaunal taxa now encountered in the sanctuary. Taken together, the 2000, 2001, 2002, and 2005 surveys have contributed to the cumulative taxa list as shown in Table 3.9.

Table 3.9. Number of taxa identified at GRNMS, and number of taxa added on each sampling occasion .

Visit	Total # of Taxa	# of Grabs (0.04m <sup>2</sup> )	Cumul. # of Taxa	# of New Taxa Added
April 2000	348	60	348	348
April 2001	305	18	444	96
April/June 2002*	265	18	508	64
May 2005	353	60	588	80

\* 15 of 18 samples (5 of 6 sites) were collected in June 2002.

Species accumulation curves (SACs) provide a means of characterizing the cumulative number of taxa encountered with increasing sample size and can serve as a useful tool for estimating levels of biodiversity within a region of interest. The jagged curve in Fig. 3.12a represents one possible ordering of samples collected in spring 2000, 2001, 2002, and 2005, respectively. Repeated re-sampling of sites, in which samples are selected in random order without replacement (Gotelli and Colwell 2001), yields the mean SAC shown in Fig. 3.12b. It is reasonable to assume that given sufficiently exhaustive field sampling, eventually most (though not all; Clench 1979) infaunal taxa in the GRNMS could be identified and that the SAC would approach its maximum horizontal asymptote. From Fig. 3.12b, it seems apparent that further sampling would be required to reach such a theoretical maximum. It also is reasonable to assume that this maximum is somewhere in excess of 600 species. Indeed, fitting an asymptotic model for SACs (Colwell and Coddington 1994; Clench 1979),

$$S(n) = \frac{S_{\max} n}{b + n}, \quad (3.1)$$

where  $S_{\max}$  and  $b$  are fitted constants and  $n$  is the number of samples collected, to the original data yields the curve depicted in Fig. 3.12c. The plot suggests that the maximum ( $S_{\max}$ ) may be in the neighborhood of 800 taxa, though a large number of samples would be required to approach such a maximum.

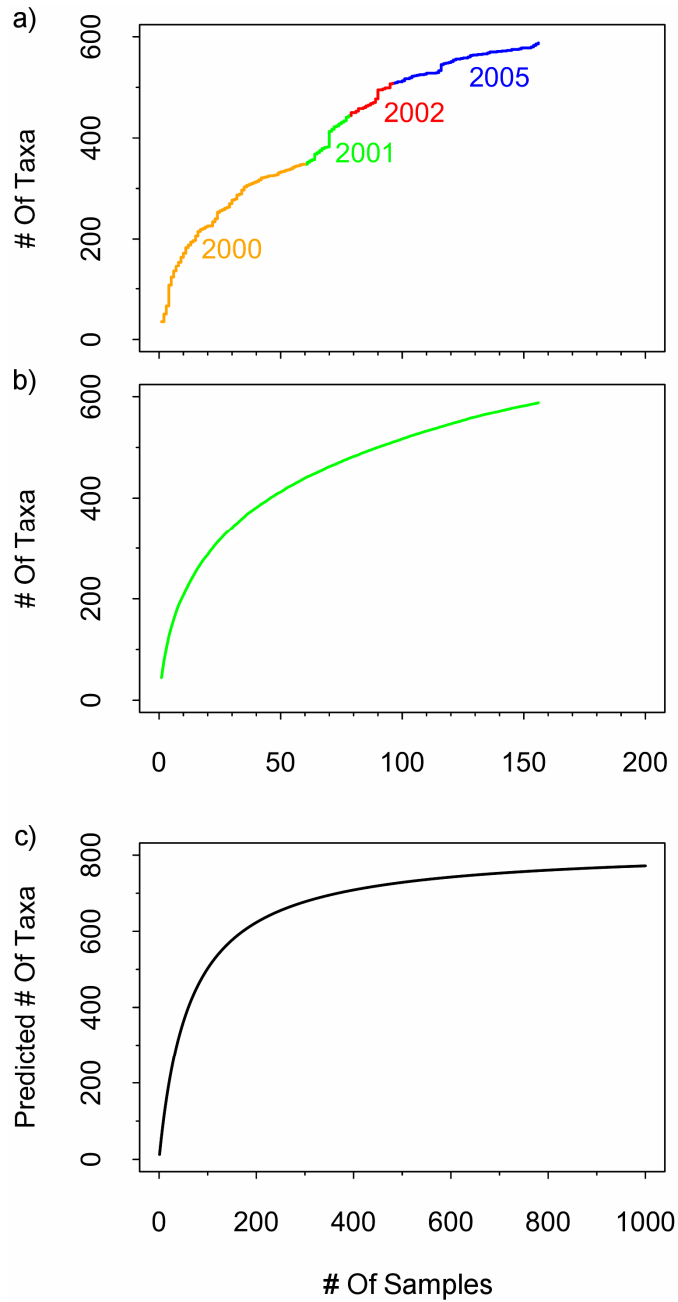


Figure 3.12. Species accumulation curves (SACs) for a) one possible ordering of samples, b) repeated random orderings of samples (green curve represents the mean of n=100 random permutations), and c) predicted number of taxa obtained by fitting an asymptotic model (Eq. 3.1) for SACs (Colwell and Coddington 1994; Clench 1979) to the original data.

## 4.0 Conclusions

Surveys of the soft-bottom benthos of GRNMS in spring of 2000 and 2005 lead to several general observations:

- Sediment concentrations of chemical contaminants measured in spring 2005 appear to be at background levels, with concentrations of many organic contaminants (PCBs, pesticides) below the limit of detection. While some PAHs were detectable in sediment – including biphenyl, dibenz[a,h]anthracene, 2,6-dimethylnaphthalene, fluoranthene, fluorene, indeno[1,2,3-c,d]pyrene, 1- and 2-methylnaphthalene, naphthalene, phenanthrene, and pyrene – the concentrations were well below sediment bioeffect guidelines. Concentrations of most metals (with the exception of copper, mercury, and silver) were above detection limits, though none of the concentrations exceeded upper (ERM) or lower (ERL) sediment quality bioeffect guidelines. The dominant metals were As, Cd, Cr, Pb, and Zn. In general, sediment contaminant concentrations at GRNMS were comparable between the two surveys, with average mERMq values of 0.0047 and 0.0051 in spring 2005 and 2000 respectively, which are well within the range typically associated with healthy benthic assemblages.
- Chemical contaminants in edible tissues of fish (*C. striatus*) and shellfish (*A. zebra*) are present at low levels (below the limit of detection, in many cases), but with none exceeding FDA guideline values. PCBs and pesticides were below the limit of detection in 2005, but metals and PAHs were detected in both species. Arsenic, iron, zinc, and aluminum were the dominant metals in both species. PAHs in tissues included acenaphthene (both species), acenaphthylene (both species), benz[a]anthracene (zebra ark), benzo[b]fluoranthene and benzo[j+k]fluoranthene (zebra ark), benzo[g,h,i]perylene (zebra ark), biphenyl (both species), chysene+triphenylene (zebra ark), dibenz[a,h]anthracene (zebra ark), 2,6-dimethylnaphthalene (both species), fluoranthene (both species), 1- and 2-methylnaphthalene (both species), naphthalene (both species), phenanthrene (both species), pyrene (zebra ark), and 1,6,7-trimethylnaphthalene (zebra ark). While some of these contaminants, particularly the trace metals, may be naturally occurring, it is clear that man-made PAHs are making their way into the sanctuary either by air/water transport from terrestrial sources or exhaust/discharges from vessels.
- The sanctuary supports highly diverse benthic assemblages, with densities and numbers of taxa being consistently high between the spring 2000 baseline survey and the spring 2005 survey. Seasonal peaks in abundance of some taxa notwithstanding (*Ervilia* sp. A), measures of abundance and diversity did not change significantly between the two sampling efforts. A total of 588 taxa (371 identified to species) have been found at GRNMS since the spring 2000 baseline survey, with new taxa being added to the total with each additional sampling effort. In the spring 2005 survey alone, 135 new taxa (84 identified to species) were added that were not found in the spring 2000 baseline study. Based on the shape of the resulting species accumulation curve (SAC), future benthic surveys can expect to find additional new taxa that have yet to be encountered at GRNMS and thus contribute to a theoretical total number predicted to be in excess of 600 species.

- While total faunal abundance (without *Ervilia* sp. A) and numbers of species were similar between years, dominance patterns and relative abundances of component species were less repeatable, reflecting the temporally dynamic nature of benthic fauna in shelf waters of the surrounding South Atlantic Bight ecosystem.
- The distribution of benthic macroinfaunal species in 2000 appeared to be influenced foremost by sediment characteristics (e.g., sediment particle size, % gravel), which may in turn be related to larger mesoscale bottom-habitat features (rippled sands, flat sands, live bottom) identified by Kendall et al (2005). In the spring 2000 survey, Hyland et al (2006) found a close association between the spatial patterns of benthic site groups and the mosaic of these bottom habitat (or benthoscape) types. However, the results of the spring 2005 survey did not appear to suggest as strong a relationship. This could be due to several factors, such as a lack of representative habitat types in the sample population (e.g., there were only two FS sites and no DC live bottom) or a change in the bottom habitat due to currents and shifting sands. Relevant to this latter point, our 2000 benthic infaunal data are tied much more closely to the timing of the sonar data (collected in summer 2001) that Kendall et al. (2005) used to develop the habitat map, than are our 2005 infaunal data. In a follow-up evaluation of the accuracy of the map, independent diver observations in 2004-2005 revealed some discrepancies between the original map and diver classifications that were attributable in part to changes in the bottom type between the two sampling periods due to physical forces such as shifting sands or bioturbation (Kendall et al. 2007). Further ground-truthing of habitat types and additional characterizations of benthic assemblages are necessary to gain a clearer understanding of such relationships.



## **5.0 Acknowledgments**

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Appendix A. Summary of abiotic environmental variables measured in spring 2005 at 20 sites in GRNMS.

Station	Latitude	Longitude	Depth (m)	Salinity (PSU)	Temperature (°C)	DO (mg L <sup>-1</sup> )	% Sand	% Gravel	% Silt-clay	Median Particle Size (Φ)	Sorting Coefficient	% TOC
45	31.41668	-80.91397	17.7	33.4	18.7	7.63	98.5	1.4	0.2	1.331	0.851	0.47
46	31.41274	-80.89441	18.4	33.5	18.7	7.63	98.2	0.6	1.1	1.224	0.867	0.56
47	31.41916	-80.86767	21.0	34.2	18.7	7.61	98.0	1.7	0.3	1.305	0.522	0.93
48	31.41331	-80.84779	21.9	34.2	18.7	7.60	97.2	2.3	0.4	1.135	1.071	0.80
49	31.41257	-80.84549	21.9	34.3	18.7	7.59	97.1	2.6	0.3	0.992	0.924	0.72
50	31.39256	-80.91606	16.0	33.4	18.8	7.62	93.0	1.6	5.5	1.215	0.957	--
51	31.39666	-80.88603	16.9	34.0	18.8	7.60	70.8	19.5	9.7	0.298	1.618	1.90
52	31.39380	-80.87104	18.2	34.0	18.7	7.60	99.2	0.4	0.5	1.899	0.791	1.64
53	31.40526	-80.85324	19.9	34.2	18.7	7.60	90.1	9.3	0.7	0.344	1.134	1.00
54	31.39961	-80.84465	20.5	34.2	18.7	7.60	99.7	0.1	0.2	1.777	0.783	0.78
55	31.37849	-80.91892	17.3	33.4	18.8	7.61	90.0	9.7	0.4	0.831	1.438	0.90
56	31.38971	-80.90113	16.8	33.4	18.8	7.62	97.3	2.5	0.2	0.996	0.935	1.56
57	31.37980	-80.88277	20.7	34.1	19.2	7.54	98.2	1.2	0.7	1.408	1.154	1.56
58	31.38821	-80.85220	19.6	34.1	18.7	7.60	93.2	6.7	0.2	0.684	1.102	0.66
59	31.38029	-80.84397	18.5	34.0	18.8	7.60	97.5	2.2	0.3	0.798	0.824	0.95
60	31.37578	-80.90475	20.3	34.0	19.1	7.55	99.0	0.6	0.4	1.930	0.783	1.11
61	31.37126	-80.88822	18.1	33.9	18.9	7.58	92.2	7.6	0.1	0.345	1.120	1.11
62	31.36569	-80.86872	18.4	33.9	18.8	7.59	98.4	1.5	0.1	0.930	1.108	0.91
63	31.37425	-80.85625	17.7	34.1	18.9	7.58	95.3	4.5	0.2	0.582	0.987	0.87
64	31.37290	-80.84424	18.9	33.3	18.7	7.64	96.7	3.1	0.2	0.767	0.908	0.61

Appendix B. Summary of sediment contaminant concentrations measured at Gray's Reef National Marine Sanctuary in May 2005 in relation to sediment quality guidelines (SQG). Concentrations below method detection limits are reported as < MDL; in these cases, a value of zero was used for data computations (e.g., averaging across stations).

Analyte	Average	Range		SQG <sup>a</sup>		# Sites > SQG	
		Min	Max	ERL	ERM	ERL	ERM
<i>Metals (ug/g dry wt., unless otherwise indicated)</i>							
Aluminum (%)	0.41	0.29	0.61	—	—	—	—
Arsenic	2.46	1.45	4.41	8.2	70	0	0
Cadmium	0.30	0.12	0.61	1.2	9.6	0	0
Chromium	7.34	5.18	13.00	81	370	0	0
Copper	< MDL	< MDL	< MDL	34	270	0	0
Iron (%)	0.12	< MDL	0.25	—	—	—	—
Lead	3.58	2.60	5.38	46.7	218	0	0
Manganese	56.28	26.40	126.00	—	—	—	—
Mercury	< MDL	< MDL	< MDL	0.15	0.71	0	0
Nickel	1.42	< MDL	2.23	—	—	—	—
Selenium	0.23	< MDL	0.48	—	—	—	—
Silver	< MDL	< MDL	< MDL	1	3.7	0	0
Tin	0.07	< MDL	0.79	—	—	—	—
Zinc	2.48	< MDL	16.60	150	410	0	0
<i>PAHs (ng/g dry wt.)</i>							
Acenaphthene	< MDL	< MDL	< MDL	16	500	0	0
Acenaphthylene	< MDL	< MDL	< MDL	44	640	0	0
Anthracene	< MDL	< MDL	< MDL	85.3	1100	0	0
Benz[a]anthracene	< MDL	< MDL	< MDL	261	1600	0	0
Benzo[a]pyrene	< MDL	< MDL	< MDL	430	1600	0	0
Benzo[e]pyrene	< MDL	< MDL	< MDL	—	—	—	—
Benzo[b]fluoranthene	< MDL	< MDL	< MDL	—	—	—	—
Benzo[g,h,i]perylene	< MDL	< MDL	< MDL	—	—	—	—
Benzo[j+k]fluoranthene	< MDL	< MDL	< MDL	—	—	—	—
Biphenyl	0.22	< MDL	0.97	—	—	—	—

## Appendix B (continued).

Analyte	Average	Range		SQGa		# Sites > SQG	
		Min	Max	ERL	ERM	ERL	ERM
Chrysene+Triphenylene	< MDL	< MDL	< MDL	384 <sup>b</sup>	2800 <sup>b</sup>	0	0
Dibenz[a,h]anthracene	0.03	< MDL	0.29	63.4	260	0	0
Dibenzothiophene	< MDL	< MDL	< MDL	—	—	—	—
2,6-Dimethylnaphthalene	0.37	0.07	1.57	—	—	—	—
Fluoranthene	< MDL	< MDL	0.03	600	5100	0	0
Fluorene	< MDL	< MDL	0.07	19	540	0	0
Indeno[1,2,3-c,d]pyrene	0.07	< MDL	0.71	—	—	—	—
1-Methylnaphthalene	0.52	< MDL	1.93	—	—	—	—
2-Methylnaphthalene	0.91	< MDL	3.22	70	670	0	0
1-Methylphenanthrene	< MDL	< MDL	< MDL	—	—	—	—
Naphthalene	0.73	< MDL	2.41	160	2100	0	0
Perylene	< MDL	< MDL	< MDL	—	—	—	—
Phenanthrene	0.18	< MDL	0.33	240	1500	0	0
Pyrene	0.02	< MDL	0.08	665	2600	0	0
1,6,7-Trimethylnaphthalene	< MDL	< MDL	< MDL	—	—	—	—
Total PAHs <sup>c</sup>	1.25	0.15	2.86	—	—	—	—
<i>PCBs (ng/g dry wt.)</i>							
Total PCBs	< MDL	< MDL	< MDL	22.7	180	0	0
<i>Pesticides (ng/g dry wt.)</i>							
Aldrin	< MDL	< MDL	< MDL	—	—	—	—
Alpha-Chlordane	< MDL	< MDL	< MDL	—	—	—	—
Chlorpyrifos	< MDL	< MDL	< MDL	—	—	—	—
Dieldrin	< MDL	< MDL	< MDL	—	—	—	—
Endosulfan I	< MDL	< MDL	< MDL	—	—	—	—
Endosulfan II	< MDL	< MDL	< MDL	—	—	—	—
Endosulfan sulfate	< MDL	< MDL	< MDL	—	—	—	—
Lindane (gamma-HCH)	< MDL	< MDL	< MDL	—	—	—	—
Heptachlor	< MDL	< MDL	< MDL	—	—	—	—
Heptachlor epoxide	< MDL	< MDL	< MDL	—	—	—	—



Appendix B (continued).

Analyte	Average	Range		SQG <sup>a</sup>		# Sites > SQG	
		Min	Max	ERL	ERM	ERL	ERM
2,4'-DDD (o,p'-DDD)	< MDL	< MDL	< MDL	—	—	—	—
4,4'-DDD (p,p'-DDD)	< MDL	< MDL	< MDL	—	—	—	—
2,4'-DDE (o,p'-DDE)	< MDL	< MDL	< MDL	—	—	—	—
4,4'-DDE (p,p'-DDE)	< MDL	< MDL	< MDL	2.2	27	0	0
2,4'-DDT (o,p'-DDT)	< MDL	< MDL	< MDL	—	—	—	—
4,4'-DDT (p,p'-DDT)	< MDL	< MDL	< MDL	—	—	—	—
Total DDT <sup>d</sup>	< MDL	< MDL	< MDL	1.58	46.1	0	0

<sup>a</sup> SQGs are the ERL and ERM values from Long et al. (1995).

<sup>b</sup> ERL/ERM values are for chrysense only.

<sup>c</sup> Without perylene.

<sup>d</sup> Total DDTs = 2,4'-DDD + 4,4'-DDD + 2,4'-DDE + 4,4'-DDE + 2,4'-DDT + 4,4'-DDT.

Appendix C. Summary of contaminant concentration ranges observed in edible tissues of black sea bass (*Centropristis striata*) and zebra ark (*Arca zebra*) at Gray's Reef National Marine Sanctuary sites in May 2005. Concentrations are reported on a dry-weight basis. FDA guideline values are included where available, and have been converted to dry weight basis by multiplying published wet-weight values by a factor of five (assumes body weight is 80% water). Concentrations below method detection limits are reported as < MDL; in these cases, a value of zero was used for data computations (e.g., averaging across all stations).

Analyte	Black Sea Bass			Zebra Ark			FDA Guideline	# Sites > Guideline
	Average	Range		Average	Range			
		Min.	Max.		Min.	Max.		
<i>Metals (ug/g dry wt.)</i>								
Aluminum	4.24	1.30	18.90	54.51	30.30	120.00	—	—
Arsenic	59.76	24.60	87.20	46.84	33.30	58.70	215.00 <sup>a</sup>	0
Cadmium	< MDL	< MDL	< MDL	1.46	0.76	4.54	15.00 <sup>a</sup>	0
Chromium	1.63	1.31	2.13	3.43	3.03	3.88	55.00 <sup>a</sup>	0
Copper	1.02	< MDL	8.61	4.90	4.05	5.58	—	—
Iron	39.17	< MDL	60.10	215.63	184.00	272.00	—	—
Lead	< MDL	< MDL	0.01	0.14	0.11	0.20	3.00 <sup>a</sup>	0
Manganese	0.04	< MDL	0.63	20.34	12.70	25.70	—	—
Mercury	0.34	0.20	0.47	0.07	0.04	0.09	5.00 <sup>b</sup>	0
Nickel	0.06	0.03	0.11	0.87	0.52	1.27	350.00 <sup>a</sup>	0
Selenium	3.25	2.57	4.67	5.98	4.46	7.75	—	—
Silver	< MDL	< MDL	< MDL	4.51	1.05	9.84	—	—
Tin	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Zinc	20.36	16.70	24.30	118.28	60.20	158.00	—	—
<i>PAHs (ng/g dry wt.)</i>								
Acenaphthene	1.08	< MDL	16.27	6.97	< MDL	55.73	—	—
Acenaphthylene	0.54	< MDL	8.16	1.77	< MDL	14.16	—	—
Anthracene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Benz[a]anthracene	< MDL	< MDL	< MDL	3.31	< MDL	26.45	—	—
Benzo[a]pyrene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Benzo[e]pyrene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Benzo[b]fluoranthene	< MDL	< MDL	< MDL	4.74	< MDL	37.94	—	—
Benzo[j+k]fluoranthene	< MDL	< MDL	< MDL	1.45	< MDL	11.64	—	—
Benzo[g,h,i]perylene	< MDL	< MDL	< MDL	5.18	< MDL	41.43	—	—
Biphenyl	3.25	0.36	7.06	4.52	1.95	7.22	—	—

Appendix C (continued).

Analyte	Black Sea Bass			Zebra Ark			FDA Guideline	# Sites > Guideline
	Average	Range		Average	Range			
		Min.	Max.		Min.	Max.		
Chrysene+Triphenylene	< MDL	< MDL	< MDL	6.08	< MDL	16.97	—	—
Dibenz[a,h]anthracene	< MDL	< MDL	< MDL	2.20	< MDL	17.60	—	—
Dibenzothiophene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
2,6-Dimethylnaphthalene	0.49	< MDL	3.18	2.11	< MDL	6.94	—	—
Fluoranthene	0.02	< MDL	0.26	1.44	< MDL	1.98	—	—
Fluorene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Indeno[1,2,3-c,d]pyrene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
1-Methylnaphthalene	3.66	< MDL	11.65	8.10	1.06	18.94	—	—
2-Methylnaphthalene	7.73	< MDL	24.31	15.04	2.91	31.70	—	—
1-Methylphenanthrene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Naphthalene	8.93	< MDL	25.69	17.38	2.11	37.63	—	—
Perylene	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Phenanthrene	1.75	0.60	3.58	4.09	1.92	5.55	—	—
Pyrene	<MDL	<MDL	<MDL	0.60	< MDL	1.00	—	—
1,6,7-Trimethylnaphthalene	<MDL	<MDL	<MDL	0.21	< MDL	1.67	—	—
Total PAHs (without Perylene)	15.58	1.24	34.69	52.18	7.49	213.09	—	—
<i>PCBs (ng/g dry wt.)</i>								
Total PCBs	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	10000.00 <sup>c</sup>	0
<i>Pesticides (ng/g dry wt.)</i>								
Aldrin	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	1500.00 <sup>b</sup>	0
Chlorpyrifos	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
alpha-Chlordane	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
DDD <sup>d</sup>	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	25000.00 <sup>b</sup>	0
DDE <sup>d</sup>	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	25000.00 <sup>b</sup>	0
DDT <sup>d</sup>	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	25000.00 <sup>b</sup>	0
Total DDTs <sup>e</sup>	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	25000.00 <sup>b</sup>	0
Dieldrin	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	1500.00 <sup>b</sup>	0
Endosulfan I	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Endosulfan II (Beta-Endosulfan)	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Endosulfan sulfate	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Heptachlor	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	1500.00 <sup>b</sup>	0

Appendix C (continued).

Analyte	Black Sea Bass			Zebra Ark			FDA Guideline	# Sites > Guideline
	Average	Range		Average	Range			
		Min.	Max.		Min.	Max.		
Heptachlor epoxide	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	1500.00 <sup>b</sup>	0
Hexachlorobenzene (HCB)	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Lindane	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
Mirex	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	500.00 <sup>b</sup>	0
trans-Nonachlor	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	—	—
<i>Lipids (% dry wt.)</i>	4.98	2.5	8.35	8.21	7.29	10.6	—	—

<sup>a</sup> FDA Level of Concern for contaminants in shellfish. Value is lowest of multiple values reported by FDA for humans of various ages consuming either crustaceans or molluscs at the 90<sup>th</sup> percentile consumption rate. Values (converted from wet weight to dry weight) are from: FDA 1993a for As, FDA 1993b for Cd, FDA 1993c for Cr, FDA 1993d for Pb, and FDA 1993e for Ni.

<sup>b</sup> FDA Action Level for poisonous or deleterious substances in human food and animal feed (level for edible portion of fish is given). FDA 1994.

<sup>c</sup> FDA Tolerance for unavoidable residues of PCBs in fish and shellfish. FDA 1984.

<sup>d</sup> DDD = 2,4'-DDD + 4,4'-DDD; DDE = 2,4'-DDE + 4,4'-DDE; DDT = 2,4'-DDT + 4,4'-DDT.

<sup>e</sup> Total DDTs = 2,4'-DDD + 4,4'-DDD + 2,4'-DDE + 4,4'-DDE + 2,4'-DDT + 4,4'-DDT.



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