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Assessment of Ecological Condition and Potential Stressor Impacts in Offshore Areas of Florida Keys National Marine Sanctuary

NOAA National Centers for Coastal Ocean Science
Stressor Detection and Impacts Division
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Executive Summary

In June 2015, the NOAA National Centers for Coastal Ocean Science conducted an assessment of the status of ecological condition of unconsolidated, soft-bottom habitat and overlying waters at 30 sites from 11 – 99 m depth in targeted shelf areas of Florida Keys National Marine Sanctuary (FKNMS). All sampling was undertaken outside of Sanctuary Preservation Areas (SPAs), Ecological Reserves, and Research-Only Areas and all coral reef and hardbottom, continuous seagrass beds and shallow areas (< 10 m) were avoided in order to minimize potential impacts of sediment grab sampling on protected areas and sensitive habitat, to accommodate sampling gear limitations, and to satisfy working requirements of the research vessel (limited to depths > 10 m). Sampling included measures of water quality, sediment quality, and benthic biological condition (taxonomic richness, diversity, and abundance of infauna; fish tissue contaminant levels). A probabilistic sampling design was used to support unbiased statistical estimates of the magnitude and areal extent of condition with respect to the various measured indicators and corresponding thresholds of interest.

Sediments from half the sites (15 stations, 50 % area) were composed of sand (< 20 % silt+clay), while sediments at the remaining sites were characterized as muddy sand (20 – 80 % silt+clay). TOC in sediments was typically low (< 2 %) with a mean of 0.7 %; levels of TOC at all sites were below the range associated with potentially harmful effects to benthic fauna (i.e., > 5 %).

Measured salinities (surface and bottom) occupied a narrow range between 36 and 37 psu, with a mean salinity of 36.4 psu. Strong vertical stratification ($\Delta\sigma_t > 2$) was observed at five sites; these stations were also some of the deepest sites along the shelf break on the southeastern Sanctuary boundary (Atlantic side). Near-surface water temperatures averaged 28.5 °C (range of 27.8 – 29.3 °C); bottom-waters tended to be colder on average (15.2 – 29.3 °C, mean of 26.2 °C), particularly at deeper sites. The lowest temperature was observed at the deepest (98.8 m) and most vertically stratified ($\Delta\sigma_t = 3.3$) site (station 12). Bottom DO was also lowest at station 12 (4.3 mg/L), while DO concentrations at the remaining 29 sites (96.7 % area) were all above 5 mg/L ($\geq$ 5.9 mg/L).

Total suspended solids (TSS) ranged from 2.4 – 15.0 mg/L (mean of 3.6 mg/L) in near-surface waters, with most stations (29 of 30 sites, 96.7 % area) having TSS $\leq$ 5.1 mg/L. Similarly, surface turbidity was low, ranging from 0.2 – 1.0 NTU (mean of 0.4 NTU).

The concentration of dissolved inorganic nitrogen (DIN: nitrogen as nitrate + nitrite + ammonium) in near-surface waters ranged from 0.006 – 0.038 mg/L and averaged 0.012 mg/L. Twenty-two stations (representing 73 % of the study area) had surface-water DIN concentrations equal to or below the EPA water quality target of 0.01 mg/L. Total phosphorus levels ranged from 4.7 – 17.6 µg/L (mean of 8.5 µg/L), with 53 % of the study area having concentrations less than or equal to the EPA water quality target of 7.7 µg/L. The ratio of DIN:DIP in surface waters averaged 18.5 (range of 10.9 – 71.1), with the majority of sites sampled (19 stations, 63 % area) having levels indicative of nitrogen limitation (DIN:DIP < 16).

Levels of chlorophyll *a* (CHL *a*) in near-surface waters varied between 0.03 µg/L and 0.68 µg/L (mean of 0.16 µg/L), with 93 % of the study area (28 of 30 sites) having CHL *a* concentrations below the EPA water quality target (for reef sites) of 0.35 µg/L.

Concentrations of chemical contaminants in sediments were generally at low background levels, below expected bioeffect ranges, although a number of metals, PAHs, PCBs, and pesticides were measured at concentrations above the minimum method detection limit (MDL). Although no contaminants were found in excess of corresponding Effects Range-Median (ERM) values, levels of Total PCBs were measured in excess of the Effects Range-Low (ERL) at one site, located approximately 3 km offshore of Conch Reef.

Calculated mean ERM quotients (mERM-Q) were well below levels associated with expected toxicity to benthic organisms. All mERM-Qs were within the lower range corresponding to a low likelihood of toxicity based on acute amphipod toxicity tests (mERM-Q < 1) and observations of benthic community-level responses in field samples (mERM-Q $\leq$ 0.020).

Results of three sediment (or sediment porewater) toxicity tests varied by assay. Although some of these tests indicated toxicity based on the criteria used for each, there was no significant correlation among results of the three tests, nor with any other measured parameters likely to cause toxicity. Hence, the observed toxicity was likely caused by other, unmeasured stressors or confounding factors.

Concentrations of a suite of metals and organic compounds (PAHs, PBDEs, PCBs, and pesticides) were measured in edible tissues (homogenized, skin-on fillets) of 52 fish specimens (representing six species) collected at 22 stations. Tissue contaminant levels were compared to risk-based EPA advisory guidelines for recreational fishers, which set recommended consumption limits based on concentration ranges of a number of contaminants with respect to risk of cancer and non-cancer (chronic systemic) human-health effects. Concentrations of inorganic arsenic (estimated as 2 % of total arsenic) in edible tissues (skin-on fillets) of grey triggerfish at one site fell within the range of values for which the USEPA suggests limiting consumption to four fish meals per week. Levels of mercury (assumed to be all methylmercury) in tissues of lane snapper, sand perch, and blackline tilefish fell within guidance limits for methylmercury at eight stations and exceeded the upper limit at an additional eight stations.

A total of 763 benthic infaunal taxa were identified, of which 570 were identified to the species level. Annelida (mostly polychaetes) was the dominant phylum, both by percent of total number of taxa (47.8 %) and percent of total density (60.7 %). Arthropods made up 30.9 % of taxa and 18.1 % total density; of these, crustaceans made up 97 % and 96 % of arthropod taxa and density, respectively. Molluscs represented 18.1 % of taxa and 10.5 % abundance. Echinoderms made up less than 2 % of total taxa (and density), consisting mainly of brittle stars (Ophiuroidea) and urchins (Echinoidea), as well as some starfish (Echinoidea) and sea cucumbers (Holothuroidea). 'Other' taxa included members of phyla Nemertea, Sipuncula, Cnidaria, and a few Brachiopoda, Phoronida, Hemichordata, and some Chordata (e.g., lancelets, *Branchiostoma* sp.). Most of these 'Other' taxa were identified only to higher taxonomic level and so represented a small percentage of total taxa, although they constituted nearly 10 % of total density. Overall, mean number of taxa ranged from 23 - 113 across the 30 stations; mean density ranged

from 1,062 - 13,075. The 10 dominant taxa, in decreasing order of abundance, included the sabellid (Family Sabellidae) polychaete *Fabricinuda trilobata*; the syllid (Family Syllidae) polychaete *Haplosyllis spongicola*; members of Phylum Nemertea ('ribbon worms'); members of the spionid polychaete genus Prionospio; Phylum Sipuncula ('peanut worms'); members of Subclass Oligochaeta; the pilargid (Family Pilargidae) polychaete *Litocorsa ewingi*; the spionid polychaete *Prionospio cristata*; members of the peanut worm Family Aspidosiphonidae (LPIL); and the sabellid polychaete *Galathowenia oculata*.

Benthic infaunal richness and abundance were higher in FKNMS compared to related studies conducted in other U.S. Atlantic and GOM shelf regions, while diversity was similar to the northeast GOM and Southwest Florida Shelf. We found no association of low values of benthic biological attributes (i.e., richness, diversity, abundance) with indicators of poor water or sediment quality. At all stations, the sediments and overlying waters in the surveyed area of FKNMS were in good condition with respect to DO, targeted contaminants, and TOC, with lower-end values of benthic biological attributes representing parts of a normal reference range controlled primarily by natural factors. However, low yet detectable levels of chemical contaminants (below bioeffect thresholds) at multiple sites and a higher concentration of total PCBs at one site (station 29), below the ERM but in excess of the ERL, indicate an increased potential for bioeffects from these or other stressors on Sanctuary resources. Four of the 30 stations in the present study also tested positive for sediment toxicity, based on both the sea-urchin and Microtox bioassays, although exact causes of the observed toxicity are not known. Such conditions could justify follow-up surveys to assess the extent and source of contamination in specific areas. Specifically, a survey to determine the local spatial extent, and possibly the source, of sediment PCB contamination off Conch Key is recommended. Also, since this study was focused mainly on deeper, offshore portions of FKNMS, a similar companion study of inshore areas would be valuable, as it would provide complementary information that could contribute to a more complete characterization of the sanctuary.

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1 Introduction

The Florida Keys are a chain of limestone islands extending approximately 220 mi (354 km) from the southern tip of the Florida mainland southwest to the Dry Tortugas (NOAA 2007, 2011). In 1990, the Florida Keys National Marine Sanctuary and Protection Act (H.R. 5909, Public Law 101-605) designated a boundary encompassing 2,800 nmi^2 (9,603 km^2) of islands, coastal waters, and coral reef tract as the Florida Keys National Marine Sanctuary (FKNMS) (Boyer and Briceño 2010). The boundaries were expanded in 2001 to include the Tortugas Ecological Reserve, bringing the total area of the sanctuary to 2,896 nmi^2 (9,933 km^2) (NOAA 2011). The sanctuary includes much of the Upper, Middle, and Lower Keys, as well as the western keys of the Marquesas and Dry Tortugas (Figure 1).

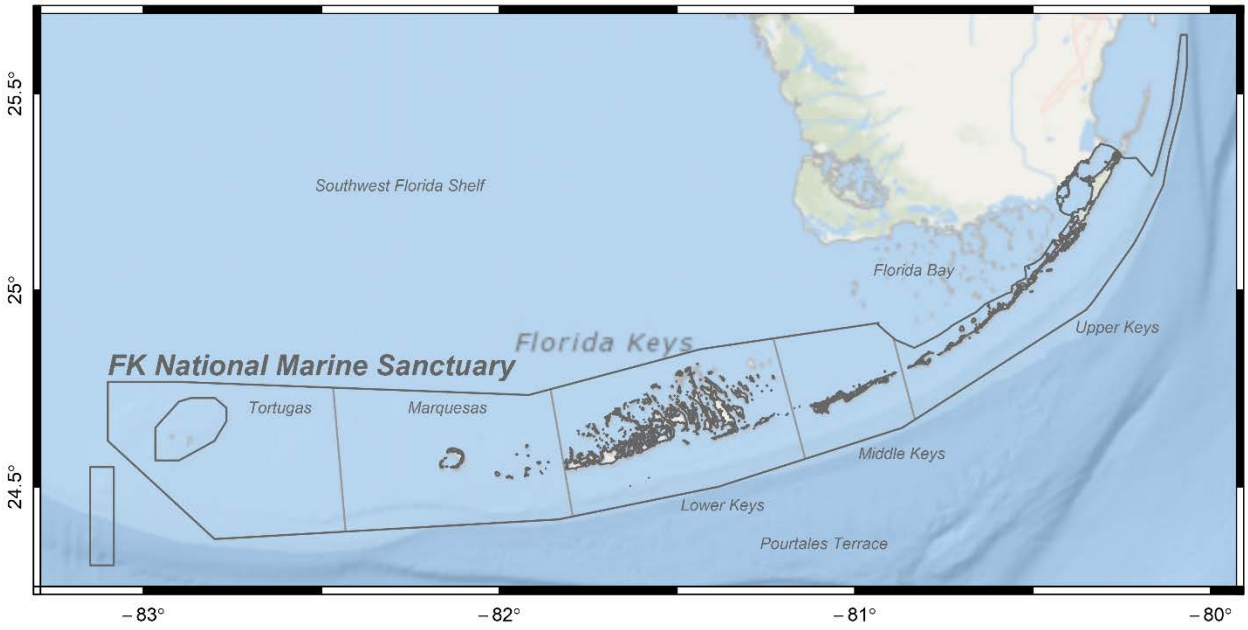

Figure 1. The Florida Keys National Marine Sanctuary (FKNMS), divided for ease of reference into the general areas of Upper Keys, Middle Keys, Lower Keys, Marquesas, and Tortugas.

Located at the convergence of subtropical and temperate climate zones, south Florida and the Florida Keys are influenced by large-scale ocean circulation patterns as well as localized tides and wind-driven currents. Off peninsular Florida, the Gulf of Mexico Loop Current and the Yucatan Current converge to form the Florida Current, which flows northeastward through the Florida Straits and is renamed the Gulf Stream off the southeastern U.S. (Lee et al. 1992). The Florida Current transports warm water from the Caribbean and is the major reason for reef development and the occurrence of tropical marine biota in the Florida Keys (Jaap 1984, NOAA 2011). The coral reefs and associated soft-sediment communities of the Florida Keys represent one of the most unique and diverse assemblages of plants and animals in North America (NOAA 2011).

The Florida Keys have a long history of exploitation, and the sanctuary continues to face many pressures including coastal development, pollution, commercial and recreational fishing, harmful algal blooms,

and vessel groundings, among others. Climate change, sea level rise, and ocean acidification may also impact sanctuary resources (NOAA 2011). Multiple species have been affected by disease outbreaks, including massive die-offs of commercial sponges in 1938-1940 and the virtual eradication of the long-spined sea urchin (Diadema antillarum) due to mortality events in 1983 and 1991 (NOAA 2011). Coral habitats have been in decline throughout the sanctuary since the 1970s, primarily due to disease and bleaching events. A particularly devastating disease outbreak occurred in 2014 and is still ongoing. An extremely high-prevalence (61%), localized outbreak of white-plague disease at 14 sites off southeastern Florida caused regional losses of 97 % of colonies for some species (Precht et al. 2016). The disease outbreak has since spread further south into the Florida Keys and is now referred to operationally as stony coral tissue loss disease.

Point and non-point sources of pollution represent another set of stressors impacting sanctuary resources. Excess nutrients from fertilizers may contribute to near-shore eutrophication in sanctuary waters. Chemical contaminants (e.g., trace metals, pesticides, PAHs, PCBs, PBDEs) can have a range of impacts, including both lethal and sub-lethal toxicity effects that can disrupt reproductive success in reef coral (Victor and Richmond 2005) or cause photoinhibition in coral symbionts (Jones 1997, Cantin et al. 2007), leading to declines in coral cover and species diversity (Downs et al. 2005, 2012).

The most recent condition report for the FKNMS identifies a lack of information, and the need to understand, the geographic extent and spatial variation in concentrations of various contaminants, the temporal variability of these concentrations, and contaminant pervasiveness and toxicity to organisms (NOAA 2011). In an effort to address some of these needs, and to provide information on abundance and diversity of soft-bottom benthic infaunal assemblages, NOAA's National Centers for Coastal Ocean Science (NCCOS) partnered with the FKNMS to design a study to assess the status of ecological condition and potential stressor impacts in unconsolidated sediments and overlying waters of the FKNMS. Using multiple indicators of general habitat characteristics, potential stressors, and biological condition, the study provides a baseline for monitoring and detecting change over time in support of NCCOS priorities including *Stressor Impacts and Mitigation* and *Coastal Change*. While unconsolidated sediments (sand and mud) are one of the dominant habitats of the FKNMS, with associated fauna playing vital roles in detrital decomposition, nutrient cycling, and energy flow to higher trophic levels, an overall assessment of environmental condition within this important habitat has never been completed. The results of this study will help to fill this gap for future sanctuary condition reports. A secondary objective is to contribute additional information on patterns of marine biodiversity in support of the Marine Biodiversity Observing Network (MBON), which is focusing on Sanctuaries including FKNMS as one of its initial demonstration sites. The project provides relevant data for FKNMS, including information to define spatial patterns of diversity in relation to various environmental controlling factors, map biodiversity hotspots, and identify areas of potential human impacts.

2 Methods

2.1 Sampling Design & Field Collections

Sampling was conducted from June 7 – June 11, 2015 at 30 stations targeting soft-bottom benthic habitats in Florida Keys National Marine Sanctuary (Figure 2). The sampling domain was developed, in

close consultation with the Sanctuary, specifically to avoid Sanctuary Preservation Areas (SPAs), Ecological Reserves, and Research-Only Areas; coral reef and hardbottom; continuous seagrass beds; and shallow areas (< 10 m). These areas were avoided in order to minimize potential impacts of sediment grab sampling on protected areas and sensitive habitat, to accommodate sampling gear limitations as dense seagrass assemblages can interfere with or prevent the collection of sediment grab samples, and to satisfy working requirements of the research vessel (limited to depths ≥ 10 m). Shapefiles delineating protected areas were provided by FKNMS staff. The Unified Florida Reef Map (FWC-FWRI 2016) was used to identify areas of coral reef, hardbottom, and seagrass beds. Bathymetric contours for the southeast U.S. (FWC-FWRI 2011) were used to eliminate areas having depth < 10 m. Sampling was carried out onboard the NOAA Ship Nancy Foster, commencing mid-day on June 7, 2015 and running as continuous 24-hour operations until mid-day on June 11, 2015. Station coordinates are provided in Appendix A.

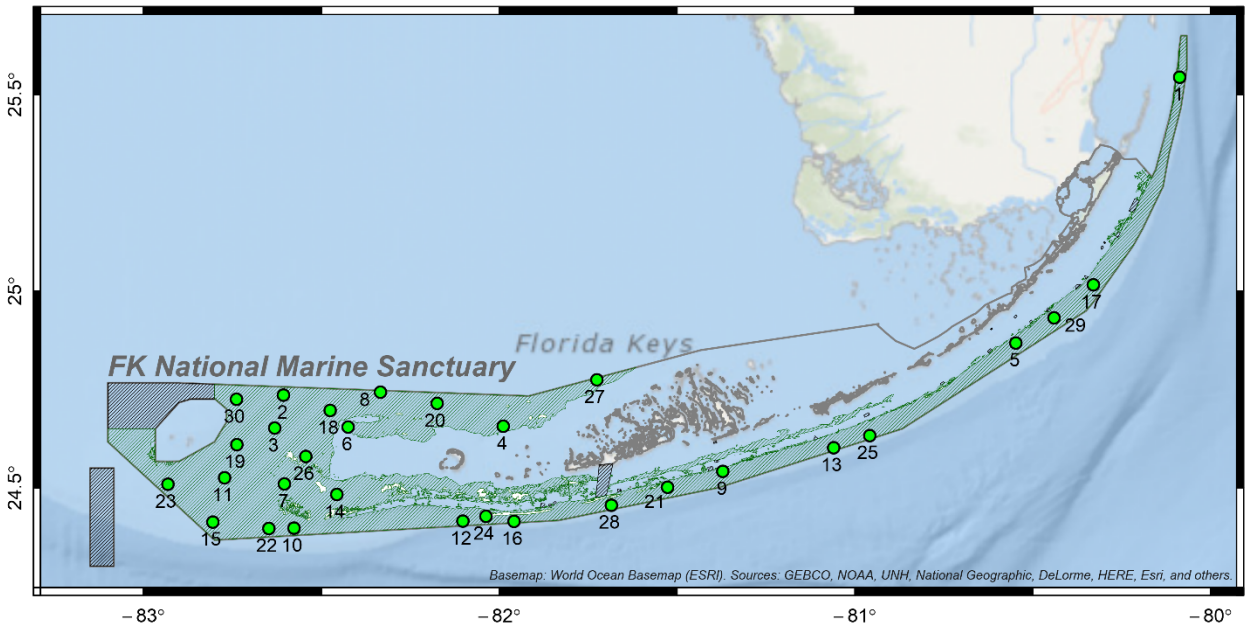

Figure 2. Locations of the 30 random sites sampled in Florida Keys National Marine Sanctuary. Green shading indicates the sampling domain.

Thirty sampling locations were selected using an unstratified, equal probability Generalized Random-Tessellation Stratified (GRTS) survey design with 100% oversampling (30 additional sites to be used as alternates, if needed). The data obtained within this probabilistic framework can be used to make unbiased statistical estimates (with confidence intervals) of the spatial extent and magnitude of condition relative to various measured indicators and corresponding management thresholds. This method has been used widely in EPA's EMAP and National Coastal Assessment programs and is used to achieve both a random and spatially balanced coverage (Stevens & Olsen 2004). The sampling design and other methods described below are also consistent with those used in similar regional and placed-

based assessments of ecological condition conducted by NCCOS in other near-coastal and continental shelf areas (Balthis et al. 2011, 2013; Cooksey et al. 2010, 2014).

Multiple ecological indicators were sampled synoptically at each station, including:

- General habitat characteristics: Water-column depth, temperature, salinity, dissolved oxygen (DO), pH, turbidity, total suspended solids (TSS), nutrients (ammonium, nitrate/nitrite, total nitrogen, orthophosphate, total phosphorus), chlorophyll *a*, and phaeophytin; sediment grain-size (% sand, % silt+clay) and total organic carbon (TOC).
- Stressor levels: Chemical contaminants in sediments (metals, pesticides, PCBs, PAHs, PBDEs), hypoxia/anoxia, and organic over-enrichment (elevated TOC).
- Sediment toxicity: Microtox solid-phase assay, reporter gene assay, and sea urchin embryo developmental assay.
- Health of resident benthic infaunal communities (animals sampled with 0.04-m^2 grab, sieved on 0.5-mm screen, and identified to lowest practical taxonomic level (species, where possible)).
- Human-health risks: Chemical contaminants in finfish (lane snapper, sand perch, gray triggerfish, yellowtail snapper, blackline tilefish, sand tilefish).
- Aesthetics: Water clarity, presence of noxious sediment odor, oily sediment, marine debris.

Vertical water-column profiles of conductivity/salinity, temperature, depth, dissolved oxygen, and pH were acquired at each station using a Sea-Bird Electronics (SBE) Conductivity-Temperature-Depth (CTD) profiler, equipped with supplemental dissolved oxygen and pH sensors. The CTD was an SBE 9Plus with an 11Plus deck unit that provided real-time data recording of the vertical profile. Only surface and bottom values for these various indicators are presented in this report. Data for all depths are included in the study database and are available on request to the authors. An index of density stratification ($\Delta\sigma_t$) was calculated as the difference between the computed bottom and surface density (σ_t) values, where σ_t is the density of a parcel of water with a given salinity and temperature relative to atmospheric pressure (Fofonoff and Millard 1983). The CTD was incorporated into a frame that included a rosette of 12 Niskin bottles used to collect near-surface (~ 1 m below surface) water samples. Water samples were analyzed for nutrients, chlorophyll *a*, total suspended solids (TSS), and turbidity. The CTD was lowered into the water until completely submerged and held just beneath the surface for three minutes while the water pump was allowed to purge any air from the system. The unit was then lowered to within one meter of the bottom at a rate of approximately 1 m/s, then returned to the surface. Four Niskin bottles were activated to collect water samples at approximately 1 m below the surface.

Sediment samples were collected using a 0.04-m^2 Young-modified Van Veen grab sampler. Two replicate grab samples were retained for analysis of benthic infaunal composition, sieved onboard through a 0.5-mm screen, and preserved in 10% buffered formalin with rose bengal stain. The upper 2 - 3 cm of sediments from additional grabs (typically 2 or 3) were combined to yield a sediment composite, which was then homogenized and sub-sampled for analysis of metals, organic contaminants (pesticides, PCBs, PAHs, PBDEs), toxicity (Microtox, reporter gene system, sea urchin development assay), grain size (% sand, % silt+clay), and total organic carbon (TOC). Porewater for the sea urchin assay was extracted from composited sediment using airstones connected with plastic airline tubing to a cleaned 30 mL syringe.

Airstones were inserted into the sediment and the syringe plunger was withdrawn to create a vacuum within the barrel. Accumulated porewater in the syringe barrel was decanted from the syringe into a clean Teflon vial (60 mL) and stored at -40 °C until return to port. Sediment samples (other than infauna) were kept refrigerated (Microtox, reporter gene) or frozen (contaminants) onboard the ship during sampling and maintained on ice during shipment to the respective analytical laboratories.

Hook-and-line fishing was attempted at all 30 stations. Targeted species included members of the families Paralichthyidae (sand flounders), Serranidae (sea basses and groupers), Lutjanidae (snappers), Balistidae (gray triggerfish), and Malacanthidae (tilefishes). Specimens from six species representing these five groups were collected from 22 of the 30 stations. Edible tissues (skin-on fillets) of 52 specimens were analyzed for metals, pesticides, PAHs, PCBs, and PBDEs.

2.2 Nutrient, Chlorophyll, Phaeophytin, TSS, and Turbidity Analysis

Initial sample preparations were performed by the field crew on the day of collection. Approximately 0.5 L of water from each station was vacuum-filtered using Filterware microfiltration glassware and a Whatman GF/F 47-mm filter. The filtered water sample was then transferred to a 120-mL polypropylene bottle, frozen (< -20°C), and analyzed within 30 days for dissolved nutrients including ammonium (NH_4^+), nitrate/nitrite ($NO_{2/3}$), and orthophosphate (PO_4^{3-}). Each filter was folded and wrapped in a foil pouch, frozen, and analyzed for chlorophyll *a* (CHL *a*) and phaeophytin (PHAEO). Whole (unfiltered) water samples were also obtained from each station, portions of which were placed in 60-mL polypropylene bottles and kept frozen until later analyzed for total nitrogen (TN) and total phosphorus (TP). A 25 mL aliquot of the unfiltered water was also removed and measured on site for turbidity using a Hach 2100P turbidity meter; resulting measurements were expressed in standard Nephelometric Turbidity Units (NTU). The remaining unfiltered water from each station was used to measure TSS.

Subsequent instrumental analyses were performed using established analytical methods. Dissolved nutrients were measured as follows: NH_4^+ (Method 804-86T, Technicon 1986), NO2/3 (Method 158-71, Technicon 1977), and PO_4^{3-} (Method 155-71W, Technicon 1973). Concentrations of TN and TP were determined by a persulfate digestion method (Valderrama 1981). The Welschmeyer method (Welschmeyer 1994) was used to determine both CHL *a* and PHAEO. Concentrations of TSS were measured on a HACH DR/2500 TSS analyzer using a photometric method (Method 8006, Hach 2003).

2.3 Sediment TOC and Grain Size Analysis

Sediment characterization included analyses of total organic carbon (TOC) and grain size distribution. Samples for grain size analysis were prepared by sieve separation followed by timed pipette extractions as described in Plumb (1981). TOC analysis followed USEPA Method 9060. A minimum of 5g (wet weight) of sediment was initially dried for 48 h. Weighed subsamples were ground to fine consistency and acidified to remove sources of inorganic carbon (e.g., shell fragments). The acidified samples were ignited at 950 °C and the carbon dioxide evolved was measured with an infrared gas analyzer.

2.4 Chemical Contaminant Analysis

2.4.1 Laboratory Sample Preparation

Sediment samples were kept frozen at approximately - 40 °C until analyzed. To thaw, samples were left in closed containers in a 4 °C cooler for approximately 24 hours. Samples were thoroughly homogenized by hand prior to any sample extraction. Fish and oyster tissue samples were frozen upon receipt in the laboratory and stored at - 40 °C. Prior to analysis, samples were removed from the freezer and stored overnight at 4 °C to partially thaw. Tissue samples (fish fillets: skin on, oysters: whole body) were homogenized using a ProScientific homogenizer in 500 mL Teflon containers. The homogenized tissue sample was divided into organic and inorganic subsamples, placed in pre-cleaned glass and polypropylene containers, respectively, and stored at - 40 °C until extraction or digestion. A percent dry-weight determination was made gravimetrically on an aliquot of the wet sediment and tissues. A list of analytes is provided in Table 1.

2.4.2 Inorganic Sample Preparation and Analysis

Dried sediment was ground with a mortar and pestle and transferred to a 20-mL plastic screw-top container. A 0.25-g subsample of the ground material was transferred to a Teflon-lined digestion vessel and digested in 5 mL of concentrated nitric acid using microwave digestion. The sample was brought to a fixed volume of 50 mL in a volumetric flask with deionized water and stored in a 50-mL polypropylene centrifuge tube for subsequent analysis of Li, Be, Al, Fe, Mg, Ni, Cu, Zn, Cd, and Ag. A second 0.25-g subsample of dried sediment was transferred to a Teflon-lined vessel and digested with 5 mL of concentrated nitric acid and 1 mL of concentrated hydrofluoric acid in a microwave digestion unit. The sample was then evaporated on a hotplate at 225 °C to near dryness and 1 mL of nitric acid was added. The sample was brought to a fixed volume of 50 mL in a volumetric flask with deionized water and stored in a 50-mL polypropylene centrifuge tube for subsequent analysis of V, Cr, Co, As, Sn, Sb, Ba, Tl, Pb, and U. Samples for selenium analysis were prepared by hotplate digestion using a 0.25-g subsample of dried sediment and 5 mL of concentrated nitric acid. Each sample was brought to a fixed volume of 50 mL in a volumetric flask with deionized water and stored in a 50-mL polypropylene centrifuge tube for subsequent analysis.

For tissue analyses, 2 – 3 grams of wet tissue were microwave-digested in Teflon-lined digestion vessels using 10 mL of concentrated nitric acid along with 2 mL of hydrogen peroxide. Digested samples were brought to a fixed volume with deionized water in graduated polypropylene centrifuge tubes and stored until analysis.

The analysis of mercury, for both sediments and tissue samples, was performed on separate aliquots of wet sediment or tissue material using a Milestone DMA-80 Direct Mercury Analyzer. All remaining elemental analyses were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Data-quality procedures included the use of blanks, spiked solutions, and standard reference materials (NRC MESS-3: marine sediments and NIST 1566b: freeze-dried mussel tissue).

2.4.3 Organic Sample Preparation and Analysis

An aliquot (10 g sediment or 5 g tissue wet weight) was extracted with anhydrous sodium sulfate using accelerated solvent extraction in either a 1:1 mixture of methylene chloride and acetone (for sediments) or 100% dichloromethane (for tissues) (Schantz 1997). Following extraction, samples were dried and cleaned using gel-permeation chromatography and solid-phase extraction to remove lipids and then solvent-exchanged into hexane for analysis. Samples were analyzed for PAHs, PBDEs, PCBs (individual congeners), and a suite of chlorinated pesticides using gas chromatography/mass spectrometry (GC/MS) technology. Data-quality procedures included the use of spiked blanks, reagent blanks, and appropriate standard reference materials (NIST 1944: sediments and NIST 1947: fish-muscle tissue).

Table 1. Analytes measured in Florida Keys National Marine Sanctuary sediments and fish tissue.

PCBs	PAHs
PCB 1 (2-Chlorobiphenyl)	1-Methylnaphthalene
PCB 2 (3-Chlorobiphenyl)	1-Methylphenanthrene
PCB 3 (4-Chlorobiphenyl)	1,6,7-Trimethylnaphthalene
PCB 8/5 Coelution	2-Methylnaphthalene
PCB 9 (2,5-Dichlorobiphenyl)	2,6+2,7-Dimethylnaphthalene
PCB 12 (3,4-Dichlorobiphenyl)	Acenaphthene
PCB 15 (4,4'-Dichlorobiphenyl)	Acenaphthylene
PCB 18 (2,2',5-Trichlorobiphenyl)	Anthracene
PCB 20 (2,3,3'-Trichlorobiphenyl)	Benz[a]anthracene
PCB 26 (2,3',5-Trichlorobiphenyl)	Benzo[a]pyrene
PCB 28/31 Coelution	Benzo[b]fluoranthene
PCB 29 (2,4,5-Trichlorobiphenyl)	Benzo[e]pyrene
PCB 37 (3,4,4'-Trichlorobiphenyl)	Benzo[g,h,i]perylene
PCB 44 (2,2',3,5'-Tetrachlorobiphenyl)	Benzo[j]fluoranthene
PCB 45 (2,2',3,6-Tetrachlorobiphenyl)	Benzo[k]fluoranthene
PCB 47/48 Coelution	Biphenyl
PCB 49 (2,2',4,5'-Tetrachlorobiphenyl)	Chrysene+Triphenylene
PCB 50 (2,2',4,6-Tetrachlorobiphenyl)	Dibenz[a,h]anthracene
PCB 52 (2,2',5,5'-Tetrachlorobiphenyl)	Dibenzothiophene
PCB 56/60 Coelution	Fluoranthene
PCB 61 (2,3,4,5-Tetrachlorobiphenyl)	Fluorene
PCB 63 (2,3,4',5-Tetrachlorobiphenyl)	Indeno[1,2,3-c,d]pyrene
PCB 66 (2,3',4,4'-Tetrachlorobiphenyl)	Naphthalene
PCB 69 (2,3',4,6-Tetrachlorobiphenyl)	Perylene
PCB 70 (2,3',4',5-Tetrachlorobiphenyl)	Phenanthrene
PCB 74 (2,4,4',5-Tetrachlorobiphenyl)	Pyrene
PCB 76 (2,3',4',5'-Tetrachlorobiphenyl)	Retene (7-Isopropyl-1-methylphenanthrene)
PCB 77 (3,3',4,4'-Tetrachlorobiphenyl)	**Pesticides**
PCB 81 (3,4,4',5-Tetrachlorobiphenyl)	2,4'-DDD (o,p'-DDD)
PCB 82 (2,2',3,3',4-Pentachlorobiphenyl)	2,4'-DDE (o,p'-DDE)
PCB 84 (2,2',3,3',6-Pentachlorobiphenyl)	2,4'-DDT (o,p'-DDT)
PCB 87/115 Coelution	4,4'-DDD (p,p'-DDD)
PCB 88 (2,2',3,4,6-Pentachlorobiphenyl)	4,4'-DDE (p,p'-DDE)
PCB 101/90/89 Coelution	4,4'-DDT (p,p'-DDT)
PCB 92 (2,2',3,5,5'-Pentachlorobiphenyl)	Aldrin
PCB 95 (2,2',3,5',6-Pentachlorobiphenyl)	alpha-Chlordane
PCB 99 (2,2',4,4',5-Pentachlorobiphenyl)	alpha-Hexachlorocyclohexane (alpha-BHC)
PCB 103 (2,2',4,5',6-Pentachlorobiphenyl)	beta-Hexachlorocyclohexane (beta-BHC)
PCB 104 (2,2',4,6,6'-Pentachlorobiphenyl)	Chlorpyrifos
PCB 105 (2,3,3',4,4'-Pentachlorobiphenyl)	cis-Nonachlor
PCB 106/118 Coelution	Dieldrin
PCB 108/107 Coelution	Endosulfan I
PCB 110 (2,3,3',4',6-Pentachlorobiphenyl)	Endosulfan II (Beta-Endosulfan)
PCB 114 (2,3,4,4',5-Pentachlorobiphenyl)	Endosulfan sulfate
PCB 119 (2,3',4,4',6-Pentachlorobiphenyl)	Endrin
PCB 123 (2,3',4,4',5'-Pentachlorobiphenyl)	gamma-Chlordane
PCB 126 (3,3',4,4',5-Pentachlorobiphenyl)	Heptachlor
PCB 128 (2,2',3,3',4,4'-Hexachlorobiphenyl)	Heptachlor epoxide
PCB 130 (2,2',3,3',4,5'-Hexachlorobiphenyl)	Hexachlorobenzene (HCB)
PCB 132 (2,2',3,3',4,6'-Hexachlorobiphenyl)	Lindane
PCB 138 (2,2',3,4,4',5'-Hexachlorobiphenyl)	Mirex
PCB 141 (2,2',3,4,5,5'-Hexachlorobiphenyl)	Oxychlordane
PCB 146 (2,2',3,4',5,5'-Hexachlorobiphenyl)	trans-Nonachlor

Table 1 (continued).

PCBs (continued)	PBDEs
PCB 149 (2,2',3,4',5',6-Hexachlorobiphenyl)	PBDE 17 (2,2',4-tribromodiphenyl ether)
PCB 151 (2,2',3,5,5',6-Hexachlorobiphenyl)	PBDE 28 (2,4,4'-tribromodiphenyl ether)
PCB 153/168 Coelution	PBDE 47 (2,2',4,4'-tetrabromodiphenyl ether)
PCB 154 (2,2',4,4',5,6'-Hexachlorobiphenyl)	PBDE 66 (2,3',4,4'-tetrabromodiphenyl ether)
PCB 156 (2,3,3',4,4',5-Hexachlorobiphenyl)	PBDE 71 (2,3',4',6-tetrabromodiphenyl ether)
PCB 157 (2,3,3',4,4',5'-Hexachlorobiphenyl)	PBDE 85 (2,2',3,4,4'-pentabromodiphenyl ether)
PCB 158 (2,3,3',4,4',6-Hexachlorobiphenyl)	PBDE 99 (2,2',4,4',5-pentabromodiphenyl ether)
PCB 159 (2,3,3',4,5,5'-Hexachlorobiphenyl)	PBDE 100 (2,2',4,4',6-pentabromodiphenyl ether)
PCB 164/163 Coelution	PBDE 138 (2,2',3,4,4',5'-hexabromodiphenyl ether)
PCB 165 (2,3,3',5,5',6-Hexachlorobiphenyl)	PBDE 153 (2,2',4,4',5,5'-hexabromodiphenyl ether)
PCB 167 (2,3',4,4',5,5'-Hexachlorobiphenyl)	PBDE 154 (2,2',4,4',5,6'-hexabromodiphenyl ether)
PCB 169 (3,3',4,4',5,5'-Hexachlorobiphenyl)	PBDE 183 (2,2',3,4,4',5',6-heptabromodiphenyl ether)
PCB 170/190 Coelution	PBDE 190 (2,3,3',4,4',5,6-heptabromodiphenyl ether)
PCB 172 (2,2',3,3',4,5,5'-Heptachlorobiphenyl)	PBDE 209 (Decabromodiphenyl ether)
PCB 174 (2,2',3,3',4,5,6'-Heptachlorobiphenyl)	Metals
PCB 177 (2,2',3,3',4,5',6'-Heptachlorobiphenyl)	Aluminum
PCB 180/193 Coelution	Antimony
PCB 183 (2,2',3,4,4',5',6-Heptachlorobiphenyl)	Arsenic
PCB 184 (2,2',3,4,4',6,6'-Heptachlorobiphenyl)	Barium
PCB 187 (2,2',3,4',5,5',6-Heptachlorobiphenyl)	Beryllium
PCB 188 (2,2',3,4',5,6,6'-Heptachlorobiphenyl)	Cadmium
PCB 189 (2,3,3',4,4',5,5'-Heptachlorobiphenyl)	Chromium
PCB 194 (2,2',3,3',4,4',5,5'-Octachlorobiphenyl)	Cobalt
PCB 195 (2,2',3,3',4,4',5,6-Octachlorobiphenyl)	Copper
PCB 198 (2,2',3,3',4,5,5',6-Octachlorobiphenyl)	Iron
PCB 200 (2,2',3,3',4,5',6,6'-Octachlorobiphenyl)	Lead
PCB 201 (2,2',3,3',4',5,5',6-Octachlorobiphenyl)	Lithium
PCB 202 (2,2',3,3',5,5',6,6'-Octachlorobiphenyl)	Manganese
PCB 203/196 Coelution	Mercury
PCB 206 (2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl)	Nickel
PCB 207 (2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl)	Selenium
PCB 208 (2,2',3,3',4,5,5',6,6'-Nonachlorobiphenyl)	Silver
PCB 209 (2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl)	Thallium
	Tin
	Uranium
	Vanadium
	Zinc

2.5 Sediment Toxicity Testing

2.5.1 Microtox Solid-Phase Assay

This Microtox assay was conducted using the standard solid-phase test protocol and a Microtox Model 500 analyzer (Modern Water, Inc., New Castle, DE). For each sample, the sediment was homogenized and a 7.0 – 7.1-g subsample was used to make a series of sediment dilutions with 3.5 % NaCl diluent, which were incubated for 10 minutes at 15 °C. Luminescent bacteria (*Vibrio fischeri*) were then added to the test concentrations. The liquid phase was filtered from the sediment phase and bacterial post-exposure light output was measured using Microtox Omni Software. An EC_{50} value (the sediment concentration that reduced light output by 50 % relative to controls) was calculated for each sample.

Triplicate samples were analyzed simultaneously. Sediment samples were classified as either toxic or nontoxic using criteria provided in Ringwood et al. (1997).

2.5.2 Reporter-Gene Assay

This assay utilizes a reporter gene system (RGS) based on cytochrome P450 to screen samples for a range of organic compounds (Denison et al. 2004, Baston et al. 2010, Brennan et al. 2015). The reporter-gene system utilizes a human cell line (101L) into which a plasmid has been integrated containing a human CYP1A1 promoter and 5'-flanking sequences fused to a reporter gene, firefly luciferase. In the presence of CYP1A1-inducing compounds, the enzyme luciferase is produced, and its reaction with luciferin can be detected by measuring relative light units (RLUs) in a luminometer. To quantify the inducing compounds in the sample, the mean response, in RLUs, of the three sample replicates is divided by the mean response of three replicates of a solvent blank, yielding a "fold induction," which is a measure of the increase of the sample response over the background response. Fold-induction values are converted to benzo[a]pyrene equivalents (B[a]PEq) for PAHs based on the fold-induction responses to standards containing benzo[a]pyrene.

2.5.3 Sea Urchin Embryo Developmental Toxicity Assay

2.5.3.1 Sediment Porewater Water Quality and Ammonia Determination

Frozen porewater samples were thawed at 4 °C (72 h). Once completely thawed, samples were brought to room temperature and salinity was measured. Sample salinity was adjusted using reagent water (Type 1, ASTM 2018) to a target of 35.0 ± 0.5 psu. Following salinity adjustment, a 5-mL aliquot was removed to a clean 20-mL glass vial and dissolved oxygen and pH were measured using probes connected to a Thermo Orion 5-Star multimeter. Total ammonia nitrogen (TAN) was determined from 400 µL of sample using a colorimetric microplate assay based on a commercial kit (Red Sea, Houston, TX). Ammonia standards for the assay were generated using 100 mg/L ammonia standard (Hach, Catalog #2406549) in a two-fold dilution series (0.13 – 8.0 mg/L) in 35 psu artificial seawater (ASW, Pro-Reef Sea Salt, Tropic Marin, Wartenberg, Germany). Un-ionized ammonia nitrogen (UAN) was calculated following the methods of Bower and Bidwell (1978). Subsequent to water quality analysis, samples of porewater (5 mL, 4 replicates) were placed in pre-cleaned, rinsed (with 5 mL ASW, 35 psu) 20-mL glass vials and held at 23.0 ± 0.5 °C.

2.5.3.2 Sediment Porewater Toxicity

Sediment porewater toxicity was determined according to the methods of Carr and Chapman (1992) and Carr et al. (1996a). Gravid sea urchins (*Lytechinus variegatus*) were acquired from the Florida Keys (vendor: Reeftopia), and held for three months at 25 °C in a shallow glass-Teflon aquarium system containing ASW (Aquarium Sea Salt Mixture, Instant Ocean, Blacksburg, VA, 35 psu). Lighting was provided by one 1500W, 5000K LED (range 7 – 26 µmol/m^2/s at depth) on a 12h:12h light:dark cycle. Urchins were fed a rotating diet of organic carrots, organic spinach, and seaweed (Julian Sprung's Sea Veggies®) three times per week.

Urchin spawning was initiated using 1 – 3 mL potassium chloride (0.5 M) injections into the coelom by inserting the needle through the peristomal membrane surrounding the mouth. Eggs were collected by inverting the female urchin over a beaker filled to the brim with artificial seawater (35 psu, 25°C). The

urchin aboral side was submerged slightly, so that the eggs were extruded directly into the seawater. After spawning was complete, the eggs were washed three times with equal volumes of fresh ASW (Tropic Marin, 35 psu) and enumerated on a Sedgewick-Rafter counting chamber. Sperm was collected dry by aspiration with a micropipet tip and placed in a sterile 0.5 mL polypropylene Eppendorf tube. Sperm was kept chilled (not directly on ice) until used. Sperm was diluted 1:250 in ASW to activate and cell concentration was determined and motility was verified from a 1:2000 dilution in ASW.

Prior to beginning the assay, optimal fertilization rates were determined using four dilutions of sperm in a fertilization pre-test. Embryos (~200 in 50 µL volume) were placed in pre-cleaned and rinsed (with 5 mL ASW, 35 psu) 20-mL glass vials containing 5 mL of sample porewater (n=4 per sample). Artificial seawater (35 psu) and 4 mg/L sodium dodecyl sulfate in ASW were included as assay controls. Embryos were incubated for 48 h at 23 ± 0.5 °C under ambient fluorescent lighting on a 12h:12h light:dark cycle. Following incubation, an equal volume of 2X zinc-formalin fixative (Anatech, Poughkeepsie, NY) in ASW was added to each vial, and embryo developmental stage and developmental aberrations were scored, with a target of 100 embryos evaluated per sample replicate.

2.5.3.3 Ammonia Dose-Response Test

To determine the effect of un-ionized ammonia in sediment porewater on *Lytechinus variegatus* embryo development, a dose response test was performed concurrently with the porewater toxicity assay. A stock solution with calculated ammonia-nitrogen of 2.08 g/L was prepared using ammonium chloride dissolved in Type 1 water. Sea urchin embryos (~200) were exposed to dilutions of the stock in ASW (0.02 – 2.0 mg/L TAN) for 48 h at 23 ± 0.5 °C under ambient lighting on a 12 h:12 h light:dark cycle. Embryos were fixed in 2X zinc-formalin fixative solution prepared in ASW (70 psu) prior to evaluation. Actual values of TAN for each test solution were verified with the modified microplate assay and un-ionized ammonia was calculated as described above.

2.5.3.4 Partial Toxicity Identification and Evaluation

Two sediment porewater samples (FK15_001, FK15_007) which resulted in the lowest rates of normal sea urchin embryo development were filtered with a Strata X 33u polymeric reversed phase solid phase extraction (SPE) column (Phenomenex, Torrence, CA) and subsequently tested for toxicity. Columns (1 mL bed volume) were charged with 1 mL pesticide-free methanol and rinsed with 1 mL Type 1 water as per the manufacturer's instructions. One milliliter of the porewater sample (previously adjusted for appropriate salinity) was used to rinse the column before applying the remaining sample for collection using a vacuum manifold (~1 drop/s). Salinity and pH for filtered, post-SPE porewater samples were verified prior to beginning this follow-up sea urchin development assay.

2.6 Benthic Community Analysis

Benthic samples were transferred from formalin to 70 % ethanol in the laboratory. Macroinfaunal invertebrates (sampled with 0.04 m^2 grab, sieved on 0.5 mm screen) were sorted from the sample debris under a dissecting microscope and identified to the lowest practical taxon (usually to species). Data-quality steps included: (1) tests of ongoing sorting proficiency on 10 % of samples by independent sorters to assure that > 95 % of animals in each sample were removed by the original sorter, (2) use of skilled taxonomists with updated standard taxonomic keys and reference collections to perform species

identifications, (3) checks for potential misidentifications on a minimum of 10 % of samples by independent qualified taxonomists, and (4) appropriate corrective actions to resolve any potential sorting or species identification errors. Resulting data were used to calculate # taxa, density (#/m^2), and diversity (Shannon H', calculated with base-2 logarithms).

2.7 Data Analysis

The probabilistic design used in this study allows estimation of the percent area of the sampling domain corresponding to specified values of a given parameter under consideration. Estimated cumulative distribution functions (CDFs), point estimates, and 95% confidence intervals were developed for water quality, sediment, and biological parameters measured in this study using formulas described in the EMAP statistical methods manual (Diaz-Ramos 1996). Calculation of CDFs was facilitated using algorithms (*spsurvey* package; Kincaid and Olsen 2016) developed for R, a language and environment for statistical computing and graphics (R Core Team 2017).

Measured parameters were compared to established thresholds of concern, where available (Table 2 - Table 4), and the corresponding percentiles of the estimated CDFs were reported. Where no such recommended levels of concern exist (e.g., benthic metrics), common distributional properties are reported (e.g., lower or upper percentiles).

Individual contaminant concentrations in sediment samples were compared to corresponding Effects Range Low (ERL) and Effects Range Median (ERM) sediment quality guidelines (SQGs, Long et al. 1995) as a means of evaluating their potential risks to benthic fauna. Mean ERM Quotients (mERM-Q, Long et al. 1998, Hyland et al. 1999) were also calculated to provide a single measure of the mixture of multiple contaminants present in a sample; values were compared to corresponding benthic-risk thresholds provided in Hyland et al. (1999). While the above bioeffect thresholds were developed with data typically from shallower-water estuarine habitats, they were applied here as reasonable surrogates to use in the absence of similar guidelines for offshore applications.

The synoptic measurement of sediment contaminants, sediment toxicity, and condition of ambient benthic fauna supports a weight-of-evidence, Sediment Quality Triad (SQT) approach to assessing any signs of pollution-induced degradation of the benthos at sampling sites (Chapman 1990).

Additional data on human-health risks from measures of chemical contaminants in targeted fish species are also reported here and compared to corresponding management thresholds.

Table 2. Thresholds used for classifying samples relative to various environmental indicators.

Indicator	Threshold	Reference
Water Quality (WQ)		
Salinity (psu)	< 5 = Oligohaline 5 – 18 = Mesohaline >18 – 30 = Polyhaline > 30 = Euhaline	Carriker 1967
$\Delta \sigma_t$	> 2 = strong vertical stratification	Nelson et al. 2008
DO (mg/L)	> 5 = High (Good) 2 – 5 = Moderate (Fair) < 2 = Low (Poor)	USEPA 2012; Diaz and Rosenberg 1995
DIN (mg/L)	<= 0.01 mg/L (Meets WQ target) > 0.01 mg/L (Does not meet WQ target)	Boyer and Briceño 2010
Ratio of DIN:DIP	> 16 = phosphorus limited < 16 = nitrogen limited	Redfield 1958; Geider and La Roche 2002
TP (µg/L)	<= 7.7 µg/L (Meets WQ target) > 7.7 µg/L (Does not meet WQ target)	Boyer and Briceño 2010
CHL *a* [a]	<= 0.35 µg/L (Meets WQ target) > 0.35 µg/L (Does not meet WQ target)	Boyer and Briceño 2010
Sediment Quality		
Silt-Clay Content (%)	> 80 = Mud 20 – 80 = Muddy Sand < 20 = Sand	USEPA 2012
TOC Content (%)	< 2 = Low (Good) 2 – 5 = Moderate (Fair) > 5 = High (Poor)	USEPA 2012
Overall chemical contamination of sediments	No ERMs exceeded and < 5 ERLs exceeded = Low (Good); ≥ 5 ERL values exceeded = Moderate (Fair); ≥ 1 ERM value exceeded = High (Poor)	USEPA 2012
Individual chemical contaminant concentrations in sediments	> ERM = High probability of bioeffects < ERL = Low probability of bioeffects	Long et al. 1995; Table 3 herein
Sediment toxicity using Microtox® assay	Silt+clay < 20 %: Toxic if EC_{50} < 0.5 % Silt+clay ≥ 20 %: Toxic if EC_{50} < 0.2 %	Ringwood et al. 1997

Indicator	Threshold	Reference
Biological Condition		
Reduced benthic taxonomic richness, diversity, or abundance	≤ lower 10th percentile of all values for corresponding variable	Nelson et al. 2008
Chemical Contaminants in Fish Tissues	All chemicals fall below the range of EPA advisory guidance values [b] = Low (Good); ≥ 1 chemical falls within the range of EPA advisory guidance values = Moderate (Fair); ≥ 1 chemical exceeds the maximum value in the range of EPA advisory guidance values = High (Poor).	USEPA 2012
Individual chemical contaminants in fish tissues	Risk endpoints based on consumption of four 8-ounce meals per month (general adult population).	USEPA 2000; Table 4 herein

[a] Water quality target for chlorophyll *a* is for reef sites.

[b] Range of concentrations of a given chemical contaminant considered safe at a consumption rate of four 8-oz fish meals/month.

Table 3. ERM and ERL guideline values in sediments (Long et al. 1995).

Chemical	ERL	ERM
Metals (µg/g)		
Arsenic	8.2	70
Cadmium	1.2	9.6
Chromium	81	370
Copper	34	270
Lead	46.7	218
Mercury	0.15	0.71
Nickel	20.9	51.6
Silver	1	3.7
Zinc	150	410
Organics (ng/g)		
Acenaphthene	16	500
Acenaphthylene	44	640
Anthracene	85.3	1,100
Fluorene	19	540
2-Methylnaphthalene	70	670
Naphthalene	160	2,100
Phenanthrene	240	1,500
Benzo[a]anthracene	261	1,600
Benzo[a]pyrene	430	1,600
Chrysene	384	2,800
Dibenz[a,h]Anthracene	63.4	260
Fluoranthene	600	5,100
Pyrene	665	2,600
Low molecular weight PAHs	552	3,160
High molecular weight PAHS	1,700	9,600
Total PAHs	4,020	44,800
4,4-DDE	2.2	27
Total DDT	1.58	46.1
Total PCBs	22.7	180

Table 4. Risk-based EPA advisory guidelines for recreational fishers corresponding to four fish meals per month (USEPA 2000).

	EPA Advisory Guidance Concentration Range[a]			Health Endpoint
Metals (µg/g)				
Arsenic (inorganic)[b]	>0.35	–	0.70	non-cancer
Cadmium	>0.35	–	0.70	non-cancer
Mercury (methylmercury)[c]	>0.12	–	0.23	non-cancer
Selenium	>5.90	–	12.00	non-cancer
Organics (ng/g)				
Chlordane	>590	–	1,200	non-cancer
Chlorpyriphos	>350	–	700	non-cancer
DDT (total)	>59	–	120	non-cancer
Dieldrin	>59	–	120	non-cancer
Endosulfan	>7,000	–	14,000	non-cancer
Heptachlor epoxide	>15	–	31	non-cancer
Hexachlorobenzene	>940	–	1,900	non-cancer
Lindane	>350	–	700	non-cancer
Mirex	>230	–	470	non-cancer
Toxaphene	>290	–	590	non-cancer
PAHs (benzo[a]pyrene)	>1.6	–	3.2	cancer[d]
PCB (total)	>23	–	47	non-cancer

[a] Range of concentrations associated with non-cancer and cancer health endpoint risk. Range of concentrations for non-cancer health endpoints are based on the assumption that consumption over a lifetime of four 8-oz meals per month would not generate a chronic, systemic health risk. Range of concentrations for cancer health endpoints are based on the assumption that consumption over a lifetime of four 8-oz meals per month would yield a lifetime cancer risk no greater than an acceptable risk of 1 in 100,000 (USEPA 2000).

[b] Inorganic arsenic, the form considered toxic, was estimated as 2% of total arsenic. (USEPA 2000).

[c] Because most mercury in fish and shellfish tissue is present primarily as methylmercury and because of the relatively high cost of analyzing for methylmercury, the conservative assumption was made that all mercury is present as methylmercury (U.S. EPA, 2000).

[d] A non-cancer concentration range for PAHs does not exist.

3 Results and Discussion

3.1 Depth and Water Quality

3.1.1 Depth

Depths throughout the sampled portion of FKNMS ranged from 11 – 99 m, with an average station depth of 41 m (Table 5). The deepest sites tended to occur along the eastern and southern boundaries of the sanctuary in association with the shelf break, where the Florida Plateau slopes sharply out to the Pourtales Terrace. Fifty percent of the area sampled had depths < 30 m, with most of these occurring in shallower areas between the Marquesas and Dry Tortugas or in association with the southwest Florida Shelf (Figure 2).

Table 5. Summary of depth and water-column characteristics for near-bottom (within 1 – 3 m of bottom) and near-surface (2 – 3 m) waters from 30 FKNMS sites.

	Near-bottom water					Near-surface water				
	Mean	Range	CDF 10th pctl	CDF 50th pctl	CDF 90th pctl	Mean	Range	CDF 10th pctl	CDF 50th pctl	CDF 90th pctl
Depth (m)	41.2	11 - 98.8	13.4	29.5	76.2	—	— —	—	—	—
$\Delta\sigma_t$	0.7	0 - 3.3	0	0.1	2.2	—	— —	—	—	—
Temperature (°C)	26.2	15.2 - 29.3	20.4	28	29	28.5	27.8 - 29.3	27.9	28.6	29
Salinity (psu)	36.4	36 - 37	36.3	36.4	36.6	36.4	36.1 - 36.9	36.3	36.4	36.6
DO (mg/L)	6.3	4.3 - 6.8	5.9	6.3	6.7	6.3	5.9 - 6.4	6.1	6.3	6.4
pH	7.8	7.6 - 7.8	7.7	7.8	7.8	7.8	7.8 - 7.9	7.8	7.8	7.8
DIN (mg/L)	—	— —	—	—	—	0.012	0.006 - 0.038	0.006	0.008	0.024
DIP (mg/L)	—	— —	—	—	—	0.002	0.002 - 0.003	0.002	0.002	0.003
DIN:DIP	—	— —	—	—	—	18.5	10.9 - 71.1	11.8	14.3	28.6
TN (µg/L)	—	— —	—	—	—	133	90 - 300	—	110	190
TP (µg/L)	—	— —	—	—	—	8.5	4.7 - 17.6	5.5	7.6	11.8
CHL a (µg/L)	—	— —	—	—	—	0.16	0.03 - 0.68	0.04	0.12	0.33
Turbidity (NTU)	—	— —	—	—	—	0.4	0.2 - 1.0	0.2	0.4	0.8
TSS (mg/L)	—	— —	—	—	—	3.6	2.4 - 15.0	2.4	2.9	4.6

3.1.2 General Water Characteristics: Temperature, Salinity, Vertical Stratification, DO, pH

Measured salinities (surface and bottom) at all 30 sites occupied a narrow range between 36 and 37 psu, with a mean salinity of 36.4 psu (Table 5). Strong vertical stratification ($\Delta\sigma_t > 2$) was observed at five of the 30 sites; these stations were also some of the deepest sites along the shelf break on the southeastern Sanctuary boundary (Atlantic side), as noted in the previous section. Near-surface water temperatures averaged 28.5 °C (range of 27.8 – 29.3 °C); bottom-waters tended to be colder (15.2 – 29.3 °C, mean of 26.2 °C), particularly at deeper sites. The lowest temperature was observed at station 12, which was the deepest site (98.8 m) and the most vertically stratified ($\Delta\sigma_t = 3.3$). Bottom DO was also lowest at station 12 (4.3 mg/L), while DO concentrations at the remaining 29 sites (96.7 % area) were all well above 5 mg/L (>= 5.9 mg/L). Similarly, bottom-water pH was lowest at station 12 (pH=7.6), and also tended to be somewhat lower at other vertically-stratified sites identified above; at most sites, however, bottom-water pH was restricted to the narrow range of 7.8 – 7.9.

The full range of values across all FKNMS stations, for the various water-quality variables discussed above, is displayed as CDF plots in Figure 3. The mean values by station (average of multiple CTD measurements for near-bottom and near-surface waters for each station) appear in Appendix B and Appendix C.

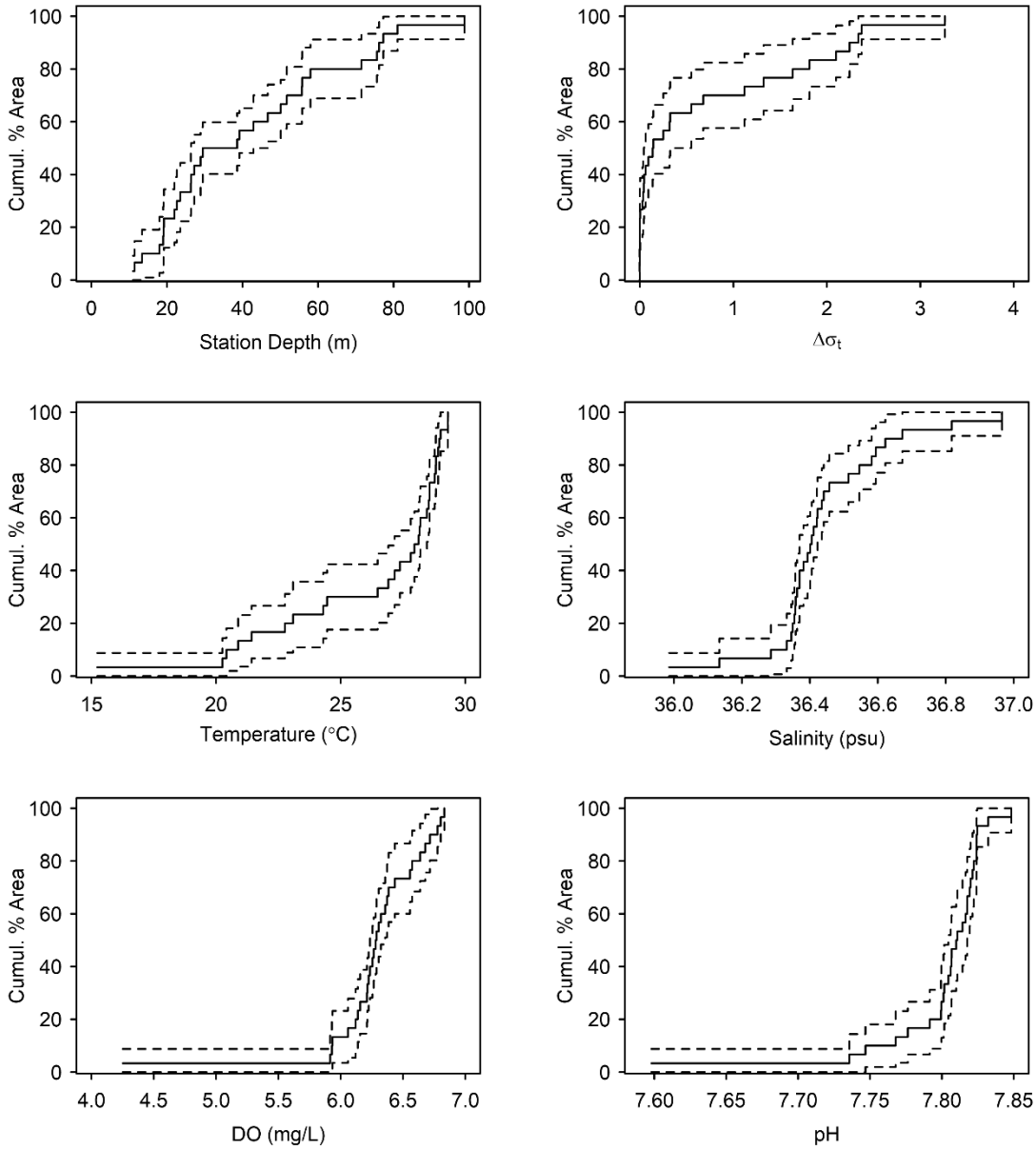

Figure 3. Estimated CDF plots representing percent area (and 95% confidence intervals) of sampled FKNMS waters vs. selected water-quality characteristics.

3.1.3 Nutrients, Chlorophyll, Turbidity, and TSS

The concentration of dissolved inorganic nitrogen (DIN: nitrogen as nitrate + nitrite + ammonium) in near-surface waters ranged from 0.006 – 0.038 mg/L and averaged 0.012 mg/L (Table 5). Twenty-two stations (representing 73 % of the study area) had surface-water DIN concentrations equal to or below the EPA water quality target (Boyer and Briceño 2010) of 0.01 mg/L. Four stations (10 % of the sampled area) had DIN levels > 0.024 (Table 5); these stations were distributed widely throughout the sampling area, with two stations in the Upper Keys (stations 1 and 5), one station in the Lower Keys (station 28),

and one station in the Tortugas (station 3). Total phosphorus levels ranged from 4.7 – 17.6 µg/L (mean of 8.5 µg/L), with 53 % of the study area having concentrations less than or equal to the EPA water quality target (Boyer and Briceño 2010) of 7.7 µg/L.

The ratio of DIN:DIP has been used as a measure of potential nutrient limitation status of phytoplankton (Redfield 1958, Geider and La Roche 2002). DIN:DIP ratios in surface waters averaged 18.5 (range of 10.9 – 71.1), with the majority of sites sampled (19 stations, 63 % area) having levels indicative of nitrogen limitation (DIN:DIP < 16). The remaining 11 of 30 stations (37 % area) had DIN:DIP ratios > 16, suggesting that phosphorus is the limiting nutrient. This may be due to higher DIN concentrations at these sites, however, as the concentration of DIP (orthophosphate) was fairly constant (0.002 – 0.003 mg/L) across all sites (Table 5). Although DIP was less variable, higher relative concentrations of dissolved inorganic forms of nitrogen were observed at station 28 (ammonium) and stations 1, 2, 3 and 5 (nitrate; Figure 4).

Levels of CHL *a* in near-surface waters varied between 0.03 µg/L and 0.68 µg/L (mean of 0.16 µg/L), with 93 % of the study area (28 of 30 sites) having CHL *a* concentrations below the EPA water quality target (for reef sites) of 0.35 µg/L. The two stations with CHL *a* levels exceeding the target were located in the northern portion of the sanctuary on the southwest Florida shelf (Figure 5); one just north of the Marquesas (station 20) and the other along the northern sanctuary boundary north of Key West (station 27). This pattern is consistent with other monitoring results, which found a gradient of decreasing CHL *a* concentrations from inshore to offshore waters of the southwest Florida shelf (Boyer and Briceño 2010).

Concentrations of TSS ranged from 2.4 – 15.0 mg/L (mean of 3.6 mg/L) in near-surface waters, with most stations (29 of 30 sites, 96.7 % area) having TSS <= 5.1 mg/L. Similarly, surface turbidity was low at FKNMS stations, ranging from 0.2 – 1.0 NTU (mean of 0.4 NTU; Table 5).

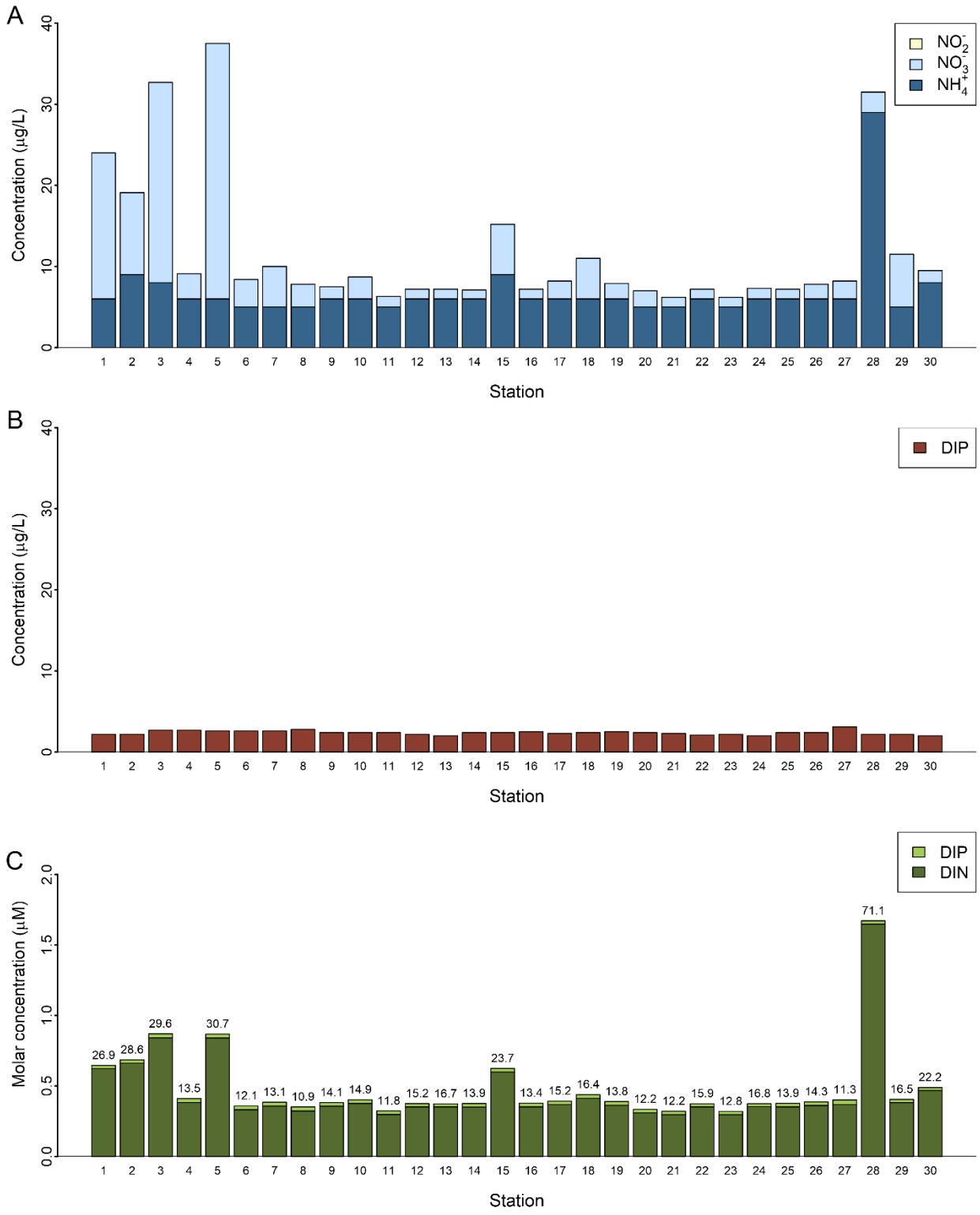

Figure 4. Relative concentrations of nitrogen and phosphorus measured in near-surface waters of FKNMS. Values of the molar ratio of DIN:DIP are shown above the bars in part C of the figure.

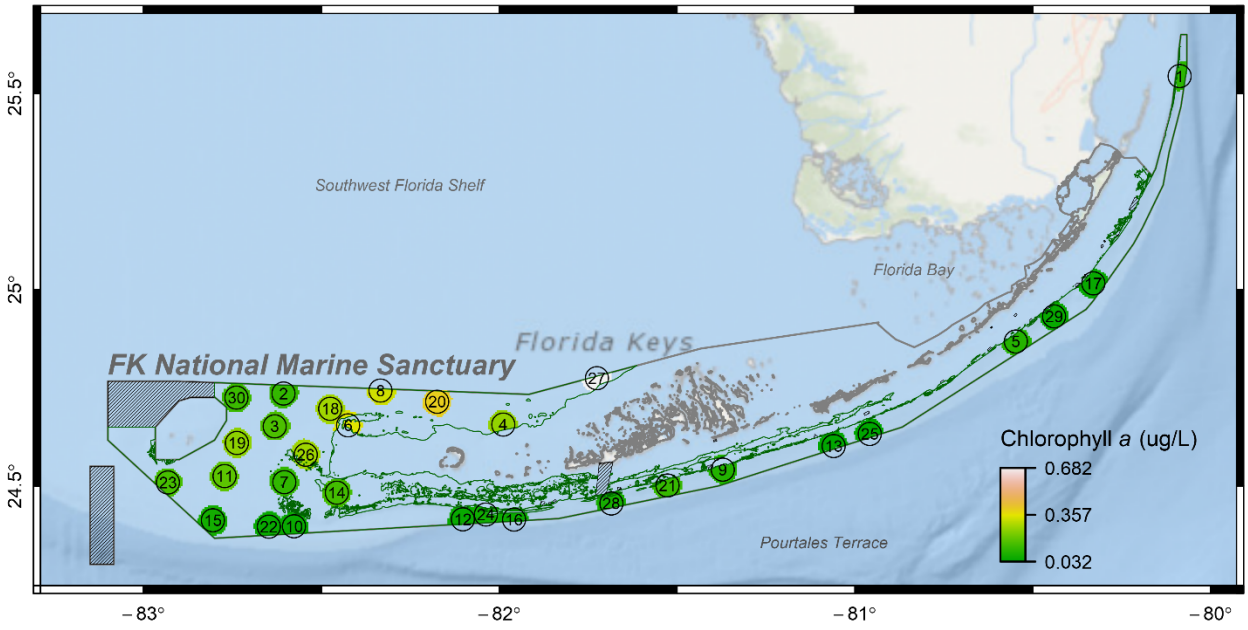

Figure 5. Concentrations of chlorophyll a measured in near-surface water samples collected at 30 stations in FKNMS.

3.2 Sediment Quality

3.2.1 Grain Size and TOC

Half of the sediments sampled in FKNMS (15 stations, 50 % area) were composed of sand (< 20 % silt+clay), while sediments at the remaining sites were characterized as muddy sand (20 – 80 % silt+clay). Silt+clay content ranged from 3.1 – 70.2 %, with a mean of 24.3 % (Table 6). Highest levels of % silt+clay were concentrated mostly in the western portion of the sanctuary (Figure 6). TOC in sediments was typically low (< 2 %) with a mean of 0.7 %, although levels of TOC at two sites (stations 19 and 26) were in the moderate range of 2 – 5 % (USEPA 2012). Although levels of TOC in sediments overall were positively correlated with percent silt+clay, interestingly, these two stations with elevated TOC did not have correspondingly high percent silt+clay content (Figures 6 - 8). In fact, the station with the highest level of TOC (station 19, TOC=3.7 %) had very low percent silt+clay (8.4 %). Values of percent sand, silt+clay, and TOC by station are listed in Appendix A.

Table 6. Summary of percent silt+clay and TOC content of sediment samples collected at 30 sites in FKNMS.

	Mean	Range	CDF 10th pctl	CDF 50th pctl	CDF 90th pctl
Silt+Clay (%)	24.3	3.1 - 70.2	3.1	18.7	53.0
TOC (%)	0.7	0.0 - 3.7	0.0	0.5	1.1

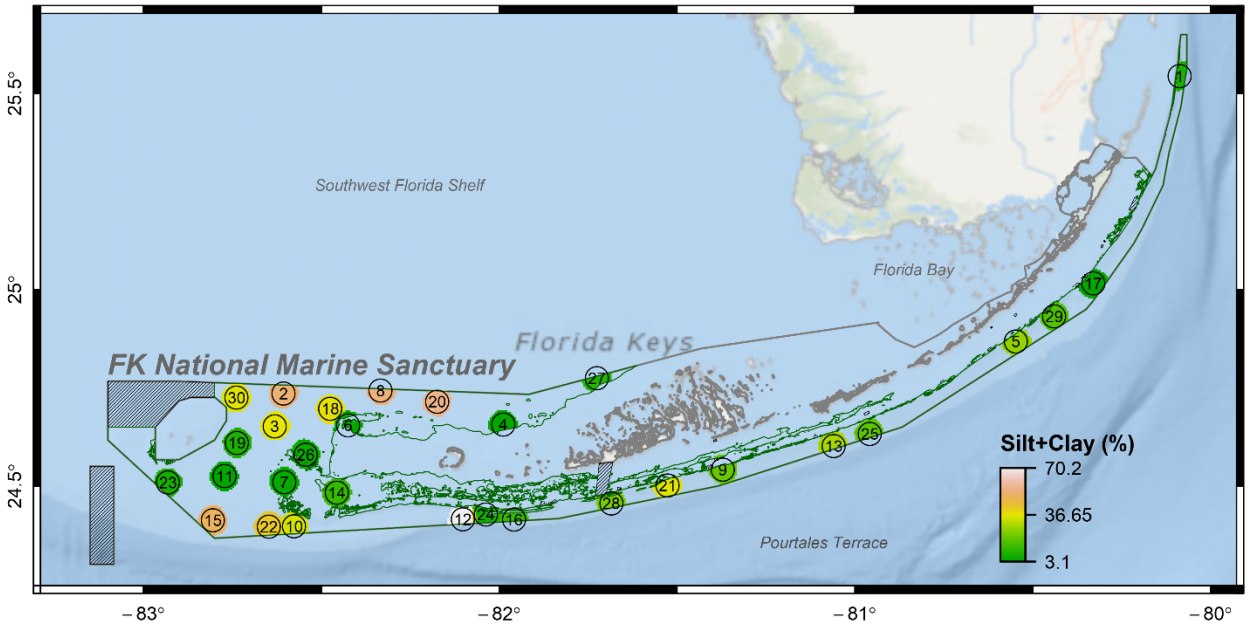

Figure 6. Levels of percent silt+clay in sediments collected at 30 sites in FKNMS.

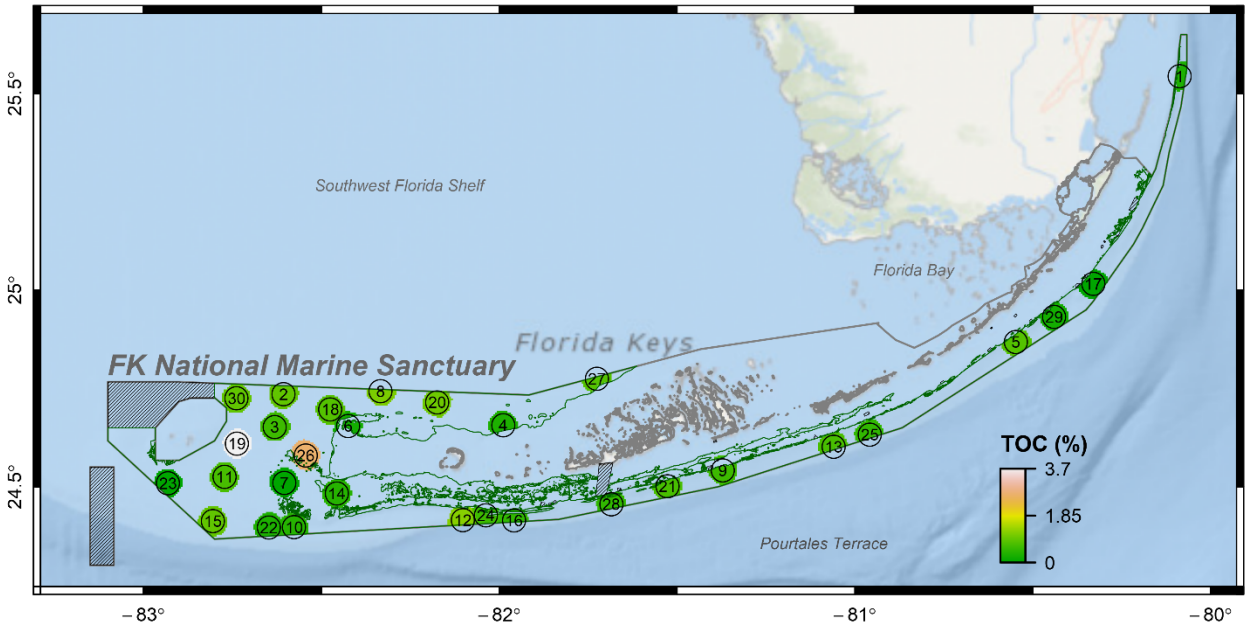

Figure 7. Levels of total organic carbon (TOC) in sediments collected at 30 sites in FKNMS.

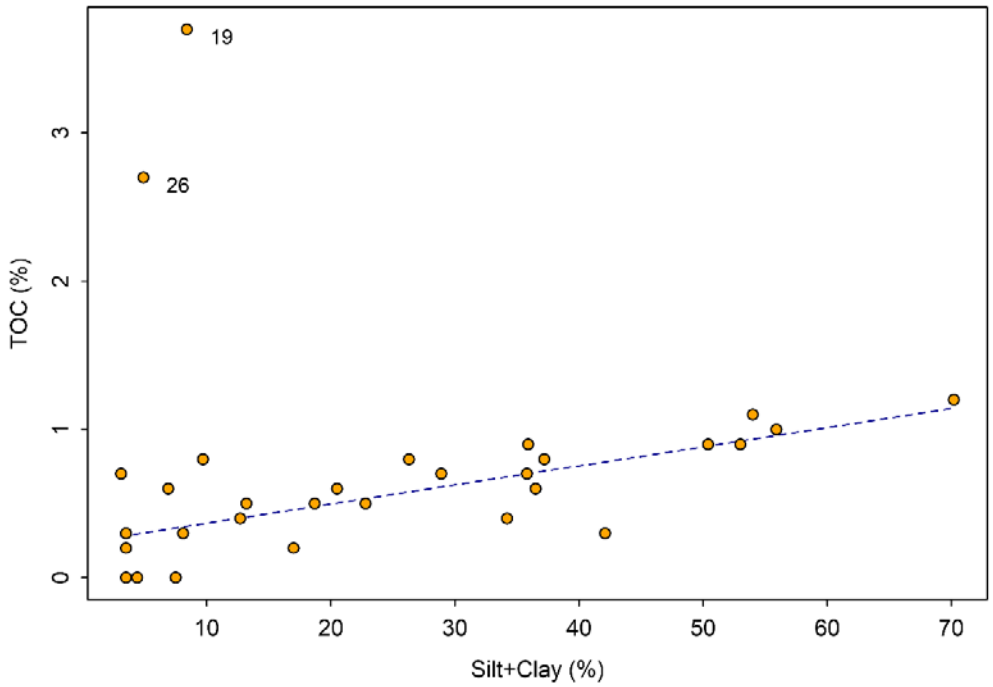

Figure 8. Relationship between TOC and silt+clay in sediment samples collected at 30 stations in FKNMS. Possible outliers are identified by station number. Dashed blue line results from linear regression using only the remaining data points (i.e., all except the two outlying values).

3.2.2 Chemical Contaminants in Sediments

Concentrations of chemical contaminants in sediments were generally at low background levels, below expected bioeffect ranges (Table 7), although a number of metals, PAHs, PCBs, and pesticides were measured at concentrations above the minimum method detection limit (MDL). Levels of Total PCBs were measured in excess of the ERL at one site (station 29, Total PCBs = 26.9 ng/g) located approximately 3 km offshore of Conch Reef. The most abundant PCB congeners and co-eluting congeners at station 29, identified by the numbering system proposed by Ballschmiter and Zell (1980) and adopted by the International Union of Pure and Applied Chemists (IUPAC), were 153/168, 180/193, 138, 149, 101/90/89, and 170/190 (Figure 9). Together, these six congener groups made up 50 % of total PCBs detected at station 29. Interestingly, a site sampled in an unrelated assessment in 2007 (Cooksey et al. 2012) and located in close proximity to station 29 had PCB concentrations (3.4 ng/g) that were elevated relative to the other sites sampled in that study, though still below the ERL for Total PCBs of 22.7 ng/g (see Table 3).

Calculated mean ERM quotients (mERM-Q) were well below levels associated with expected toxicity to benthic organisms, and in fact all mERM-Qs were within the lower range corresponding to a low likelihood of toxicity based on acute amphipod toxicity tests (mERM-Q < 1; Long et al. 1998) and observations of benthic community-level responses in field samples (mERM-Q <= 0.020; Hyland et al. 1999). Although mERM-Qs were below levels associated with expected bioeffects, the contribution of

individual contaminants or classes of contaminants to the overall mERM-Q varied among stations (Figure 10). At station 29, for example, Total PCBs made up a large proportion of the total ERM quotient (prior to dividing by the number of ERMs to obtain the mean; Figure 9-B). Individual mean ERM-Qs and ERL/ERM exceedances are listed for all sites in Appendix D.

Spatial trends in concentrations of these contaminants are shown in Figure 11 a - f. There was a significant positive association between trace metals (sum of eight trace metals for which ERMs are available: silver, arsenic, cadmium, chromium, copper, mercury, lead, and zinc) and percent silt+clay content of sediments ($r=0.55$, $p=0.001$). While the highest concentrations of trace metals occurred mostly in the western portion of the sanctuary where sediment silt+clay fraction also tended to be highest, the single highest concentration was found at a site having low percent silt+clay (station 19, Figure 12). As noted above, station 19 also had the highest measured concentration of sediment TOC (3.7 %). With few exceptions, trace metals generally tracked with the concentration of aluminum (shown as grey symbols in Figure 12), which is the most abundant naturally-occurring metal and whose concentration is generally not influenced by anthropogenic sources (Schropp and Windom 1988). These results do not argue strongly in favor of anthropogenic sources of trace metals, with the possible exception of station 19.

Table 7. Summary of chemical contaminant concentrations in FKNMS sediment samples ('N.D.' = not detected; '-' = no corresponding ERL or ERM available).

						ERL < Conc. < ERM		Conc. > ERM	
Analyte	Mean	(Std. Dev.)	Min.		Max.	# Stations	% Area	# Stations	% Area
Metals (% dry mass)									
Aluminum	0.098	0.053	0.029	-	0.220	–	–	–	–
Iron	0.135	0.051	0.081	-	0.280	–	–	–	–
Trace Metals (µg/g dry mass)									
Antimony	0.124	0.379	N.D.	-	1.372	–	–	–	–
Arsenic	1.867	1.393	0.609	-	7.833	0	0	0	0
Barium	16.309	5.092	10.064	-	33.078	–	–	–	–
Beryllium	0.076	0.079	N.D.	-	0.321	–	–	–	–
Cadmium	0.067	0.137	N.D.	-	0.635	0	0	0	0
Cobalt	1.054	0.246	0.401	-	1.602	–	–	–	–
Chromium	9.786	2.547	5.524	-	14.410	0	0	0	0
Copper	1.427	0.458	0.764	-	2.719	0	0	0	0
Mercury	0.006	0.003	0.002	-	0.015	0	0	0	0
Lithium	3.420	1.575	1.059	-	6.055	–	–	–	–
Manganese	19.391	5.527	9.839	-	30.166	–	–	–	–
Nickel	7.951	0.897	5.922	-	9.844	–	–	–	–
Lead	1.467	0.498	0.785	-	3.177	0	0	0	0
Selenium	0.423	0.054	0.340	-	0.562	–	–	–	–
Silver	0.008	0.042	N.D.	-	0.231	0	0	0	0
Tin	0.313	0.279	0.213	-	1.787	–	–	–	–
Thallium	0.048	0.096	N.D.	-	0.463	–	–	–	–
Uranium	2.496	0.685	1.233	-	3.870	–	–	–	–
Vanadium	4.247	1.661	1.811	-	8.562	–	–	–	–
Zinc	6.074	1.238	4.378	-	8.749	0	0	0	0
PAHs (ng/g dry mass)									
Acenaphthene	0.142	0.125	N.D.	-	0.405	0	0	0	0
Acenaphthylene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
Anthracene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
Benz[a]anthracene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
Benzo[a]pyrene	0.020	0.076	N.D.	-	0.303	0	0	0	0
Benzo[e]pyrene	0.013	0.072	N.D.	-	0.395	–	–	–	–
Benzo[a]fluoranthene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Benzo[b]fluoranthene	0.048	0.146	N.D.	-	0.495	–	–	–	–
Benzo[j]fluoranthene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Benzo[k]fluoranthene	0.009	0.050	N.D.	-	0.272	–	–	–	–
Benzo[g,h,i]perylene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Biphenyl	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Chrysene+Triphenylene	0.083	0.191	N.D.	-	0.604	0	0	0	0

Analyte	Mean	(Std. Dev.)	Min.		Max.	ERL < Conc. < ERM # Stations	ERL < Conc. < ERM % Area	Conc. > ERM # Stations	Conc. > ERM % Area
Dibenz[a,h]anthracene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
Dibenzothiophene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Fluoranthene	0.107	0.335	N.D.	-	1.397	0	0	0	0
Fluorene	0.026	0.079	N.D.	-	0.286	0	0	0	0
Indeno[1,2,3-c,d]pyrene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Naphthalene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
1-Methylnaphthalene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
2-Methylnaphthalene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
2,6+2,7-Dimethylnaphthalene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
1,6,7-Trimethylnaphthalene	0.068	0.223	N.D.	-	0.996	–	–	–	–
Perylene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Phenanthrene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
1-Methylphenanthrene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Pyrene	N.D.	N.D.	N.D.	-	N.D.	0	0	0	0
Retene	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Low molecular weight PAHs	0.236	0.278	N.D.	-	1.210	0	0	0	0
High molecular weight PAHs	0.267	0.540	N.D.	-	1.998	0	0	0	0
Total PAHs	0.516	0.697	N.D.	-	2.403	0	0	0	0
PCBs (ng/g dry mass)									
Total PCBs	0.996	4.897	N.D.	-	26.920	1	3.3	0	0
Pesticides (ng/g dry mass)									
Aldrin	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
alpha-Chlordane	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
gamma-Chlordane	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Oxychlordane	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Chlorpyrifos	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
cis-Nonachlor	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
2,4'-DDD (o,p'-DDD)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
4,4'-DDD (p,p'-DDD)	0.000	0.002	N.D.	-	0.012	–	–	–	–
2,4'-DDE (o,p'-DDE)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
4,4'-DDE (p,p'-DDE)	0.001	0.005	N.D.	-	0.028	0	0	0	0
2,4'-DDT (o,p'-DDT)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
4,4'-DDT (p,p'-DDT)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Dieldrin	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Endosulfan I	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Endosulfan II (Beta-Endosulfan)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Endosulfan sulfate	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Endrin	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Hexachlorobenzene (HCB)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Heptachlor	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Heptachlor epoxide	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–

Analyte	Mean	(Std. Dev.)	Min.		Max.	ERL < Conc. < ERM		Conc. > ERM	
						# Stations	% Area	# Stations	% Area
alpha-HCH	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
beta-HCH	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
gamma-HCH (Lindane)	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Mirex	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
trans-Nonachlor	N.D.	N.D.	N.D.	-	N.D.	–	–	–	–
Total DDTs	0.001	0.007	N.D.	-	0.040	0	0	0	0

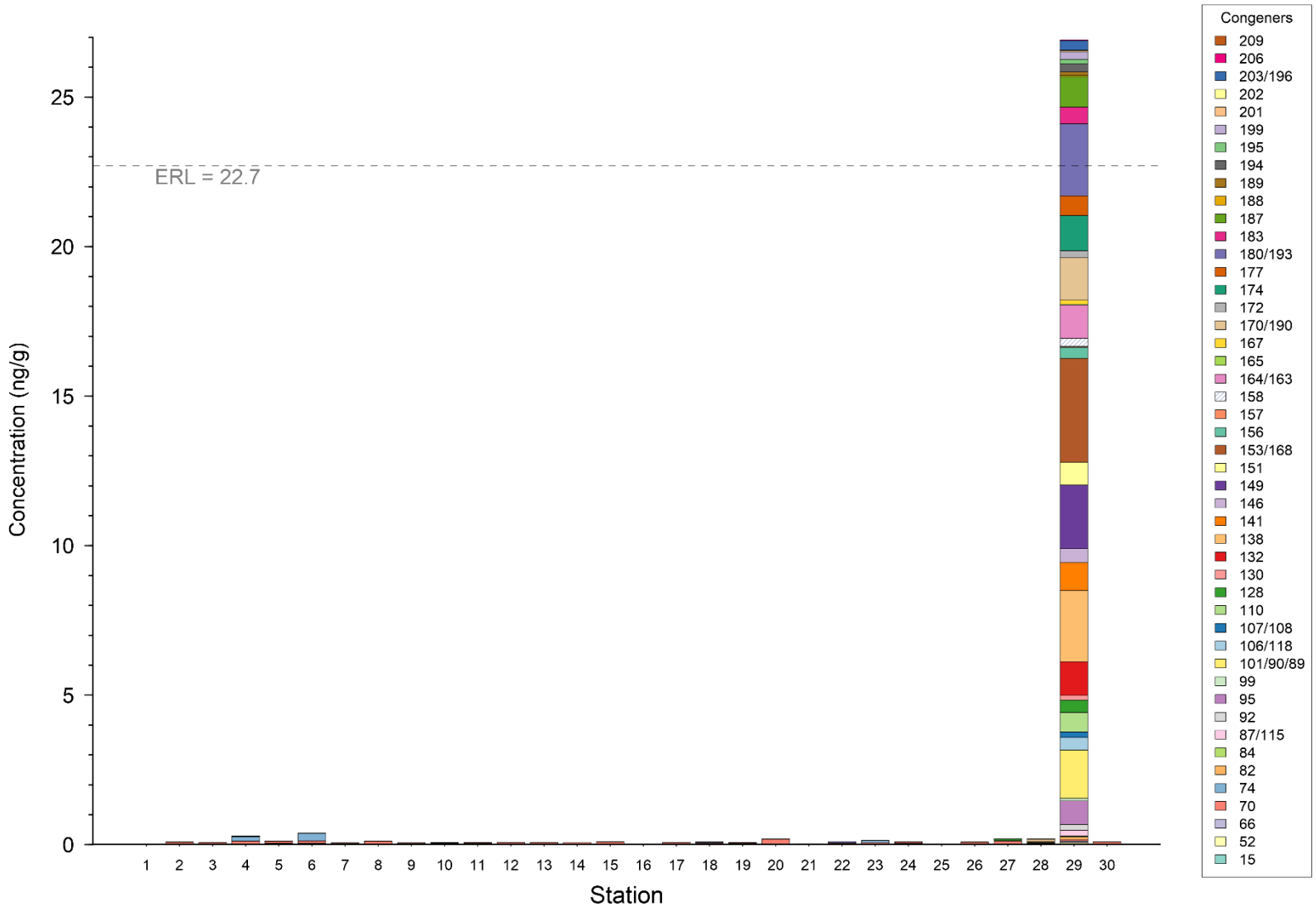

Figure 9. PCBs measured in sediments from 30 sites in FKNMS. Individual congeners are listed according to the International Union of Pure and Applied Chemists (IUPAC) numbering system. Overall bar height represents the total PCB concentration at each site.

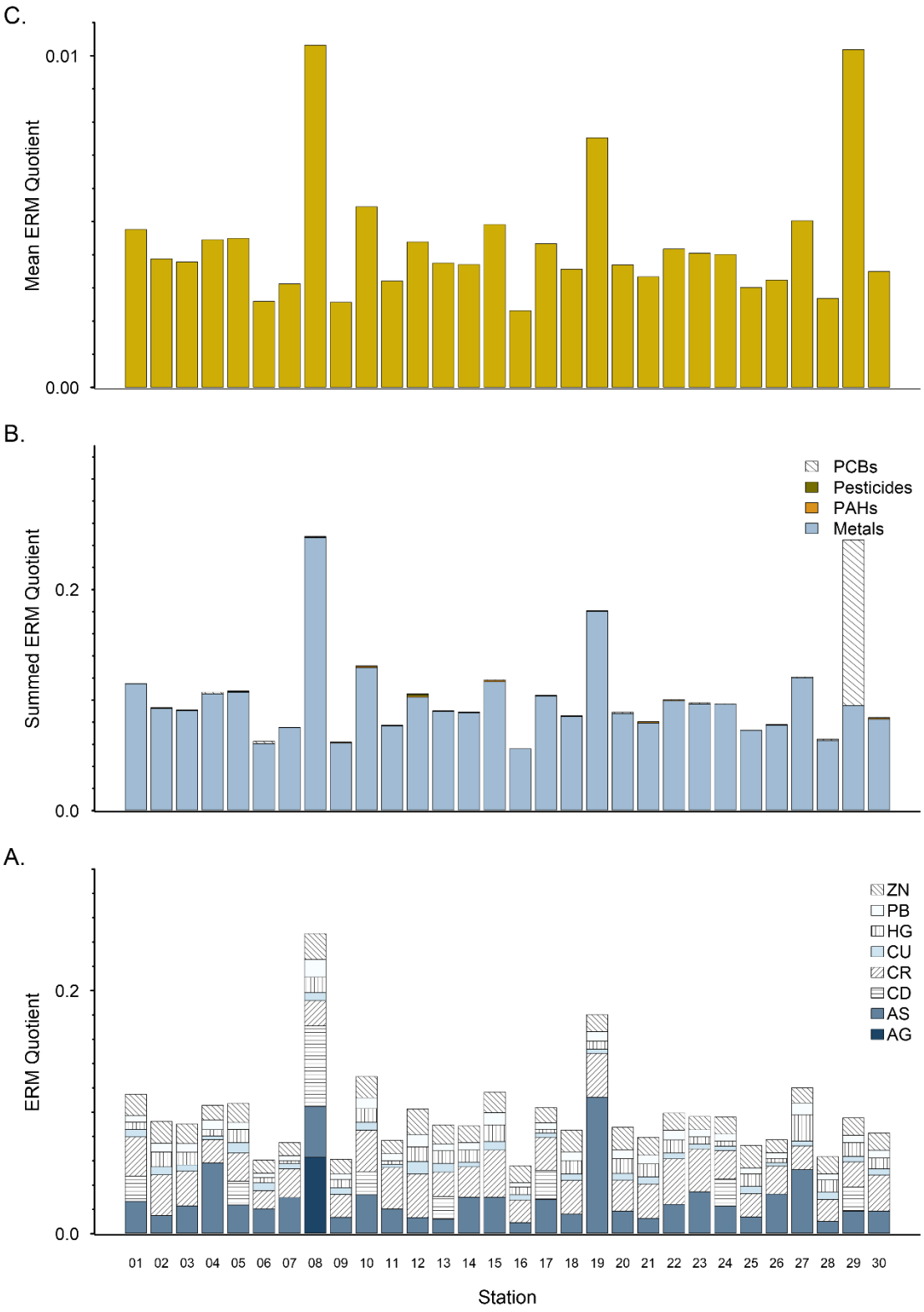

Figure 10. ERM quotients for eight individual trace metals (A), summed ERM quotients for four chemical contaminant classes (B), and overall mean ERM quotients calculated for sediments collected at 30 stations in FKNMS.

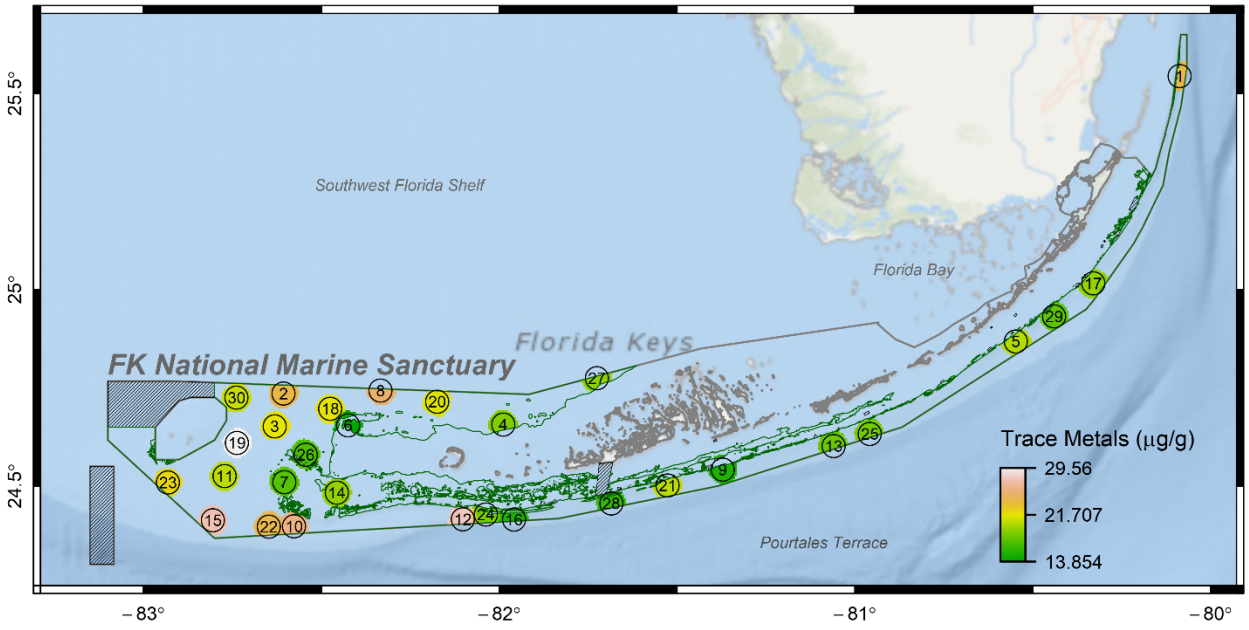

Figure 11a. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Trace metals (sum of silver, arsenic, cadmium, chromium, copper, mercury, lead, and zinc).

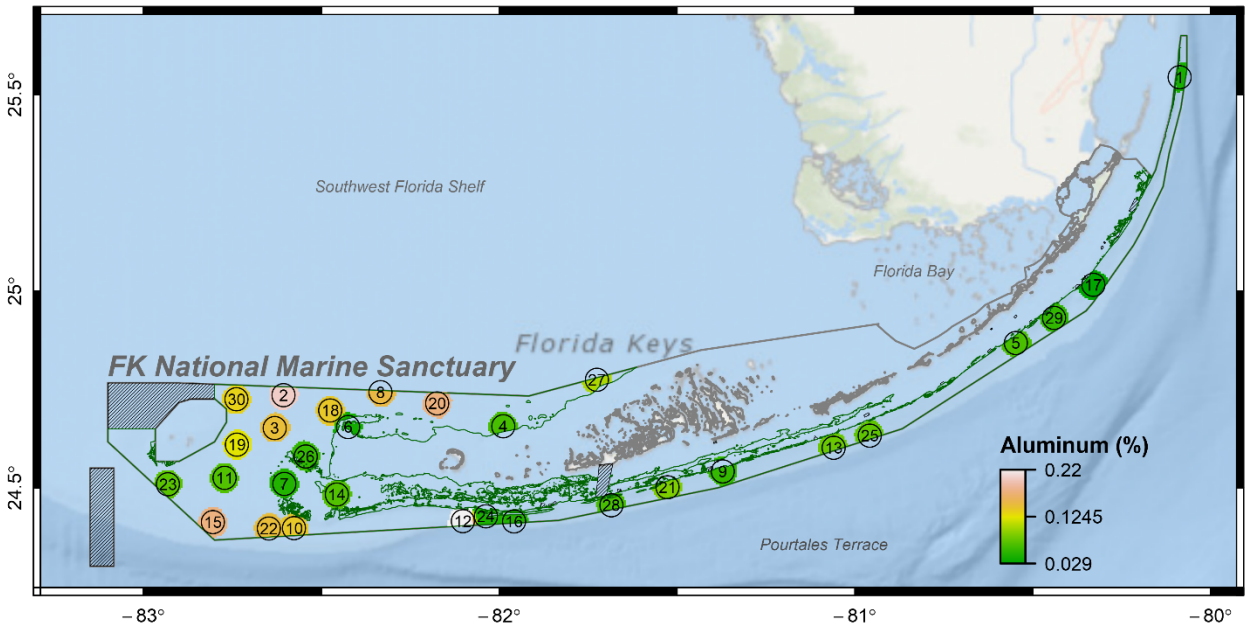

Figure 11b. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Aluminum.

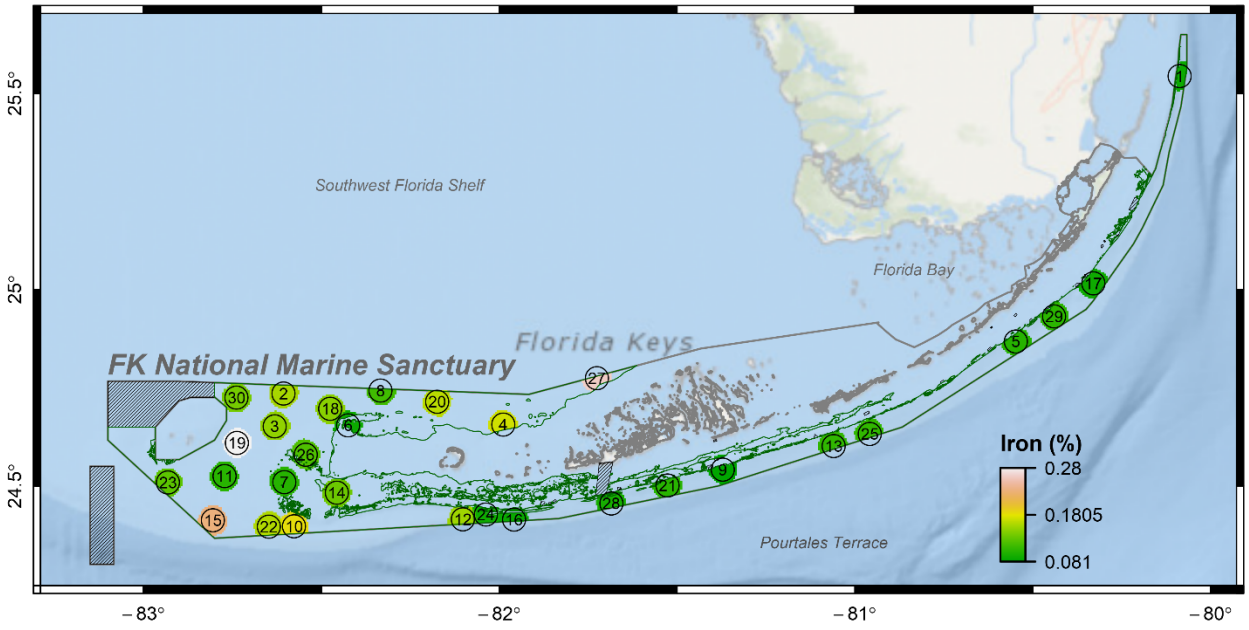

Figure 11c. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Iron.

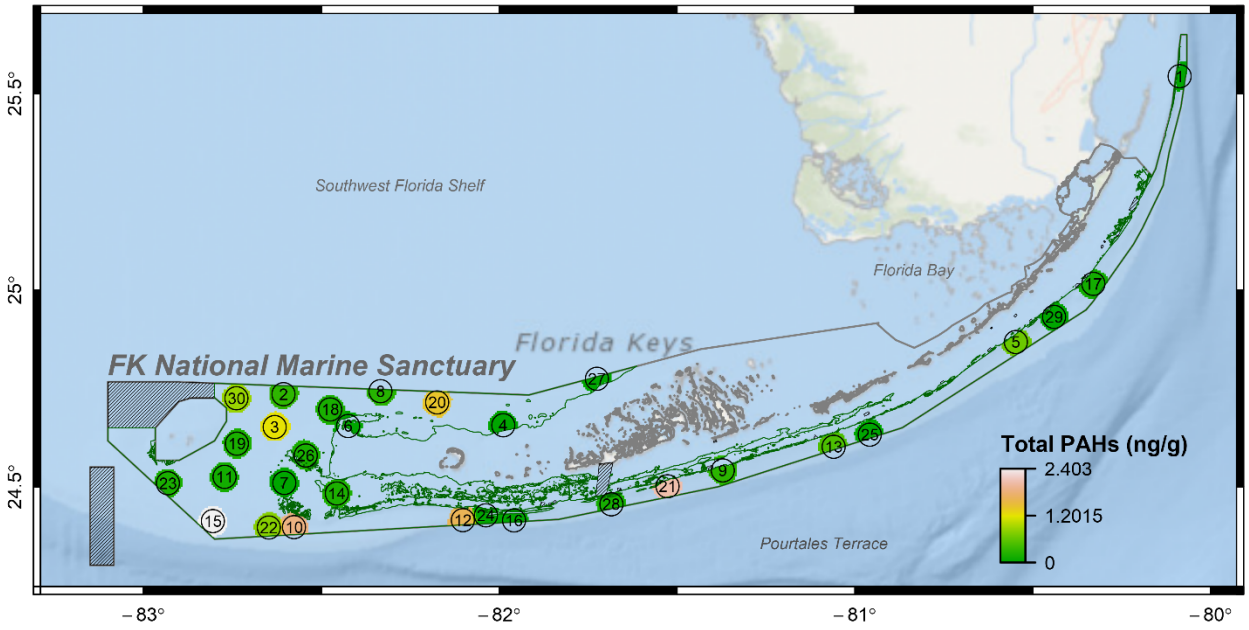

Figure 11d. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Total PAHs.

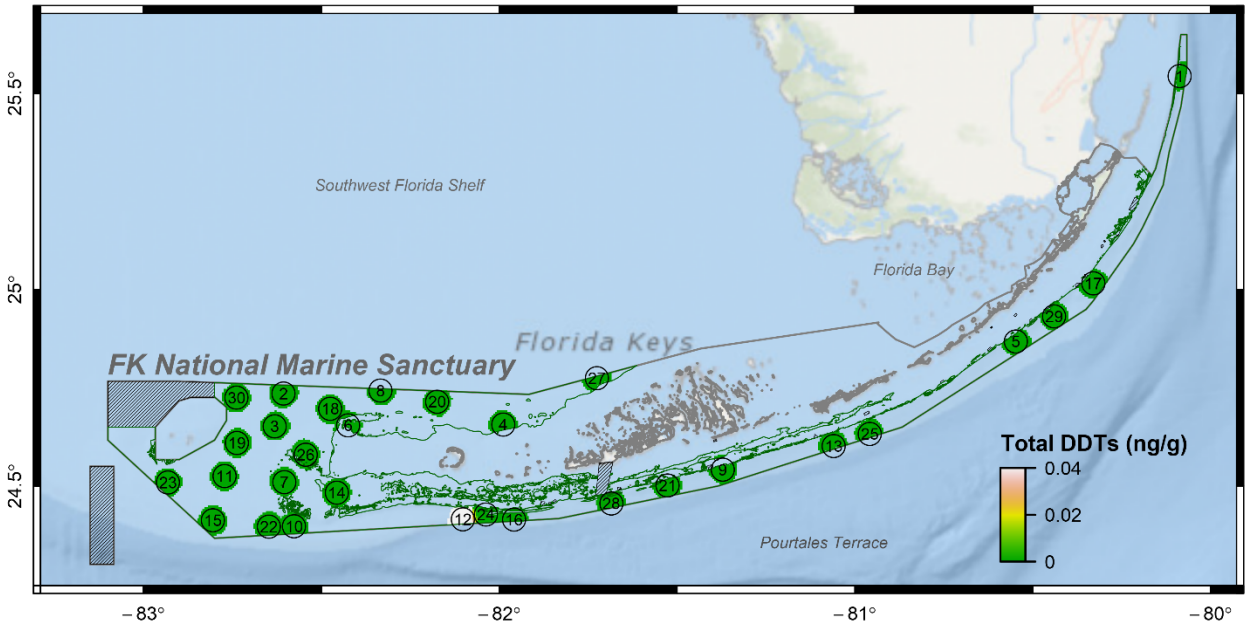

Figure 11e. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Total DDTs.

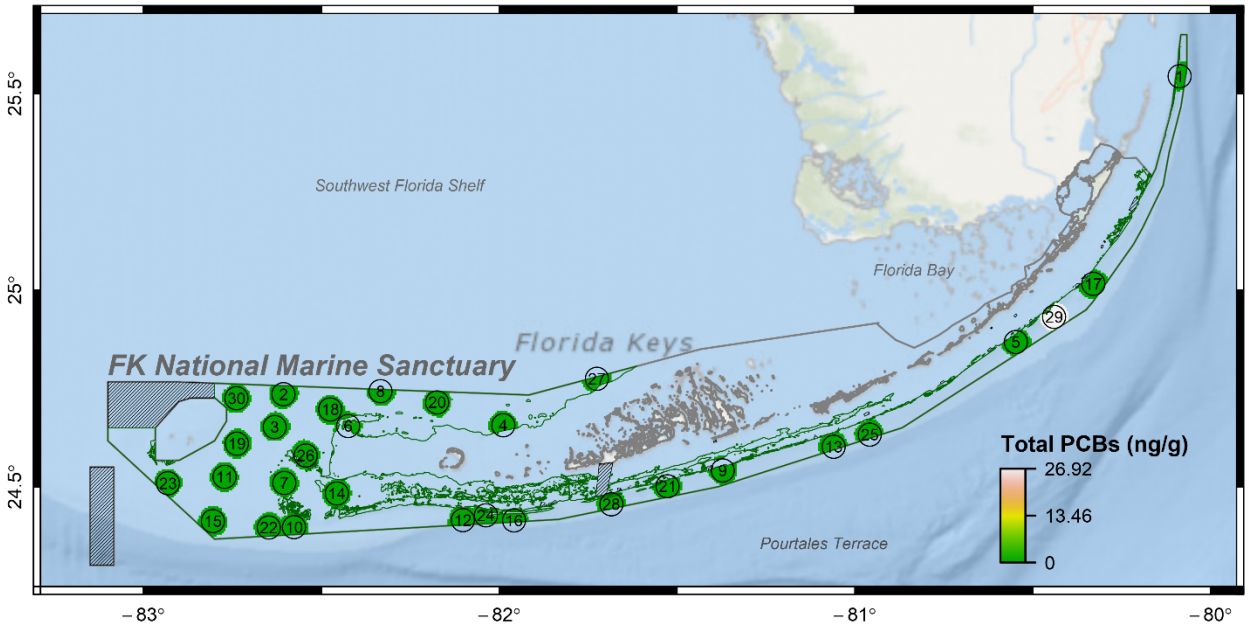

Figure 11f. Concentrations of chemical contaminants measured in sediments at 30 stations in FKNMS: Total PCBs.

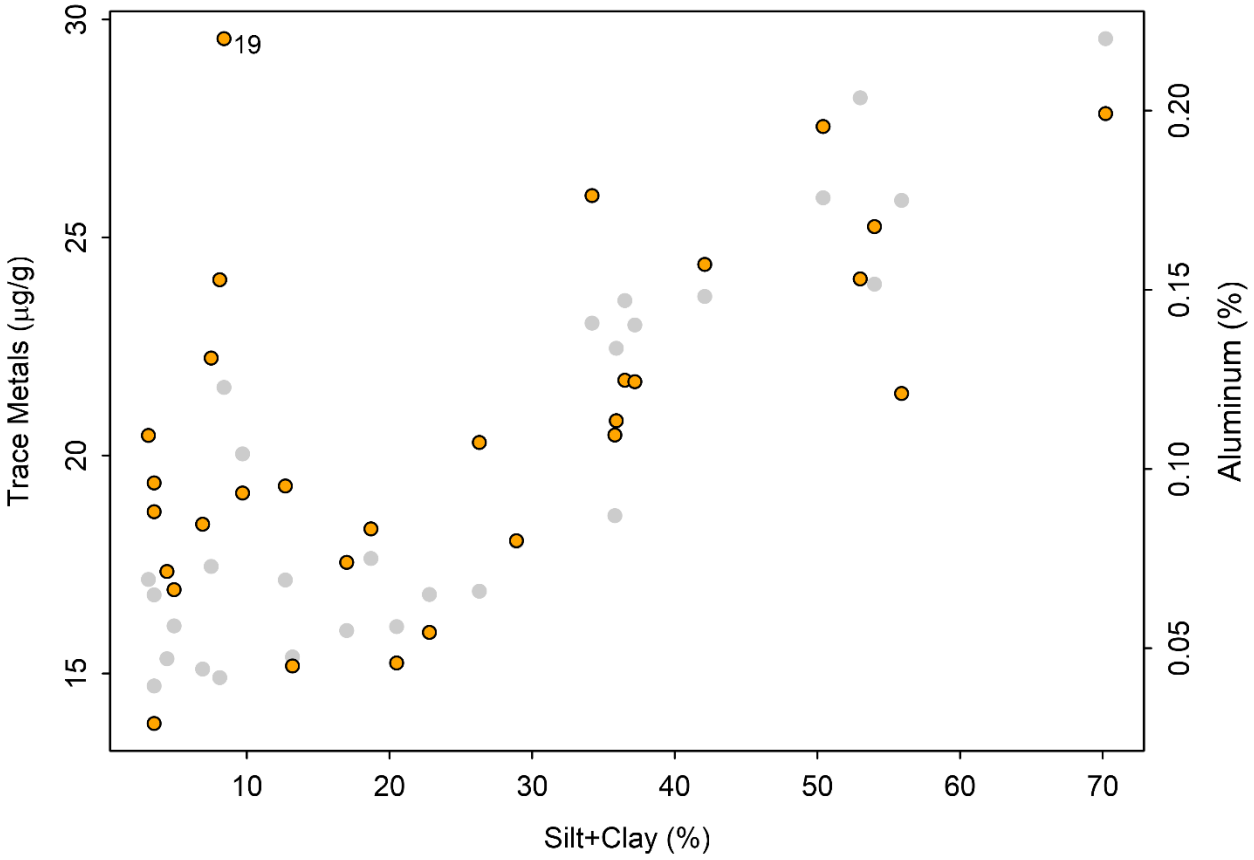

Figure 12. Trace metals (sum of silver, arsenic, cadmium, chromium, copper, mercury, lead, and zinc) versus percent silt+clay content of sediments at 30 sites in FKNMS (orange symbols). The relationship of trace metals to silt+clay is similar to that of aluminum (grey symbols), a naturally-occurring metal whose concentration is generally not influenced by anthropogenic inputs.

3.2.3 Sediment Toxicity

3.2.3.1 Microtox Assay

All 30 stations were identified as toxic by the Microtox solid-phase sediment toxicity assay. Following the criteria shown in Table 2 (Ringwood et al. 1997), samples were classified as toxic for EC_{50} < 0.5 % where silt+clay < 20%, or EC_{50} < 0.2 % for sediments with silt+clay $\geq$ 20 % (Figure 13).

Microtox hits at all 30 FKNMS stations and no co-occurring evidence of the presence of targeted stressors at high bioeffect levels suggest that results of this assay should be used with caution to avoid potential misinterpretations. The relationship between EC_{50} and percent silt+clay was statistically significant (generalized additive model, likelihood ratio test of adding smooth term for silt+clay, $p < 0.0001$). Some authors have noted that the Microtox® assay may be affected by the silt-clay content of sediments, and hence may yield false positive results (Sanger et al. 2018). If the bacteria adsorb onto clay particles and do not remain in the liquid phase, then light output would be reduced due to physical effects rather than toxicity (Ringwood et al. 1997). Benton et al. (1995) found that Microtox® toxicity of

clean sediments was significantly correlated with percent silt-clay content. Ringwood et al. (1997) also demonstrated the relationship between Microtox® EC_{50} and sediment silt-clay content using artificially prepared sediment mixtures composed of varying concentrations of sand and clay as well as natural sediments from uncontaminated reference sites. In comparative studies, the Microtox® assay gave a larger proportion of positive responses than lethality tests with *Rhepoxynius abronius* (Williams et al. 1986) or the freshwater cladoceran, *Daphnia magna* (Geisy et al. 1988). Such studies reveal potential over-sensitivity and a high risk of false-positive conclusions based on results of this assay alone. Moreover, because of uncertainty about the bioavailability of extracted chemicals and the irrelevance of bacterial bioluminescence to benthic ecosystems, any evidence of toxicity based on the Microtox® test may not necessarily reflect a potential for broader ecological degradation (Swartz 1989).

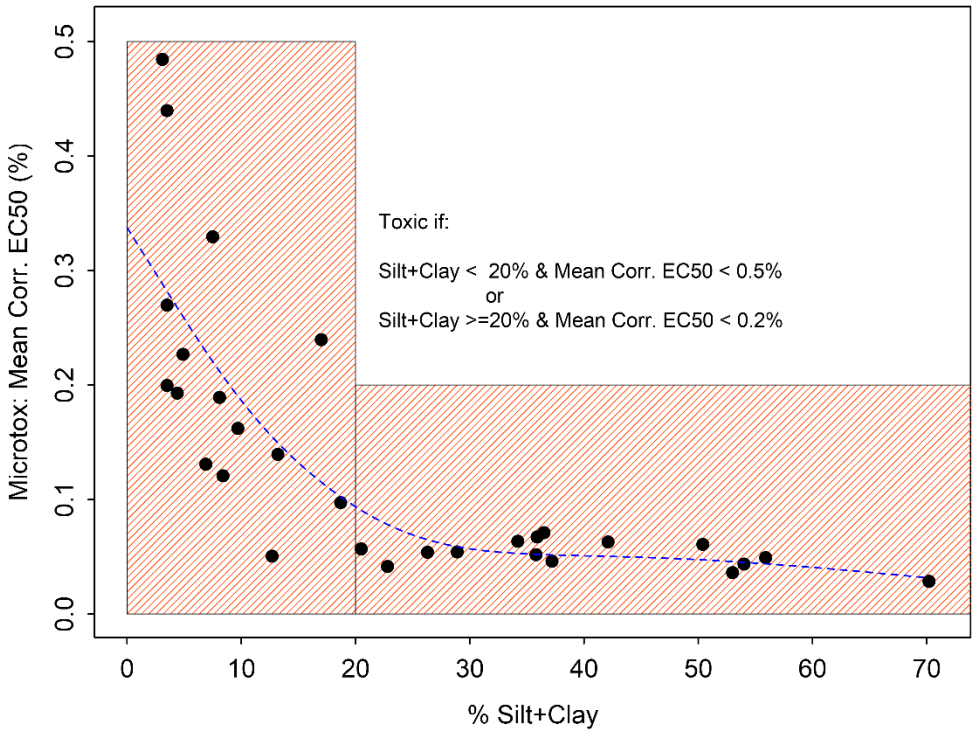

Figure 13. Relationship between Microtox mean corrected EC_{50} and sediment silt+clay content. The dark dashed line represents the fit from a generalized additive model using thin plate regression splines.

3.2.3.2 Reporter Gene Assay

There were no significant differences in reporter gene assay results (B[a]PEq) among stations. In the absence of reference data for this assay or other objective criteria for deciding whether or not a sample was toxic, reporter-gene assay results were not used to assess toxicity at these sampling sites. While there was a clear positive relationship between B[a]PEq and sediment percent silt+clay (Figure 14), results of regression were non-significant ($p = 0.151$) until removal of an apparent outlier (station 25), after which the regression was significant ($p = 0.004$). No significant correlation was observed between reporter gene assay results and Microtox® EC_{50}.

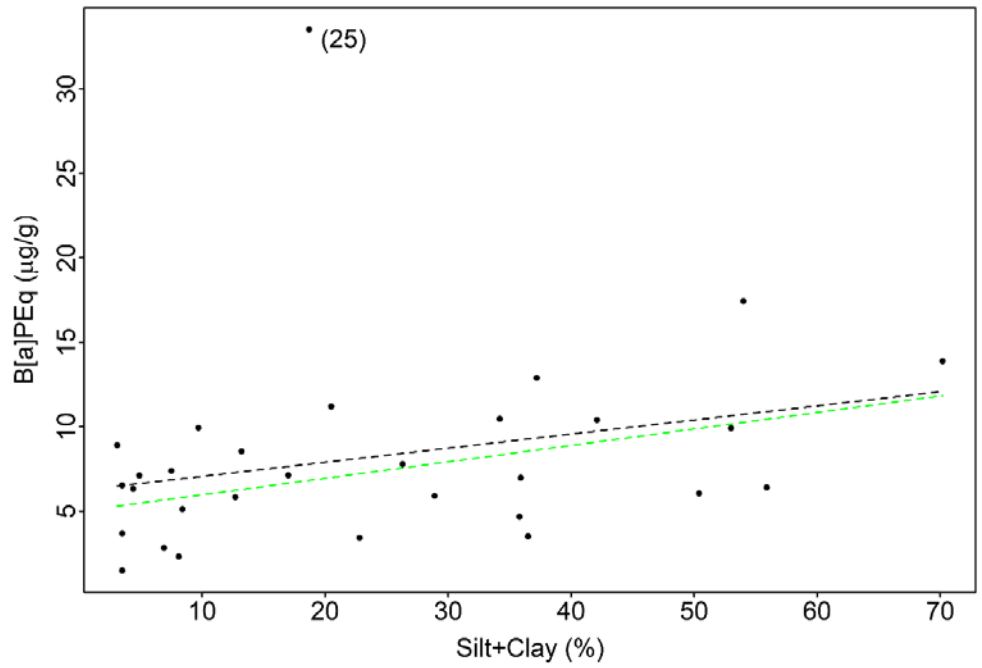

Figure 14. Plot of results of the reporter gene assay (B[a]PEq) versus percent silt+clay. The dark dashed line represents a linear regression fit using all 30 observations; the light green dashed line is from a regression excluding the possible outlier (station 25).

3.2.3.3 Sea Urchin Assay

Results of the 48 h sea urchin embryo development assay with *Lytechinus variegatus*, as well as measured concentrations of TAN and UAN, are shown in Table 8. Two samples (stations 17 and 24) were unavailable for analysis. Significant differences (compared to control) in arcsine square root-transformed mean percent normal development of *L. variegatus* exposed to site porewater were observed for stations 1, 5, 7, and 11 (Figure 15). Examples of *L. variegatus* 48 h embryo development following FKNMS sediment porewater treatment are shown in Figure 16. Despite the lack of normal development in sea urchin embryos at some sites, no significant correlations were observed between results of this assay and either the reporter gene or Microtox® assay. It is unlikely that the observed toxicity was due to porewater ammonia (UAN), given that all measured concentrations of UAN were well below the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) for *L. variegatus* (123.9 µg/L and 148.9 µg/L, respectively) obtained from the dose-response experiment described previously, and also below the reported LOEC of 90 µg/L for *Arbacia punctulata* (Carr et al. 1996b). Furthermore, none of the stations with significant toxicity had elevated levels of the chemical contaminants measured in this study.

Results of a single-step fractionation of porewater samples from stations exhibiting the highest levels of toxicity (stations 1 and 7) indicate that approximately 40 – 70 % of the toxicity is due to neutral analytes or compounds with aromatic or hydrophobic residues. This sorbent relies on three mechanisms of

retention: pi-pi bonding, hydrogen bonding (dipole-dipole interactions), and hydrophobic interaction. Examples of compounds retained on the stationary phase can include some pesticides, herbicides, steroid hormones, and certain pharmaceuticals, but the sorbent does not bind charged compounds, metals, or hydrophilic compounds.

Many benthic organisms produce and exude a wide variety of haloaromatic compounds (e.g., brominated phenols), presumably to inhibit recruitment by other species or for defensive purposes (Carr et al. 2006). Common polychaete species (e.g., capitellids, glycerids, cirratullids, pectinarids, spionids, terebellids, and nereids) have been shown to produce haloaromatics, which could be responsible for observed toxicity in sea urchin porewater toxicity tests (Fielman et al. 1999). Many of these polychaete species were present in the FKNMS samples (in fact, polychaetes were the dominant taxa overall, see benthic infauna section below). Polychaetes were dominant at three of the four stations with samples found to be toxic to sea urchin embryos; however, they were the dominant taxa at other stations as well. It is possible that the haloaromatics produced by these organisms may have at least contributed to the observed toxicity in the sea urchin development assay. It is possible, however, that toxicity was caused by other, unmeasured stressors or confounding factors.

Table 8. Results of sea urchin development assay using *Lytechinus variegatus* (% normal development, mean of four replicates), total ammonia nitrogen (TAN), and unionized ammonia nitrogen (UAN) in sediment porewater samples from FKNMS and control sample (Tropic Marin artificial seawater). Two samples (stations 17 and 24) were not available for the development assay.

Station	Mean % Normal Development	TAN (µg/L)	UAN (µg/L)
1	0.0	1,040	19.5
2	86.0	350	20.5
3	93.3	220	4.8
4	92.5	690	23.0
5	59.0	970	37.2
6	92.5	330	8.0
7	12.8	770	11.0
8	91.3	360	19.6
9	92.8	770	13.7
10	93.3	300	5.1
11	70.4	1,400	23.9
12	87.0	210	2.3
13	91.5	460	11.9
14	78.8	600	21.4
15	95.8	360	4.2
16	93.8	740	16.7
17	–	1,830	29.2
18	89.5	340	14.2
19	78.5	470	8.9
20	93.3	480	20.2
21	90.8	280	13.1
22	94.0	450	19.1
23	97.0	660	25.9
24	–	1,320	34.7
25	92.0	500	10.8
26	82.0	620	18.7
27	90.0	780	32.7
28	90.8	720	27.0
29	85.3	570	21.0
30	90.8	370	11.8
Control	93.5	48	3.1

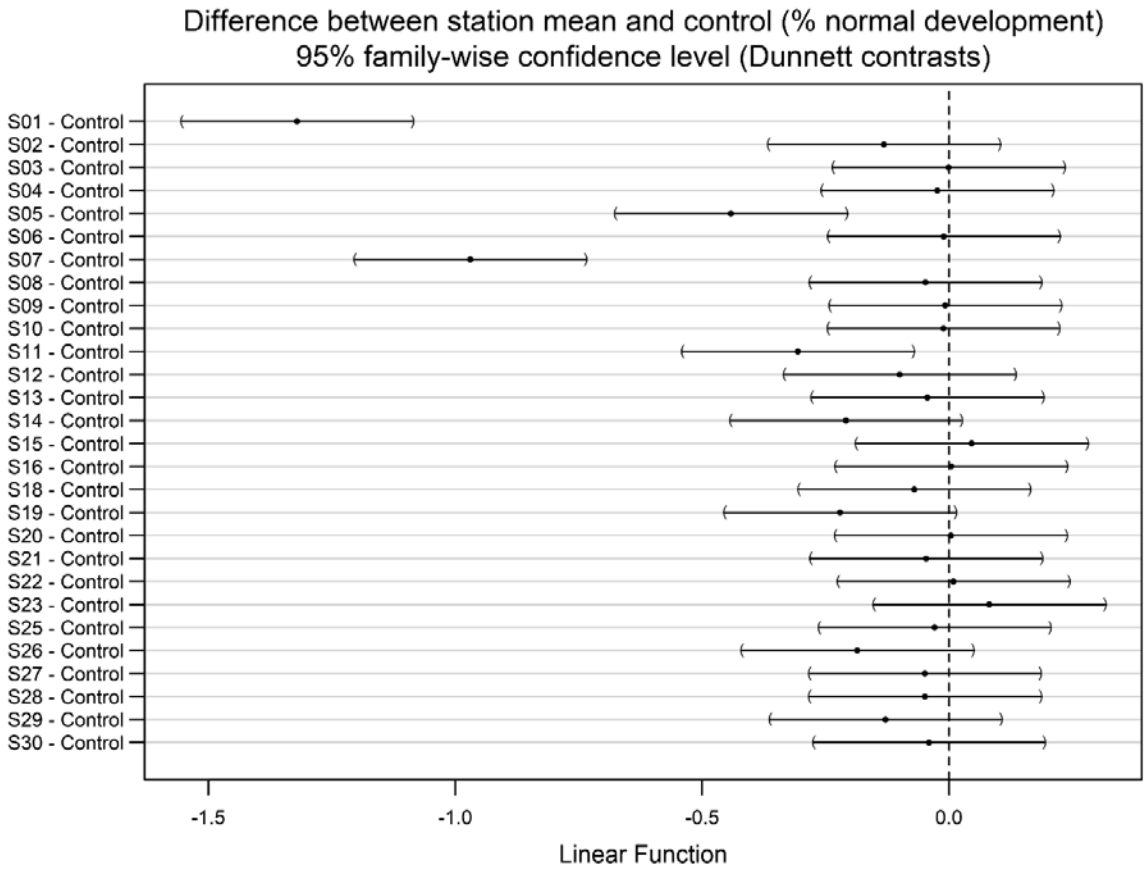

Figure 15. Results of Dunnett's multiple comparison test (comparison with control) for the sea urchin developmental assay. Comparisons having confidence intervals that do not include zero were significantly different from control.

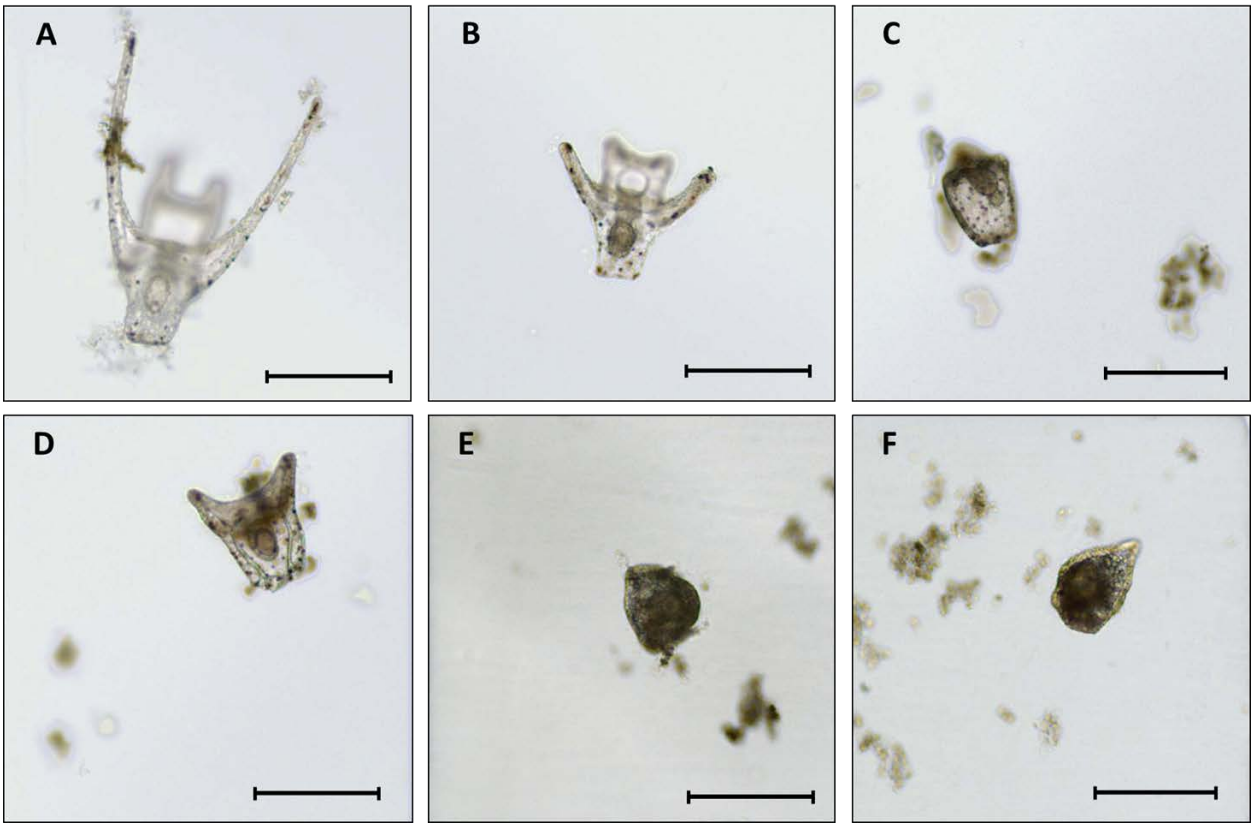

Figure 16. Examples of *Lytechinus variegatus* 48-h embryo development following FKNMS sediment porewater treatment. Panel A: Tropic Marin artificial sea water (negative control); Panel B: Sodium dodecyl sulfate, 4 mg/L (positive control); Panel C: Station 1; Panel D: Station 5; Panel E: Station 7; Panel F: Station 11. None of the remaining treatments were significantly different from the negative control. Scale bars = 200 µm, magnification = 100X.

3.3 Chemical Contaminants in Fish Tissues

Collection of fish specimens by hook-and-line fishing was successful at 22 of the 30 stations sampled in this study. At most four specimens of any given species from each station were retained, resulting in 52 individual specimens (representing six distinct species) that were analyzed for chemical contamination of tissues. Species retained for analysis and the corresponding stations where they were collected are displayed in Table 9.

Table 9. Finfish specimens retained for tissue chemical contaminant analysis.

Station	Common Name	Scientific Name	Number of Specimens
1	Lane snapper	*Lutjanus synagris*	2
3	Lane snapper	*Lutjanus synagris*	4
4	Sand perch	*Diplectrum formosum*	4
5	Grey triggerfish	*Balistes capriscus*	3
7	Yellowtail snapper	*Ocyurus chrysurus*	1
9	Lane snapper	*Lutjanus synagris*	2
10	Sand perch	*Diplectrum formosum*	3
11	Lane snapper	*Lutjanus synagris*	3
12	Blackline tilefish	*Caulolatilus cyanops*	1
14	Sand perch	*Diplectrum formosum*	3
16	Blackline tilefish	*Caulolatilus cyanops*	3
18	Lane snapper	*Lutjanus synagris*	3
19	Yellowtail snapper	*Ocyurus chrysurus*	3
20	Lane snapper	*Lutjanus synagris*	1
22	Sand perch	*Diplectrum formosum*	2
23	Yellowtail snapper	*Ocyurus chrysurus*	1
24	Sand tilefish	*Malacanthus plumieri*	1
25	Blackline tilefish	*Caulolatilus cyanops*	3
27	Sand perch	*Diplectrum formosum*	1
28	Blackline tilefish	*Caulolatilus cyanops*	4
29	Blackline tilefish	*Caulolatilus cyanops*	1
30	Sand perch	*Diplectrum formosum*	3

Concentrations of a suite of metals and organic compounds (PAHs, PBDEs, PCBs, and pesticides) were measured in edible tissues (homogenized, skin-on fillets) of fish specimens listed in Table 9. Multiple specimens of distinct species collected at a station were combined into a single composite sample prior to homogenization. Contaminants in fish tissues were present at detectable levels for 17 of 22 trace metals, 10 of 28 PAHs, 1 of 14 PBDEs, 2 of 86 PCB congeners, and 11 of 25 pesticides measured. Mean concentrations (and one standard error) of metals, PAHs, PCBs, and DDTs, averaged across the 22 stations where fish were caught are illustrated in Figure 17 for each of the six fish species.

Tissue contaminant levels were compared to risk-based EPA advisory guidelines for recreational fishers (Table 4). These guidelines set recommended consumption limits based on concentration ranges of a number of contaminants with respect to risk of cancer and non-cancer (chronic systemic) human-health effects. The results are summarized in Table 10. Concentrations of inorganic arsenic (estimated as 2 % of total arsenic) in edible fish tissues (skin-on fillets) fell within the range of values for which the USEPA (2000) suggests limiting consumption to four fish meals per week at one site (station 5). Levels of mercury (assumed to be all methylmercury) in fish tissues fell within guidance limits for methylmercury at eight stations (3, 4, 10, 11, 16, 20, 25, 28); concentrations exceeded the upper limit of the guidance range for methylmercury at an additional eight stations (1, 9, 14, 18, 19, 23, 24, 30). Investigators have found the proportion of methylmercury in fish muscle tissue typically to be 80 – 100 % of total mercury (Andersen and Deplege 1997, Wagemann et al. 1997, Kannan et al. 1998).

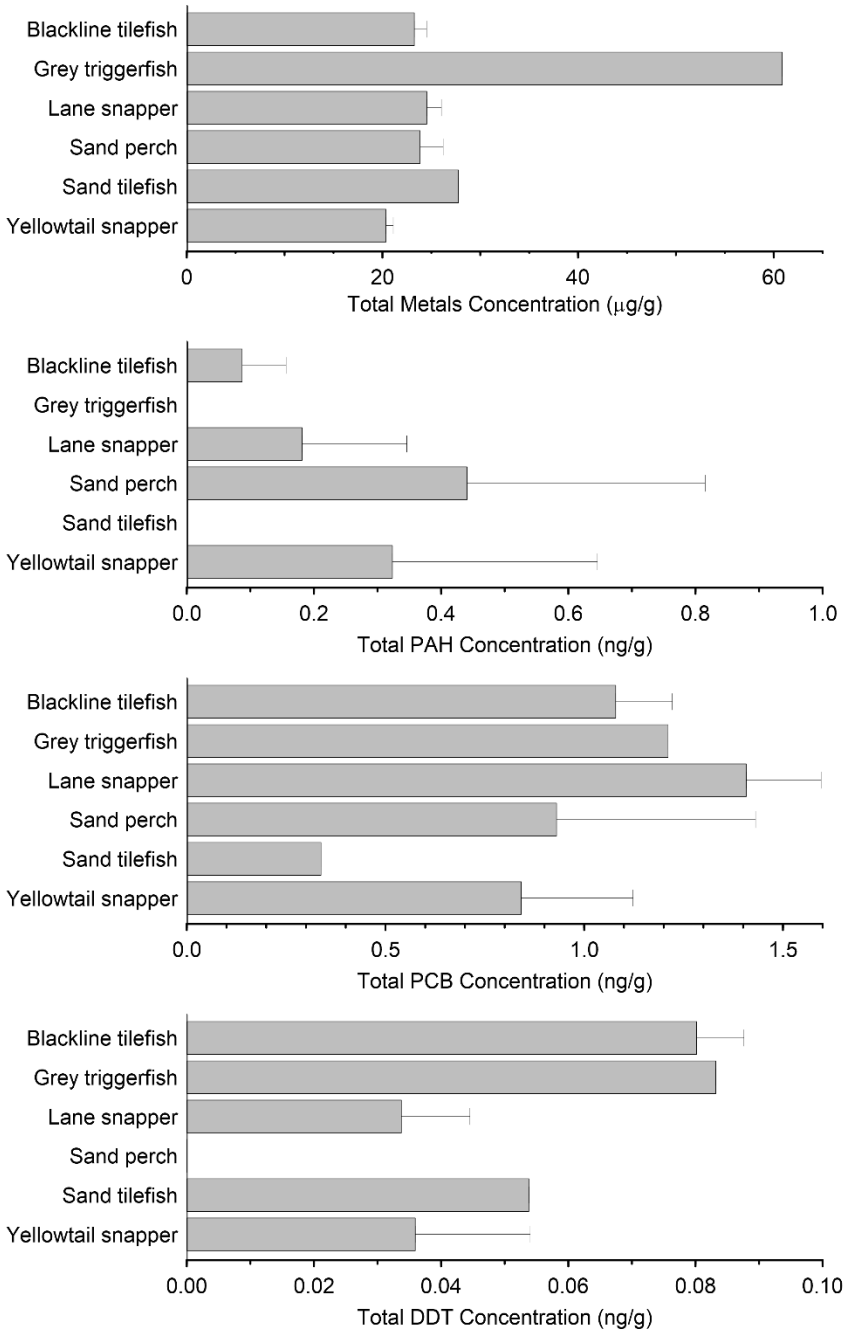

Figure 17. Mean (plus one standard error) of total metals, PAH, PCB, and DDT concentrations measured in each of six finfish species collected in FKNMS.

Table 10. Summary of contaminant concentrations (wet weight) measured in fish tissues. A total of 52 fish from 22 stations were analyzed. All measured contaminants are included. Concentrations are compared to human-health guidelines where available (from U.S. EPA 2000, also see Table 4 herein).

			No. of fish	
Analyte	Mean	Range	> Lower, ≤ Upper	> Upper
Silver	0.000	0.000 – 0.000	-	-
Aluminum	5.121	2.028 – 14.062	-	-
Arsenic	3.424	0.726 – 20.067	-	-
Inorganic Arsenic[a]	0.068	0.015 – 0.401	1	0
Barium	0.109	0.015 – 0.904	-	-
Beryllium	0.000	0.000 – 0.000	-	-
Cadmium	0.000	0.000 – 0.000	0	0
Cobalt	0.017	0.000 – 0.050	-	-
Chromium	0.411	0.140 – 0.679	-	-
Copper	0.246	0.148 – 0.343	-	-
Iron	7.441	4.583 – 13.204	-	-
Mercury[b]	0.204	0.051 – 0.405	8	8
Lithium	0.028	0.000 – 0.098	-	-
Manganese	0.313	0.083 – 1.848	-	-
Nickel	0.087	0.000 – 0.320	-	-
Lead	0.028	0.000 – 0.172	-	-
Antimony	0.000	0.000 – 0.000	-	-
Selenium	0.866	0.433 – 1.440	0	0
Tin	0.010	0.000 – 0.024	-	-
Thallium	0.000	0.000 – 0.000	-	-
Uranium	0.000	0.000 – 0.000	-	-
Vanadium	0.306	0.015 – 0.985	-	-
Zinc	6.729	4.243 – 22.480	-	-
2,6+2,7-Dimethylnaphthalene	0.000	0.000 – 0.000	-	-
Acenaphthene	0.000	0.000 – 0.000	-	-
Acenaphthylene	0.000	0.000 – 0.000	-	-
Anthracene	0.000	0.000 – 0.000	-	-
Benz[a]anthracene	0.008	0.000 – 0.089	-	-
Benzo[a]pyrene	0.007	0.000 – 0.077	-	-
Benzo[e]pyrene	0.000	0.000 – 0.000	-	-
Benzo[a]fluoranthene	0.000	0.000 – 0.000	-	-
Benzo[b]fluoranthene	0.018	0.000 – 0.157	-	-
Benzo[j]fluoranthene	0.006	0.000 – 0.072	-	-
Benzo[k]fluoranthene	0.010	0.000 – 0.083	-	-
Benzo[g,h,i]perylene	0.000	0.000 – 0.000	-	-
Biphenyl	0.000	0.000 – 0.000	-	-
Chrysene+Triphenylene	0.020	0.000 – 0.161	-	-
Dibenz[a,h]anthracene	0.000	0.000 – 0.000	-	-
Dibenzothiophene	0.000	0.000 – 0.000	-	-
Fluoranthene	0.011	0.000 – 0.231	-	-
Fluorene	0.000	0.000 – 0.000	-	-
Indeno[1,2,3-c,d]pyrene	0.006	0.000 – 0.116	-	-
1-Methylnaphthalene	0.000	0.000 – 0.000	-	-
2-Methylnaphthalene	0.000	0.000 – 0.000	-	-
1-Methylphenanthrene	0.000	0.000 – 0.000	-	-
Naphthalene	0.000	0.000 – 0.000	-	-
Perylene	0.000	0.000 – 0.000	-	-
Phenanthrene	0.000	0.000 – 0.000	-	-
Pyrene	0.000	0.000 – 0.000	-	-
Retene	0.136	0.000 – 1.863	-	-
1,6,7-Trimethylnaphthalene	0.000	0.000 – 0.000	-	-

Analyte	Mean	Range	No. of fish > Lower, ≤ Upper	No. of fish > Upper
TOT_PAH	0.223	0.000 – 1.923	-	-
Total PBDEs	0.006	0.000 – 0.035	-	-
TOT_PCB	1.074	0.029 – 2.842	0	0
Aldrin	0.000	0.000 – 0.000	-	-
alpha-Hexachlorocyclohexane	0.003	0.000 – 0.055	-	-
alpha-Chlordane	0.000	0.000 – 0.000	-	-
beta-Hexachlorocyclohexane	0.016	0.000 – 0.168	-	-
Chlorpyrifos	0.000	0.000 – 0.000	-	-
cis-Nonachlor	0.000	0.000 – 0.000	-	-
DDD	0.000	0.000 – 0.000	-	-
2,4'-DDD (o,p'-DDD)	0.000	0.000 – 0.000	-	-
4,4'-DDD (p,p'-DDD)	0.000	0.000 – 0.000	-	-
DDE	0.040	0.000 – 0.097	-	-
2,4'-DDE (o,p'-DDE)	0.000	0.000 – 0.000	-	-
4,4'-DDE (p,p'-DDE)	0.040	0.000 – 0.097	-	-
DDT	0.000	0.000 – 0.000	-	-
2,4'-DDT (o,p'-DDT)	0.000	0.000 – 0.000	-	-
4,4'-DDT (p,p'-DDT)	0.000	0.000 – 0.000	-	-
Dieldrin	0.000	0.000 – 0.000	0	0
Endosulfan I	0.000	0.000 – 0.000	-	-
Endosulfan II (Beta-Endosulfan)	0.000	0.000 – 0.000	0	0
Endrin	0.000	0.000 – 0.000	-	-
Endosulfan sulfate	0.000	0.000 – 0.000	-	-
gamma-Chlordane	0.000	0.000 – 0.004	-	-
Hexachlorobenzene (HCB)	0.000	0.000 – 0.000	0	0
Heptachlor	0.021	0.000 – 0.061	-	-
Heptachlor epoxide	0.000	0.000 – 0.000	0	0
gamma-Hexachlorocyclohexane	0.000	0.000 – 0.000	0	0
Mirex	0.000	0.000 – 0.004	0	0
Oxychlordane	0.005	0.000 – 0.027	-	-
TOT_CHL	0.012	0.000 – 0.041	0	0
TOT_DDT	0.040	0.000 – 0.097	0	0
trans-Nonachlor	0.006	0.000 – 0.016	-	-

3.4 Status of Benthic Communities

Macrobenthic infauna (those retained on a 0.5-mm sieve) were collected at all 30 stations. Two grabs (0.04 m^2 each) were collected at each station, resulting in a total of 60 grabs. Measures of taxonomic diversity and abundance were calculated separately for each of the 60 grabs and averaged by station where indicated in Table 11 (e.g., mean # taxa/0.04 m^2, mean H'/0.04 m^2). The resulting data were used to assess the status of benthic community characteristics (taxonomic composition, diversity, abundance, and dominant taxa), the incidence of non-indigenous species, and potential linkages to ecosystem stressors throughout the FKNMS study area.

Table 11. Mean, range, and selected distributional properties of key benthic variables. The benthic measures represent 60 0.04-m^2 grabs collected at 30 sites (2 replicate grabs at each station) in FKNMS.

				Area-based Percentiles			Frequency-based Percentiles				
Parameter	Overall Mean	Overall Range		CDF 10^{th} pctl	CDF 50^{th} pctl	CDF 90^{th} pctl	10^{th}	25^{th}	50^{th}	75^{th}	90^{th}
Total # Taxa/0.08 m^2	111	37 –	167	58	114	150	60	87	115	145	153
Mean # Taxa/0.04 m^2	73	23 –	113	37	71	106.5	38.5	57.5	71.8	93	107.8
Mean Density (#/m^2)	7,041	1,062 –	13,075	2,412	5,937	12,525	2,719	4,475	5,950	10,137	12,581
Mean H/0.04 m^2	5.1	2.9 –	6	4.3	5.1	5.7	4.3	4.8	5.2	5.5	5.7

3.4.1 Taxonomic Composition

A total of 763 taxa were identified throughout the study area, of which 570 were identified to the species level. Annelida was the dominant phylum, both by percent of total number of taxa (47.8 %) and percent of total density (60.7 %). The overwhelming majority of annelids was represented by polychaetes (99 % of annelid taxa and 96 % of annelid abundance), while the remaining annelids were oligochaetes. Arthropods made up 30.9 % of taxa and 18.1 % total density; of these, crustaceans made up 97 % and 96 % of arthropod taxa and density, respectively. Molluscs represented 18.1 % of taxa and 10.5 % abundance. Although bivalves made up slightly more than half (54 %) of molluscan taxa (gastropods 41 %), they represented the majority of molluscan abundance (bivalves and gastropods 81.6 % and 12.8 % of molluscan abundance, respectively). Echinoderms made up less than 2 % of total taxa (and density), consisting mainly of brittle stars (Ophiuroidea) and urchins (Echinoidea), as well as some starfish (Echinoidea) and sea cucumbers (Holothuroidea). The 'Other' category included members of phyla Nemertea, Sipuncula, Cnidaria, and a few Brachiopoda, Phoronida, Hemichordata, and some Chordata (e.g., lancelets, *Branchiostoma* sp.). Most of the 'Other' taxa were identified only to higher taxonomic level and so represented a small percentage of total taxa (Figure 18, Table 12), although they constituted nearly 10 % of total density.

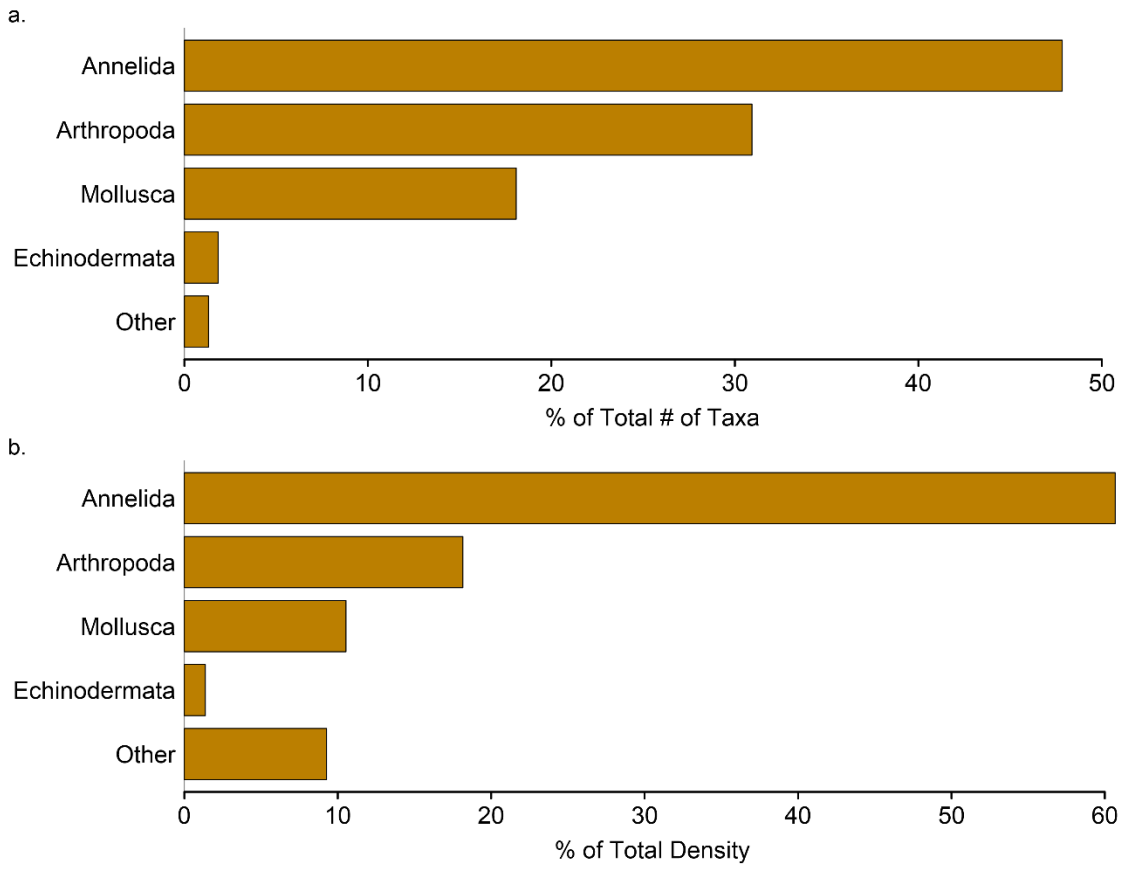

Figure 18. Taxonomic composition of benthic infauna as (a) percent of total number of taxa and (b) percent of total density.

Table 12. Summary of major taxonomic groups of benthic infauna and corresponding numbers of identifiable taxa based on 60 0.04-m^2 grab samples.

Taxonomic Group	Number identifiable taxa	% Total identifiable taxa
Phylum Annelida		
Class Polychaeta	363	47.6
Class Clitellata	2	0.3
Phylum Arthropoda		
Subphylum Chelicerata		
Class Pycnogonida	6	0.8
Subphylum Crustacea		
Class Malacostraca		
Order Amphipoda	111	14.5
Order Cumacea	27	3.5
Order Decapoda	45	5.9
Order Isopoda	17	2.2
Order Leptostraca	1	0.1
Order Mysida	2	0.3
Order Stomatopoda	3	0.4
Order Tanaidacea	23	3.0
Subphylum Hexapoda		
Class Insecta	1	0.1
Phylum Brachiopoda*	1	0.1
Phylum Chordata*	2	0.3
Phylum Cnidaria*	1	0.1
Phylum Echinodermata		
Class Asteroidea	1	0.1
Class Echinoidea	6	0.8
Class Holothuroidea	1	0.1
Class Ophiuroidea	6	0.8
Phylum Hemichordata*	1	0.1
Phylum Mollusca		
Class Bivalvia	75	9.8
Class Caudofoveata	1	0.1
Class Gastropoda	57	7.5
Class Polyplacophora	2	0.3
Class Scaphopoda	3	0.4
Phylum Nemertea*	1	0.1
Phylum Phoronida*	1	0.1
Phylum Platyhelminthes*	1	0.1
Phylum Sipuncula*	2	0.3
Total	*763*	*100*

3.4.2 Abundance and Dominant Taxa

A total of 16,903 individuals were collected across the 30 stations (60, 0.04 m^2 grabs) sampled for benthos. Mean densities at each site ranged from 1,062 – 13,080 ind/m^2 and averaged 7,041 ind/m^2 (Table 11, Appendix E). On an area-weighted basis, 10 % of the survey area (lower 10^{th} percentile) had mean densities $\leq$ 2,412 ind/m^2 and 50 % of the area had mean densities $\leq$ 5,937 ind/m^2 (Table 11, Figure 19).

The 50 most abundant taxa collected in the FKNMS study area are listed in Table 13. The top 10 dominants, in decreasing order of abundance, included the sabellid (Family Sabellidae) polychaete *Fabricinuda trilobata*; the syllid (Family Syllidae) polychaete *Haplosyllis spongicola*; members of Phylum Nemertea ('ribbon worms'); members of the spionid polychaete genus *Prionospio*; Phylum Sipuncula ('peanut worms'); members of Subclass Oligochaeta; the pilargid (Family Pilargidae) polychaete *Litocorsa ewingi*; the spionid polychaete *Prionospio cristata*; members of the peanut worm Family Aspidosiphonidae (LPIL); and the sabellid polychaete *Galathowenia oculata*.

Table 13. Fifty most abundant benthic taxa. Mean density (#/m^2), and percent frequency of occurrence are based on 60 0.04-m^2 grabs.

Taxon	Group	Density	Frequency (% of samples)
Fabricinuda trilobata	Annelida	14500	70.0
Haplosyllis spongicola	Annelida	12200	18.3
Nemertea	Other	7338	93.3
Prionospio (LPIL)	Annelida	7250	68.3
Sipuncula (LPIL)	Other	5200	85.0
Oligochaeta	Annelida	4663	65.0
Litocorsa ewingi	Annelida	4400	65.0
Prionospio cristata	Annelida	4050	66.7
Aspidosiphonidae (LPIL)	Other	3938	73.3
Galathowenia oculata	Annelida	3763	63.3
Exogone dispar	Annelida	3188	68.3
Maldanidae (LPIL)	Annelida	2838	70.0
Psammokalliapseudes granulosus	Arthropoda	2813	21.7
Netamelita brocha	Arthropoda	2750	23.3
Parvilucina crenella	Mollusca	2575	48.3
Gammaropsis (LPIL)	Arthropoda	2275	15.0
Notomastus (LPIL)	Annelida	2250	73.3
Parvilucina pectinella	Mollusca	2125	25.0
Ophiopsila vittata	Echinodermata	1875	58.3
Chone (LPIL)	Annelida	1800	51.7
Lumbrineridae (LPIL)	Annelida	1750	31.7
Prionospio sp. A	Annelida	1713	40.0
Alloleptochelia longimana	Arthropoda	1675	46.7
Sabellidae (LPIL)	Annelida	1638	60.0
Xenanthura brevitelson	Arthropoda	1625	46.7
Armandia maculata	Annelida	1600	53.3
Sphaerosyllis perkinsi	Annelida	1550	53.3
Anthozoa (LPIL)	Other	1500	46.7
Arichlidon gathofi	Annelida	1475	45.0
Syllidae (LPIL)	Annelida	1388	53.3
Abra aequalis	Mollusca	1375	61.7
Ameritella sybaritica	Mollusca	1325	45.0
Sigambra tentaculata	Annelida	1250	48.3
Paradialychone americana	Annelida	1188	18.3
Chironomidae	Arthropoda	1188	38.3
Goniadides carolinae	Annelida	1175	16.7
Capitellidae (LPIL)	Annelida	1163	53.3
Crassinella dupliniana	Mollusca	1150	15.0
Thyasira trisinuata	Mollusca	1125	15.0
Gouldia cerina	Mollusca	1125	48.3
Lembos (LPIL)	Arthropoda	1125	46.7
Prionospio fallax	Annelida	1088	35.0
Bispira melanostigma	Annelida	1088	33.3
Ceratocephale oculata	Annelida	1063	45.0
Paramicrodeutopus myersi	Arthropoda	1038	11.7
Dentatisyllis carolinae	Annelida	950	31.7
Protodorvillea kefersteini	Annelida	888	31.7
Scyphoproctus platyproctus	Annelida	875	38.3
Scoletoma (LPIL)	Annelida	875	43.3
Bivalvia (LPIL)	Mollusca	850	48.3

3.4.3 Diversity

A total of 763 taxa were identified (570 to species) in 60 grabs collected throughout the study area. Means, ranges, and other distributional properties are displayed in Table 11, with the full distribution of area-weighted estimates illustrated in Figure 19. Taxonomic richness, expressed as the mean number of taxa present in replicate 0.04 m^2 grabs at a station, ranged from 23 to 113 taxa/grab, with an overall mean of 73 taxa/grab. Fifty % of the survey area had at least 71 taxa/grab and 10 % of the survey area had > 107 taxa/grab (Table 11). Shannon H' diversity (base-2 logarithms) varied between 2.9 and 6 (mean of 5.1), and was inversely correlated with sediment percent fines (i.e., percent silt+clay; Table 11, Figure 20).

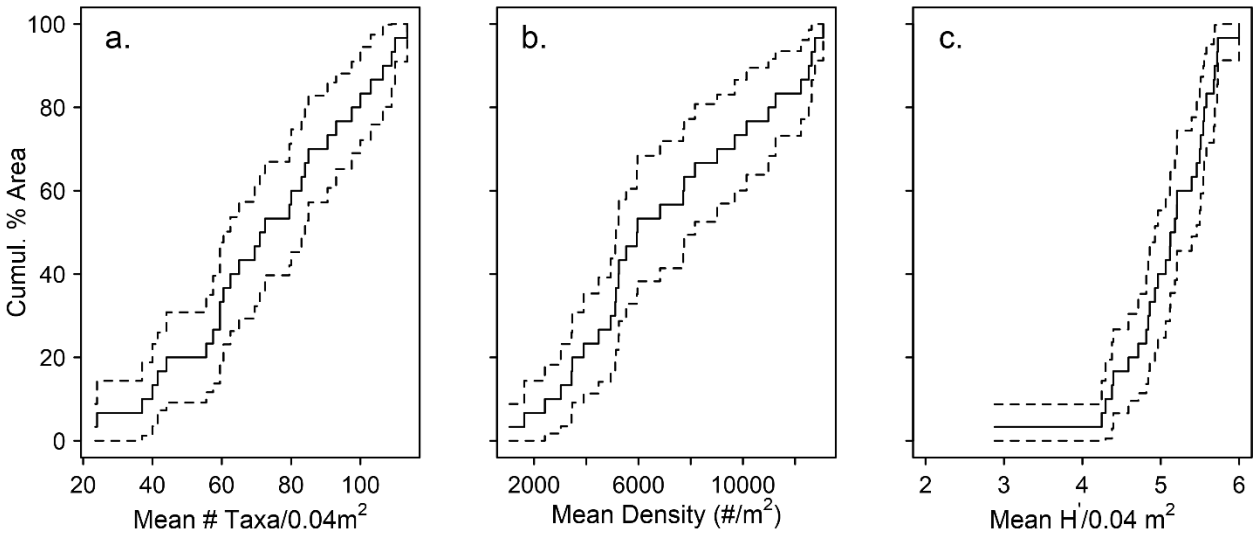

Figure 19. Percent area (and 95% confidence intervals) of FKNMS study area vs. benthic infaunal taxonomic richness (a), density (b), and H' diversity (c).

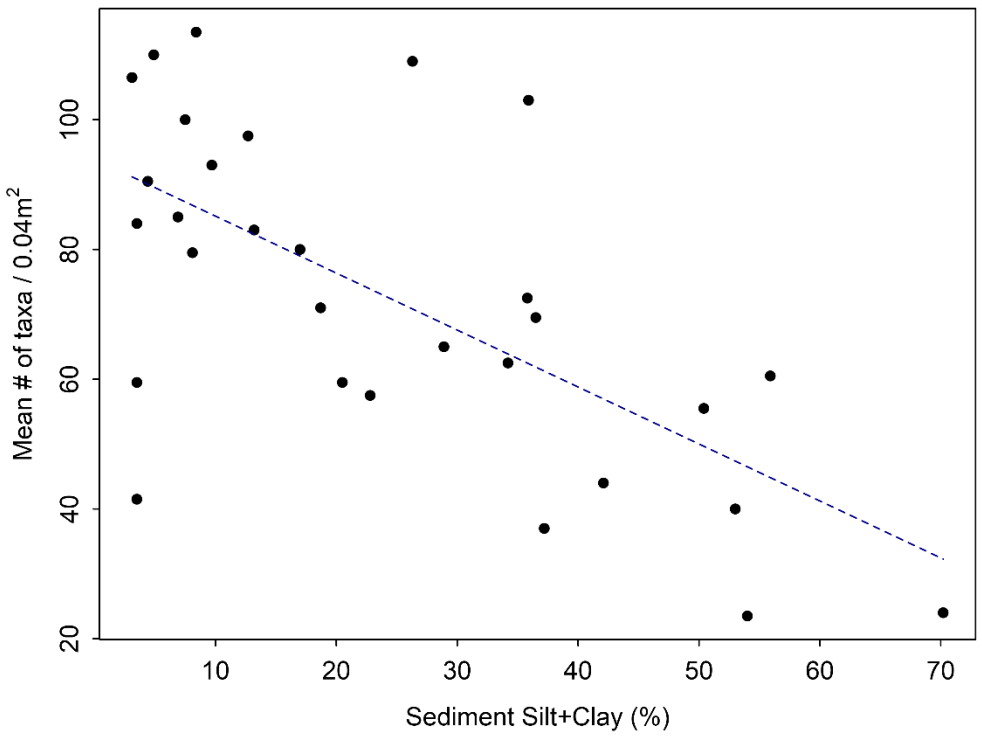

Figure 20. Plot of mean number of taxa per grab (0.04 m^2) versus sediment percent silt+clay. The dark dashed line is a linear least squares fit regression line.

3.4.4 Patterns of Benthic Infaunal Distributions

Benthic ecological community data were analyzed for patterns in faunal distributions using hierarchical cluster analysis of Bray-Curtis dissimilarities (unweighted pair-group method with arithmetic mean, or UPGMA) and analysis of similarity profiles (SIMPROF) to identify significant site groups. Non-metric multidimensional scaling (NMDS) was also used to confirm the site groups obtained from hierarchical cluster analysis. Environmental variables were fit onto the ordination (i.e., by finding vectors that were maximally correlated with the ordination configuration) in order to help explain the observed groupings based on measured abiotic factors. Analyses were performed on a species-by-station matrix of square root-transformed abundances after removing rare species (those occurring in less than 10 % of all samples) and a station-by-variable matrix of environmental (abiotic) factors.

Four overall site groupings emerged from the hierarchical cluster and SIMPROF analyses (Figure 21). Significant clusters were identified by comparing the observed similarity profiles to the mean of 1,000 permuted profiles (performed across sites for each species) at a significance level of 0.1 % (α=0.001). Results of NMDS ordination confirmed the site groupings identified in the cluster analysis, as shown in Figure 22. Results of both the cluster and NMDS analyses yielded the same site groupings, regardless of whether taxa were left at their original lowest practical identification level (usually to species) or aggregated to family level.

Vectors of significant environmental variables fit to the NMDS ordination help to illustrate several features of the infaunal assemblage data. Positive values of the first ordination axis (NMDS1) were associated with four sites (i.e., Group 2) having higher concentrations of sediment percent silt+clay (SC), increased Microtox toxicity (i.e., lower mean corrected EC_{50} values, TOX), and low numbers of taxa (NTAXA). Group 1 (station 12) had sediments with the highest percent silt+clay (70 %), it was the deepest site (99 m), and had low numbers of taxa. Group 3 consisted of the two shallowest sites sampled (stations 4 and 6), and had sediments with low percent silt+clay. Group 4 comprised the remaining 23 sites.

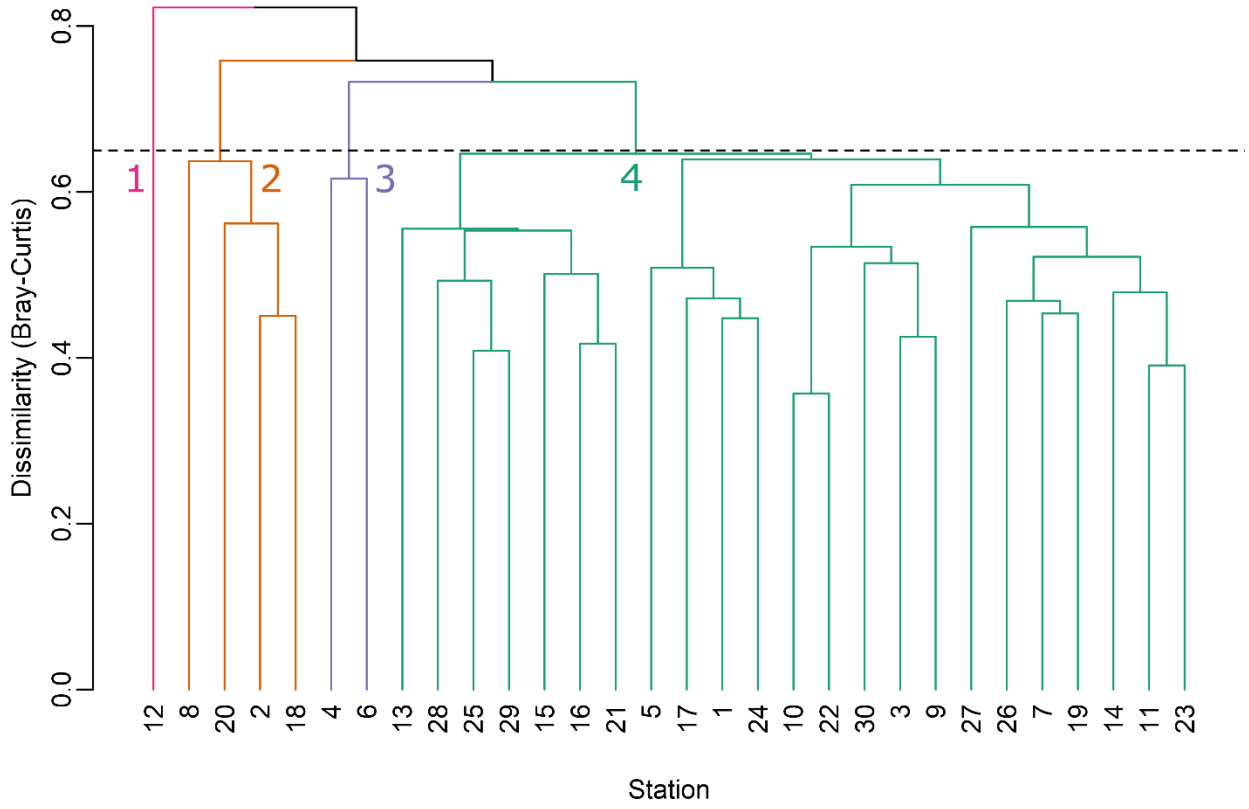

Figure 21. Dendrogram resulting from hierarchical cluster analysis of Bray-Curtis dissimilarities, calculated from square-root transformed infaunal abundance (after removing rare species), from 30 sites in FKNMS.

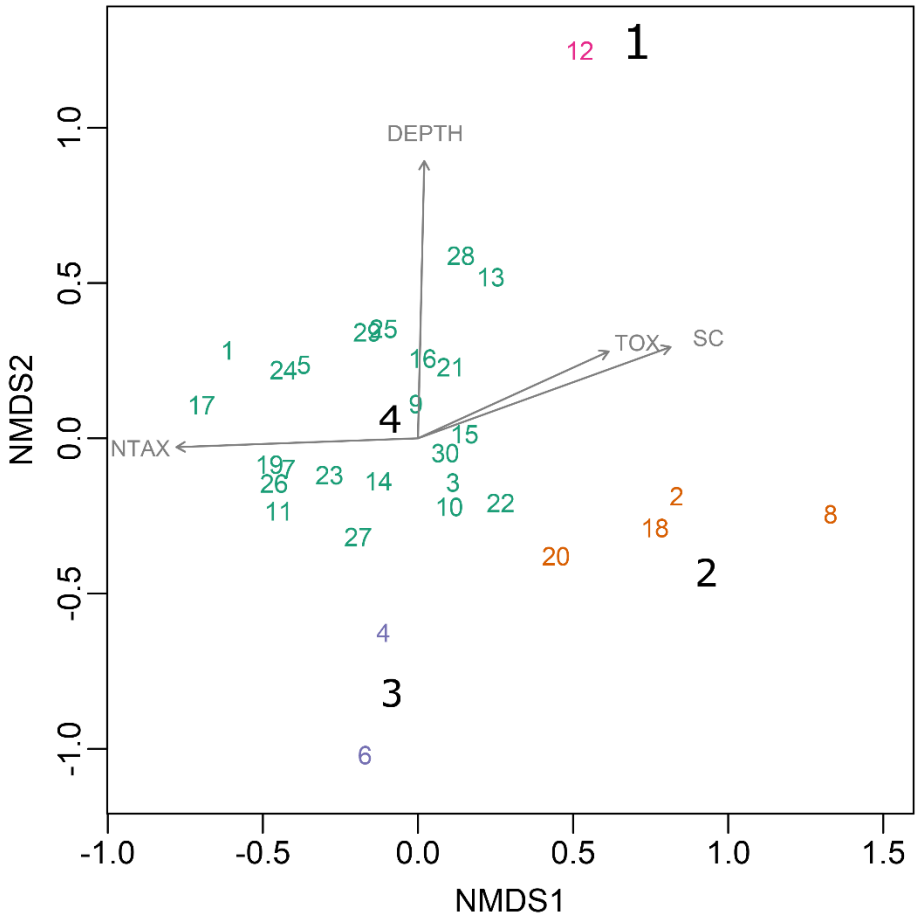

Figure 22. Ordination plot derived from non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities calculated from square-root transformed infaunal abundance (after removing rare species) from 30 sites in FKNMS.

3.5 Potential Linkage of Biological Condition to Stressor Impacts

Multi-metric benthic indices are commonly used to summarize and classify benthic habitat conditions along the continuum from non-degraded to degraded (see review by Diaz et al. 2004) and have been developed for a variety of estuarine applications (Engle et al. 1994, Weisberg et al. 1997, Van Dolah et al. 1999, Llansó et al. 2002a, 2002b, Hale and Heltshe 2008). A desired characteristic of these indices is the ability to discriminate between impaired versus unimpaired benthic condition, based on key biological attributes (e.g., numbers of species, diversity, abundance, biomass, relative proportion of pollution-sensitive or pollution-tolerant species), while taking into account natural controlling factors. As examples, such indices have been developed for estuaries of the mid-Atlantic states and Chesapeake Bay (Weisberg et al. 1997, Llansó et al. 2002a, 2002b), southeastern estuaries (Van Dolah et al. 1999), estuaries of the northern Gulf of Mexico (Engle et al. 1994, Engle and Summers 1999), the southern California mainland shelf (Smith et al. 2001), nearshore Gulf of Maine (Hale and Heltshe 2008), and

near-coastal waters off NJ (Strobel et al. 2008). More recently, a benthic index has been developed for estuarine and near-coastal waters of the entire GOM (Tetra Tech 2011), but to our knowledge no such index exists that would be directly applicable to offshore waters of the FKNMS.

In the absence of a benthic index, we attempted to assess potential stressor impacts in the present study by evaluating linkages between reduced values of biological attributes (numbers of taxa, diversity, and abundance) and synoptically measured indicators of poor sediment or water quality. Using the lower 10th percentile as a basis for defining 'low' values, we looked for co-occurrences of low values of biological attributes with indications of poor sediment or water quality defined as follows (U.S. EPA 2012): ≥ 1 chemical in excess of ERMs (from Long et al. 1995), TOC > 5 %, and DO in near-bottom water < 2.0 mg/L.

In the present study, average station values for measures of benthic infaunal richness and abundance were higher in comparison to related studies conducted in other U.S. Atlantic and GOM shelf regions (Figure 23). Measures of diversity (H' diversity) were similar to (though slightly lower than) those for the Northeast Gulf of Mexico (NE GOM) and Southwest Florida Shelf (SWFL Shelf), but higher than those for other surveyed offshore regions. Such results suggest that the FKNMS and neighboring offshore waters of the eastern GOM support diverse and abundant benthic assemblages and have important implications for programs such as MBON focused on understanding patterns of marine biodiversity.

We found no association of low values of the above biological attributes with indicators of poor water or sediment quality, since none of the measures of water or sediment quality fell within the poor range (as defined here). The lowest DO value observed (4.2 mg/L) was well above the threshold of 2 mg/L. The highest TOC concentration was 3.7 % (Appendix A), which is below the 5 % bioeffect threshold used here (from EPA 2012) and only slightly in excess of the more conservative bioeffect threshold of 3.5 % (35 mg/g) TOC published by Hyland et al. (2005). It was not, however, associated with low values (as defined here) of benthic attributes, which appear to be more closely linked to stations with higher sediment silt+clay content, as well as those in deeper, possibly depositional, areas (e.g., station 12). Also, no ERM exceedances were observed at any of the 30 sites sampled in this study (Appendix D). Lastly, sediment toxicity was not a likely cause of observed benthic community patterns and may be related more to the influence of confounding factors other than the targeted stressors including possible unmeasured chemical contaminants, natural biogenic compounds (e.g., haloaromatic compounds), or other natural abiotic environmental factors (e.g., % silt+clay content of sediment). In particular, the high incidence of sediment toxicity based on Microtox results, with significant hits at all 30 stations and low levels of chemical contaminants, suggests over-sensitivity of this assay and that it was not a good indicator of benthic infaunal health.

These results suggest that sediments and overlying waters in the surveyed area of FKNMS seem to be in good condition with respect to DO, targeted contaminants, and TOC, with lower-end values of benthic biological attributes (Appendix E) representing parts of a normal reference range controlled primarily by natural factors. However, low yet detectable levels of chemical contaminants (below bioeffect thresholds) at multiple sites and a higher concentration of total PCBs at one site (station 29), below the ERM but in excess of the ERL, indicate an increased potential for bioeffects from these or other stressors

on Sanctuary resources. As noted previously, a site sampled in 2007, which is nearby station 29, also had elevated concentrations of total PCBs (though not in excess of the lower ERL). Four of the 30 stations in the present study also tested positive for sediment toxicity, based on both the sea-urchin and Microtox bioassays, although exact causes of the observed toxicity are not known. Such conditions could justify follow-up surveys to assess the extent and source of contamination in some areas. Specifically, a survey to determine the local spatial extent, and possibly the source, of sediment PCB contamination off Conch Key is recommended. Also, since this study was focused mainly on deeper, offshore portions of FKNMS, a similar companion study of inshore areas would be valuable, as it would provide complementary information that could contribute to a more complete characterization of the sanctuary.

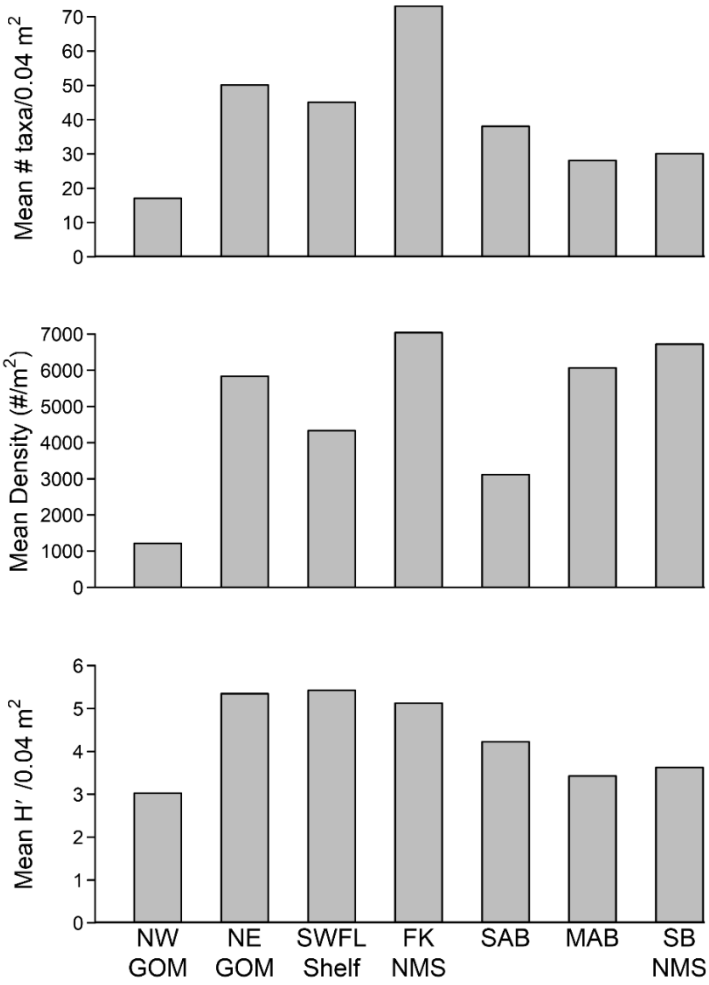

Figure 23. Comparison of measures of benthic infaunal abundance and diversity for FKNMS and other surveyed regions of the U.S. Atlantic and Gulf coastal shelf: NW GOM (Northwest Gulf of Mexico, Balthis et al. 2013), NE GOM (Northeast Gulf of Mexico, Cooksey et al. 2014), SWFL Shelf (Southwest Florida Shelf, Cooksey et al. 2012), FK NMS (Florida Keys National Marine Sanctuary, this study), SAB (South Atlantic Bight, Cooksey et al. 2010), MAB (Mid-Atlantic Bight, Balthis et al. 2009), SBNMS (Stellwagen Bank National Marine Sanctuary, Balthis et al. 2011).

4 Literature Cited

Andersen, J.L. and M.H. Deplege. 1997. A survey of total mercury and methylmercury in edible fish and invertebrates from Azorean waters. *Mar. Environ. Res.* 44(3):331-350.

[ASTM] American Society for Testing and Materials. 2018. *ASTM D1193 - 06(2018), Standard Specification for Reagent Water*. ASTM International, West Conshohocken, PA, 2018 [cited 2018 May 02] American Society for Testing and Materials

Ballschmiter, K. and M. Zell. 1980. Analysis of polychlorinated biphenyls (PCB) by glass capillary gas chromatography. Composition of technical Aroclor- and Clophen-PCB mixtures. Fresen. Z. Anal. Chem. 302: 20–31.

Balthis, W.L., J.L. Hyland, M.H. Fulton, E.F. Wirth, J.A. Kiddon, J. Macauley. 2009. *Ecological Condition of Coastal Ocean Waters Along the U.S. Mid-Atlantic Bight: 2006.* NOAA Technical Memorandum NOS NCCOS 109, NOAA National Ocean Service, Charleston, SC 29412-9110. 63 pp.

Balthis, W. L., J. L. Hyland, C. Cooksey, M. H. Fulton, E. F. Wirth, D. Cobb, D. N. Wiley. 2011. *Ecological Condition of Coastal Ocean Waters within Stellwagen Bank National Marine Sanctuary: 2008.* NOAA Technical Memorandum NOS NCCOS 129, NOAA National Ocean Service, Charleston, SC 29412-9110. 59 pp.

Balthis, W. L., J. L. Hyland, C. Cooksey, M. H. Fulton, E. F. Wirth. 2013. *Ecological Condition of Coastal Ocean Waters of the Western Gulf of Mexico: 2011.* NOAA Technical Memorandum NOS NCCOS 171, NOAA National Ocean Service, Charleston, SC 29412-9110. 63 pp.

Baston, D.S. and M.S. Denison. 2010. Considerations for potency equivalent calculations in the Ah receptor-based CALUX bioassay: Normalization of superinduction results for improved sample potency estimation. *Talanta* 83:1415-1421.

Benton, M.J., M.L. Malott, S.S. Knight, C.M. Cooper and W.H. Benson. 1995. Influence of sediment composition on apparent toxicity in a solid-phase test using bioluminescent bacteria. *Environ. Toxicol. Chem.* 14:411–414.

Bower, C.E. and J.P. Bidwell. 1978. Ionization of ammonia in seawater—Effects of temperature, pH, and salinity. *J. Fish. Res. Board Can.* 35:1012-1016.

Boyer, J.N. and H.O. Briceño. 2010. FY2009 Annual Report of the Water Quality Monitoring Project for the Florida Keys National Marine Sanctuary. US EPA Agreement #X7-96410604-5. Florida International University. Southeast Environmental Research Center Technical Report #T-497. 91 pp.

Brennan, J.C., G. He, T. Tsutsumi, J. Zhao, E. Wirth, M. H. Fulton, and M.S. Denison. 2015. Development of species-specific Ah receptor-responsive third generation CALUX cell lines with increased sensitivity and responsiveness. *Environ. Sci. Tech.* 49(19):11903-11912.

Cantin, N.E., A.P. Negri, and B.L. Willis. 2007. Photoinhibition from chronic herbicide exposure reduces reproductive output of reef-building corals. *Mar. Ecol. Prog. Ser.* 344:81-93.

Carr, S.R. and D.C. Chapman. 1992. Comparison of whole sediment and pore-water toxicity tests for assessing the quality of estuarine sediments. *Chem. Ecol.* 7:19-30.

Carr, S.R., D.C. Chapman, E.R. Long, H.L. Windom, G. Thursby, G. M. Sloane, et al. 1996a. Sediment quality assessment studies of Tampa Bay, Florida. *Environ. Toxicol. Chem.* 15:1218-1231.

Carr, R.S., D.C. Chapman, C.L. Howard, and J.M. Biedenbach. 1996b. Sediment quality triad assessment survey of the Galveston Bay, Texas system. *Ecotox.* 5: 341–364.

Carr, R.S., J.M. Biedenbach, and M. Nipper. 2006. Influence of Potentially Confounding Factors on Sea Urchin Porewater Toxicity Tests. *Arch. Environ. Contam. Toxicol.* 51:573–579.

Carriker, M. R. 1967. *Ecology of estuarine benthic invertebrates: A perspective.* In: G. Lauff (ed), Estuaries (pp. 442–487). American Association for the Advancement of Science, Washington, D.C.

Chapman, PM. 1990. The sediment quality triad approach to determining pollution-induced degradation. *Sci. Tot. Environ.* 97/98:815–825.

Cooksey, C., J. Harvey, L. Harwell, J. Hyland, J.K. Summers. 2010. *Ecological Condition of Coastal Ocean and Estuarine Waters of the U.S. South Atlantic Bight: 2000 – 2004.* NOAA Technical Memorandum NOS NCCOS 114, NOAA National Ocean Service, Charleston, SC 29412-9110; and EPA/600/R-10/046, U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze FL, 32561. 88 pp.

Cooksey, C., J. Hyland, M.H. Fulton., E. Wirth, L. Balthis. 2012. *Ecological Condition of Coastal Ocean Waters of the U.S. Continental Shelf off South Florida: 2007*. NOAA Technical Memorandum NOS NCCOS 159, NOAA National Ocean Service, Charleston, SC 29412-9110. 68 pp.

Cooksey, C., J. Hyland, M. H. Fulton, L. Balthis, E. Wirth, T. Wade. 2014. *Ecological Condition of Coastal Ocean Waters along the U.S. Continental Shelf of Northeastern Gulf of Mexico: 2010.* NOAA Technical Memorandum NOS NCCOS 188, NOAA National Ocean Service, Charleston, SC 29412-9110. 68 pp.

Denison, M.S., B. Zhao, D.S. Baston, G.C. Clark, H. Murata, and D.H. Han. 2004. Recombinant cell bioassay systems for the detection and relative quantitation of halogenated dioxins and related chemicals. *Talanta* 63:1123-1133.

Diaz, R.J. and R. Rosenberg. 1995. Marine benthic hypoxia: A review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanogr. Mar. Biol.* 33:245-303.

Diaz, R.J., M. Solan, and R.M. Valente. 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. *J. Environ. Manag.* 73:165-181.

Diaz-Ramos, S., D. L. Stevens, Jr., and A. R. Olsen. 1996. *EMAP Statistical Methods Manual.* EPA/620/R-96/002. Corvallis, OR: U.S. Environmental Protection Agency, Office of Research and Development, National Health Effects and Environmental Research Laboratory, Western Ecology Division.

Downs, C.A., J.E. Fauth, C.E. Robinson, R Curry, B. Lanzendorf, J.C. Halas, J. Halas, C.M. Woodley. 2005. Cellular diagnostics and coral health: declining coral health in the Florida Keys. *Mar. Poll. Bull.* 51:558-569.

Downs, C.A., G.K. Ostrander, L. Rougee, T. Rongo, S. Knutson, D.E. Williams, W. Mendiola, J Holbrook, R.H. Richmond. 2012. The use of cellular diagnostics for identifying sub-lethal stress in reef corals. *Ecotoxicology* 21:768-782.

Engle, V. D., J. K. Summers, and G. R. Gaston. 1994. A benthic index of environmental condition of Gulf of Mexico estuaries. *Estuaries* 17:372-384.

Engle, V. D. and J. K. Summers. 1999. Refinement, validation, and application of a benthic condition index for northern Gulf of Mexico estuaries. *Estuaries* 22(3A):624-635.

Fielman K.T., S.A. Woodin, M.D. Walla, D.E. Lincoln. 1999. Widespread occurrence of natural halogenated organics among temperate marine infauna. *Mar. Ecol. Prog. Ser.* 181:1-12.

Fofonoff, N. P. and R. C. Millard, Jr. 1983. *Algorithms for computation of fundamental properties of seawater.* Unesco Technical Papers in Marine Science, No. 44. 53 pp.

[FWC-FWRI] Florida Fish and Wildlife Conservation Commission – Fish and Wildlife Research Institute. 2016. *Users Guide to the Unified Florida Reef Tract Map Version 2.0*. Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, FL [cited 2018 March 01] Unified Florida Reef Tract Map

[FWC-FWRI] Florida Fish and Wildlife Conservation Commission – Fish and Wildlife Research Institute. 2011. *Bathymetry Contours Southeast United States*. Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, FL [cited 2018 March 27] Bathymetry Contours Southeast United States

Geider, R. J. and J. La Roche. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37:1-17.

Giesy, J.P., R.L. Graney, J.L. Newsted, C.J. Rosiuand, A. Benda, R.G. Kreis Jr., et al. 1988. Comparison of three sediment bioassay methods using Detroit river sediments. *Environ. Toxicol. Chem.* 7(6): 483–498.

Hach. 2003. *DR/2500 Spectrophotometer Procedures Manual.* Method 8006 Hach Chemical Company, Loveland, CO.

Hale, S. S. and J. F. Heltshe. 2008. Signals from the benthos: Development and evaluation of a benthic index for the nearshore Gulf of Maine. *Ecol. Indic.* 8:338–350.

Hyland, J. L., R. F. Van Dolah, and T. R. Snoots. 1999. Predicting stress in benthic communities of southeastern U. S. estuaries in relation to chemical contamination of sediments. *Environ. Toxicol. Chem.* 18(11): 2557-2564.

Hyland, J., L. Balthis, I. Karakassis, P. Magni, A. Petrov, J. Shine, O. Vestergaard, and R. Warwick. 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. *Mar. Ecol. Prog. Ser.* 295:91-103.

Jaap, W.C. 1984. The ecology of the south Florida coral reefs: a community profile. U.S. Fish and Wildlife Service and Minerals Management Service Publication FWS/OBS 82/08 and MMS 84-0038. 138pp.

Jones, R.J. 1997. Zooxanthellae loss as a bioassay for assessing stress in corals. *Mar. Ecol. Prog. Ser.* 149:163-171.

Kannan, K., R.G. Smith, Jr., R.F. Lee, H.L. Windom, P.T. Heitmuller, J.M. Macauley, and J.K. Summers. 1998. Distribution of total mercury and methyl mercury in water, sediment, and fish from South Florida estuaries. *Arch. Environ. Contam. Toxicol.* 34:109–118.

Kincaid, T. M. and A.R. Olsen. 2016. spsurvey: Spatial Survey Design and Analysis. R package version 3.3.

Lee, T.N., C. Rooth, E. Williams, M. McGowan, A.F. Szmant, and M.E. Clarke. 1992. Influence of Florida Current, gyres, and wind-driven circulation on transport of larvae and recruitment in the Florida Keys coral reefs. *Cont. Shelf Res.* 12(7/8):971-1002.

Llansó, R. J., L. C. Scott, D. M. Dauer, J. L. Hyland, and D. E. Russell. 2002a. An estuarine benthic index of biotic integrity for the Mid-Atlantic region of the United States. I. Classification of assemblages and habitat definition. *Estuaries* 25:1219-1230.

Llansó, R. J., L. C. Scott, J. L. Hyland, D. M. Dauer, D. E. Russell, and F. W. Kutz. 2002b. An estuarine benthic index of biological integrity for the Mid-Atlantic region of the United States. II. Index development. *Estuaries* 25:1231-1242.

Long, E. R., D. D. MacDonald, S. L. Smith, and F. D. Calder. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manage.* 19:81–97.

Long, E. R., L. J. Field, D. D. MacDonald. 1998. Predicting toxicity in marine sediments with numerical sediment quality guidelines. *Environ. Toxicol. Chem.* 17:714-727.

Nelson, W. G., J. L. Hyland, H. Lee II, C. L. Cooksey, J. O. Lamberson, F. A. Cole, and P. J. Clinton. 2008. *Ecological Condition of Coastal Ocean Waters along the U.S. Western Continental Shelf: 2003.* EPA 620/R-08/001, U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division, Newport OR, 97365; and NOAA Technical Memorandum NOS NCCOS 79, NOAA National Ocean Service, Charleston, SC 29412-9110. 137 pp.

[NOAA] National Oceanic and Atmospheric Administration. 2007. *Florida Keys National Marine Sanctuary revised management plan.* U.S. Dept. of Commerce, National Oceanic and Atmospheric Administration, National Ocean Service. Silver Spring, MD. 382 pp. Available from: Florida Keys National Marine Sanctuary

[NOAA] National Oceanic and Atmospheric Administration. 2011. *Florida Keys National Marine Sanctuary Condition Report 2011.* U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries, Silver Spring, MD. 105 pp.

Plumb, R. H. 1981. *Procedure for handling and chemical analysis of sediment and water samples.* Prepared for the U.S. Environmental Protection Agency/Corps of Engineers Technical Committee on Criteria for Dredge and Fill Material. Published by Environmental Laboratory, U.S. Army Waterways Experiment Station, Vicksburg, MS. Technical Report EPA/CE-81-1. 501 pp.

Precht, W.F., B.E. Gintert, M.L. Robbart, R. Fura, and R. van Woesik. 2016. Unprecedented disease-related coral mortality in southeastern Florida. *Sci. Rep.* 6:31374.

R Core Team. 2017. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria [cited 2018 May 07] R Foundation for Statistical Computing

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *Am. Sci.* 46(3):230A, 205-221.

Ringwood, A., DeLorenzo, M., Ross, P., Holland, A. 1997. Interpretation of Microtox® solid-phase toxicity tests: the effects of sediment composition. *Environ. Toxicol. Chem.* 16(6):1135-1140.

Sanger, D.M., S.P. Johnson, A.W. Tweel, S.E. Crowe, D.E. Chestnut, B. Rabon, M.H. Fulton, and E. Wirth. 2018. *The Condition of South Carolina's Estuarine and Coastal Habitats During 2015-2016: Technical Report.* Charleston, SC: South Carolina Marine Resources Division. Technical Report No. 109. 52 pp.

Schantz, M. M., J. J. Nichols, and S. A. Wise. 1997. Evaluation of pressurized fluid extraction for the extraction of environmental matrix reference materials. *Anal. Chem.* 69:4210-4219.

Schiewe, M.H., E.G. Hawk, D.I. Actor, and M.M. Krahn. 1985. Use of bacterial bioluminescence assay to assess toxicity of contaminated marine sediments. *Can. J. Fish. Aq. Sci.* 42: 1244-1248.

Schropp, S.J. and H.L. Windom. 1988. A Guide to the Interpretation of Metal Concentrations in Estuarine Sediments. Florida Department of Environmental Protection, United States. Available from: http://www.dep.state.fl.us/Waste/quick_topics/publications/documents/sediment/estuarine.pdf

Smith, R.W., M. Bergen, S.B. Weisberg, D. Cadien, A. Dalkey, D. Montagne, J.K. Stull, and R.G. Velarde. 2001. Benthic response index for assessing infaunal communities on the southern California mainland shelf. *Ecol. Appl.* 11:1073–1087.

Stevens, D. L. and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. *J. Am. Stat. Ass.* 99(465):262-278.

Strobel, C. J., H. A. Walker, D. Adams, R.Connell, N. Immesberger, and M. Kennish. 2008. *Development of benthic indicators for nearshore coastal waters of New Jersey - a REMAP project.* Presentation May 18 – 22, 2008 meeting of the National Water Quality Monitoring Council, Sixth National Monitoring Conference, Atlantic City, NJ. Available from http://acwi.gov/monitoring/conference/2008/sessione.html

Swartz, R.C. 1989. Marine Sediment Toxicity Tests. In: *Contaminated Marine Sediments: Assessment and Remediation*, pp. 115-129. National Research Council, Washington, D.C., National Academy Press.

Technicon, 1973. *Technicon Industrial Method No. 155-71W/Tentative.* Technicon Industrial Systems. Tarrytown, New York, 10591.

Technicon. 1977. *Technicon Industrial Method No. 158-71 W/A Tentative.* Technicon Industrial Systems. Tarrytown, New York, 10591.

Technicon. 1986. *Technicon Industrial Method No. 804-86T, August 1986.* Technicon Industrial Systems. Tarrytown, New York, 10591.

Tetra Tech. 2011. *Benthic Index of Biological Integrity for Estuarine and Near-Coastal Waters of the Gulf of Mexico.* Report to Gulf of Mexico Alliance, Mississippi Department of Environmental Quality Contract No. MDEQ-06-ID-01TT. 123 pp.

[USEPA] U. S. Environmental Protection Agency. 2000. *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 2: Risk Assessment and Fish Consumption Limits.* EPA-823-B-00-008. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 383 pp. Available from: Fish Consumption Limits

[USEPA] U. S. Environmental Protection Agency. 2012. *National Coastal Condition Report IV.* EPA-842-R-10-003. Office of Research and Development/Office of Water, Washington, D.C. 298 pp. Available from: National Coastal Condition Report IV

Valderrama, J. C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10: 109-122.

Van Dolah, R. F., J. L. Hyland, A. F. Holland, J. S. Rosen, and T. R. Snoots. 1999. A benthic index of biological integrity for assessing habitat quality in estuaries of the southeastern USA. *Mar. Environ. Res.* 48: 269-283.

Victor, S. and R.H. Richmond. 2005. Effect of copper on fertilization success in the reef coral *Acropora surculosa*. *Mar. Poll. Bull.* 50:1433-1456.

Wagemann, R., E. Trebacz, R. Hunt, and G. Biola. 1997. Percent methylmercury and organic mercury in tissues of marine mammals and fish using different experimental and calculation methods. *Environ. Toxicol. Chem.* 16(9):1859-1866.

Weisberg, S. B., J. A. Ranasinghe, D. M. Dauer, L. C. Schaffner, R. J. Diaz, and J. B. Frithsen. 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. *Estuaries* 20:149-158.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and phaeopigments. *Limnol. Oceanogr.* 39: 1985-1992.

Williams, L.G., P.M. Chapman, and T.C. Ginn. 1986. A comparative evaluation of marine sediment toxicity using bacterial luminescence, oyster embryo, and amphipod sediment bioassays. *Mar. Environ. Res.* 19(3): 225-249.

5 Appendices

Appendix A. Locations (latitude, longitude), depth, and sediment characteristics of sampling stations in FKNMS.

Station	Latitude	Longitude	Depth (m)	Sand (%)	SiltClay (%)	TOC (%)
1	25.5444	-80.0870	58.0	91.9	8.1	0.3
2	24.7355	-82.6063	29.5	47.0	53.0	0.9
3	24.6518	-82.6310	26.4	63.6	36.5	0.6
4	24.6564	-81.9885	11.0	96.5	3.5	0.3
5	24.8681	-80.5475	55.8	73.8	26.3	0.8
6	24.6544	-82.4246	11.4	96.6	3.5	0.2
7	24.5096	-82.6038	18.0	95.6	4.4	0.0
8	24.7430	-82.3336	22.6	46.0	54.0	1.1
9	24.5415	-81.3714	42.9	79.5	20.5	0.6
10	24.3971	-82.5767	39.2	65.8	34.2	0.4
11	24.5251	-82.7719	22.0	96.8	3.1	0.7
12	24.4149	-82.1020	98.8	29.8	70.2	1.2
13	24.6016	-81.0596	77.3	71.0	28.9	0.7
14	24.4827	-82.4562	19.0	87.4	12.7	0.4
15	24.4125	-82.8049	50.1	49.5	50.4	0.9
16	24.4143	-81.9581	81.1	86.8	13.2	0.5
17	25.0162	-80.3296	51.8	96.5	3.5	0.0
18	24.6966	-82.4751	23.5	62.8	37.2	0.8
19	24.6094	-82.7373	26.3	91.5	8.4	3.7
20	24.7146	-82.1743	19.1	44.2	55.9	1.0
21	24.5011	-81.5266	55.7	64.2	35.8	0.7
22	24.3962	-82.6474	38.6	57.9	42.1	0.3
23	24.5088	-82.9312	27.2	92.5	7.5	0.0
24	24.4272	-82.0372	46.7	93.1	6.9	0.6
25	24.6325	-80.9582	76.2	81.3	18.7	0.5
26	24.5795	-82.5437	19.2	95.1	4.9	2.7
27	24.7745	-81.7252	13.4	90.2	9.7	0.8
28	24.4553	-81.6845	75.6	77.2	22.8	0.5
29	24.9321	-80.4401	71.5	83.0	17.0	0.2
30	24.7250	-82.7385	28.9	64.1	35.9	0.9

Appendix B. Near-bottom water characteristics by station.

Station	Temperature (°C)	Salinity (psu)	DO (mg/L)	pH
1	27.4	36.4	6.4	7.8
2	28.5	36.4	6.3	7.8
3	28.5	36.4	6.3	7.8
4	29.3	36.6	6.2	7.8
5	24.3	36.4	6.8	7.8
6	29.3	37.0	5.9	7.8
7	28.9	36.5	6.3	7.8
8	28.8	36.6	6.2	7.8
9	28.2	36.8	6.3	7.8
10	27.2	36.4	6.7	7.8
11	28.6	36.4	6.4	7.8
12	15.2	36.0	4.3	7.6
13	21.4	36.5	6.6	7.8
14	28.2	36.3	6.2	7.8
15	23.1	36.3	6.8	7.8
16	20.4	36.4	5.9	7.7
17	26.9	36.4	6.4	7.8
18	28.8	36.6	6.1	7.8
19	28.6	36.4	6.2	7.8
20	29.0	36.7	6.2	7.8
21	24.5	36.4	6.8	7.8
22	26.5	36.3	6.7	7.8
23	28.1	36.3	6.4	7.8
24	28.0	36.4	6.3	7.8
25	20.9	36.4	6.3	7.8
26	28.8	36.5	6.1	7.8
27	29.0	36.1	6.1	7.8
28	20.3	36.4	5.9	7.7
29	22.8	36.4	6.6	7.8
30	27.8	36.4	6.6	7.8

Appendix C. Near-surface water characteristics by station.

Station	Temperature (°C)	Salinity (psu)	DO (mg/L)	pH	DIP (mg/L)	DIN (mg/L)	Nitrate+ Nitrite (µg/L)	Ammonium (µg/L)	N:P	Silicate (µg/L)	Chlorophyll *a* (µg/L)	Turbidity (NTU)	TSS (mg/L)
1	27.8	36.3	6.4	7.8	0.002	0.024	18.0	6.0	26.9	290.5	0.109	0.2	4.6
2	28.8	36.4	6.3	7.8	0.002	0.019	10.1	9.0	28.6	130.0	0.122	0.4	2.5
3	28.6	36.4	6.2	7.8	0.003	0.033	24.7	8.0	29.6	278.4	0.160	0.5	3.0
4	29.3	36.6	6.2	7.8	0.003	0.009	3.1	6.0	13.5	181.5	0.256	0.4	2.4
5	28.4	36.3	6.4	7.8	0.003	0.038	31.5	6.0	30.7	84.7	0.099	0.4	4.0
6	29.3	36.9	5.9	7.8	0.003	0.008	3.4	5.0	12.1	254.2	0.347	0.6	2.5
7	28.9	36.5	6.2	7.8	0.003	0.010	5.0	5.0	13.1	145.2	0.118	0.3	3.0
8	28.8	36.6	6.2	7.8	0.003	0.008	2.8	5.0	10.9	217.8	0.329	1.0	2.7
9	28.1	36.5	6.4	7.8	0.002	0.008	1.5	6.0	14.1	119.4	0.092	0.3	5.1
10	28.8	36.3	6.3	7.8	0.002	0.009	2.7	6.0	14.9	84.7	0.049	0.2	2.7
11	28.6	36.4	6.3	7.8	0.002	0.006	1.3	5.0	11.8	96.8	0.183	0.4	3.2
12	28.0	36.3	6.4	7.8	0.002	0.007	1.2	6.0	15.2	266.3	0.047	0.5	3.1
13	28.1	36.4	6.4	7.8	0.002	0.007	1.2	6.0	16.7	217.8	0.035	0.6	2.4
14	28.2	36.3	6.2	7.8	0.002	0.007	1.1	6.0	13.9	169.4	0.147	0.4	2.8
15	28.9	36.3	6.4	7.8	0.002	0.015	6.2	9.0	23.7	314.7	0.068	0.3	2.4
16	27.9	36.3	6.4	7.8	0.003	0.007	1.2	6.0	13.4	411.5	0.054	0.3	3.5
17	27.9	36.3	6.4	7.9	0.002	0.008	2.2	6.0	15.2	399.4	0.047	0.2	4.1
18	28.8	36.6	6.2	7.8	0.002	0.011	5.0	6.0	16.4	181.5	0.275	0.8	2.9
19	28.7	36.4	6.2	7.8	0.003	0.008	1.9	6.0	13.8	193.6	0.250	0.4	2.5
20	29.0	36.7	6.1	7.8	0.002	0.007	2.0	5.0	12.2	181.5	0.396	1.0	2.8
21	28.1	36.5	6.4	7.8	0.002	0.006	1.2	5.0	12.2	110.0	0.126	0.6	4.7
22	28.6	36.3	6.4	7.8	0.002	0.007	1.2	6.0	15.9	145.2	0.048	0.2	3.1
23	28.4	36.3	6.4	7.8	0.002	0.006	1.2	5.0	12.8	60.5	0.151	0.3	3.3
24	27.9	36.3	6.4	7.8	0.002	0.007	1.3	6.0	16.8	181.5	0.062	0.5	2.8
25	28.1	36.4	6.4	7.8	0.002	0.007	1.2	6.0	13.9	60.5	0.032	0.5	3.1
26	28.8	36.5	6.1	7.8	0.002	0.008	1.8	6.0	14.3	363.1	0.273	0.4	3.2
27	29.0	36.1	6.1	7.8	0.003	0.008	2.2	6.0	11.3	278.4	0.682	0.8	2.7
28	28.0	36.4	6.4	7.8	0.002	0.032	2.5	29.0	71.1	145.2	0.073	0.4	15.0
29	28.0	36.4	6.4	7.8	0.002	0.012	6.5	5.0	16.5	108.9	0.048	0.2	4.6
30	28.9	36.4	6.3	7.8	0.002	0.010	1.5	8.0	22.2	72.6	0.134	0.2	2.6

Appendix D. Summary by station of mean ERM quotients and the number of contaminants that exceeded corresponding ERL or ERM values (from Long et al. 1995).

Station	# of ERLs Exceeded	# of ERMs Exceeded	Mean ERM-Q
1	0	0	0.005
2	0	0	0.004
3	0	0	0.004
4	0	0	0.004
5	0	0	0.004
6	0	0	0.003
7	0	0	0.003
8	0	0	0.010
9	0	0	0.003
10	0	0	0.005
11	0	0	0.003
12	0	0	0.004
13	0	0	0.004
14	0	0	0.004
15	0	0	0.005
16	0	0	0.002
17	0	0	0.004
18	0	0	0.004
19	0	0	0.008
20	0	0	0.004
21	0	0	0.003
22	0	0	0.004
23	0	0	0.004
24	0	0	0.004
25	0	0	0.003
26	0	0	0.003
27	0	0	0.005
28	0	0	0.003
29	1	0	0.010
30	0	0	0.003

*Appendix E. Summary by station of benthic macroinfaunal (>0.5mm) characteristics. Two replicate benthic grabs (0.04m^2 each) were processed from each station. H' derived using base 2 logarithms. (*Values within lower 25th percentile of all values of a specific benthic variable; **values within lower 10th percentile.)*

Station	Mean # Taxa per Grab	Total # Taxa	Mean Density (#/m^2)	Mean H' per Grab
1	80	122	7713	4.9
2	40 *	58 **	1625 **	5.1
3	70	114	9688	4.8 *
4	60	99	4475 *	5.2
5	109	167	11250	5.5
6	42 *	71 *	3438 *	4.4 *
7	91	143	5938	5.7
8	24 **	38 **	1063 **	4.2 **
9	60	96	7750	4.7 *
10	63	88	10138	4.6 *
11	107	150	12525	5.6
12	24 **	37 **	3025 *	2.9 **
13	65	104	3900 *	5.5
14	98	145	12638	5.1
15	56 *	80 *	5138	4.9
16	83	134	5250	5.7
17	84	131	5963	5.7
18	37 **	66 *	2413 **	4.4 *
19	114	163	12763	5.1
20	61	92	5525	5.2
21	73	117	5238	5.4
22	44 *	62 *	6825	4.3 **
23	100	147	8163	6.0
24	85	128	9013	4.8
25	71	109	5113	5.5
26	110	157	12225	5.5
27	93	148	10975	5.6
28	58 *	87 *	3463 *	5.2
29	80	121	4938	5.7
30	103	146	13075	5.0

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