Extent and Toxicity of Contaminated Marine Sediments in Southeastern Florida

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Extent and Toxicity of Contaminated Marine Sediments in Southeastern Florida

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Extent and Toxicity of Contaminated Marine Sediments in Southeastern Florida

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ABSTRACT

Thirty sites were sampled in southern Biscayne Bay and Manatee Bay in December 1999 to determine the extent of toxicity in sediments. Analyses and assays included: pesticides and phenols in seawater; chemical contaminants in sediment; amphipod mortality, HRGS P450, sea urchin sperm fertilization and embryology, MicrotoxTM, MutatoxTM, grass shrimp AChE and juvenile clam mortality assays; sea urchin sperm, amphipod and oyster DNA damage; and benthic community assessment. Sediment sites near the mouth of canals showed evidence of contamination. Contaminant plumes and associated toxicity do not appear to extend seaward of the mouth of the canals in an appreciable manner. Concentrations of contaminants in the sediments in open areas of Biscayne and Manatee Bays are generally low.

1. INTRODUCTION

The "Biological Effects" component of the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends (NS&T) Program for Marine Environmental Quality conducts intensive regional surveys to describe the incidence, severity, and spatial extent of adverse biological effects associated with chemical contamination. These studies are conducted in specific coastal areas based on a number of considerations, including: high levels of contamination found in mussels and oyster tissues samples under the "Mussel Watch" component of NS&T program; likelihood or documentation of adverse biological effects of contamination based on state and local environmental data; and possible collaboration with other Federal, state and local agencies. Typically, the studies are designed to obtain data simultaneously on levels of chemical contaminants in sediment and biota, results of multiple toxicity tests, analysis of biomarker responses, and changes in benthic biological community structure. By combining and synthesizing data from field observations, chemical analyses, toxicity tests, and measures of benthic community structure, NOAA's "biological effects" studies provide a holistic understanding of regional environmental quality and the spatial extent of contamination-related adverse biological effects. To date, NOAA has performed "biological effects" studies in over 30 different estuaries and other coastal waters throughout the United States, often in close cooperation with coastal states. In Florida, NOAA has performed such studies in Tampa Bay, four bays of the Florida Panhandle (Pensacola, Choctawhatchee, St. Andrew and Apalachicola), and Biscayne Bay.

Comprehensive bay-wide sampling was conducted in Biscayne Bay over two years (1995 and 1996) to determine the incidence, severity and spatial extent of sediment toxicity. It was based on a stratified-random sampling design that comprised more than 200 sites covering an area of 484 sq km. As in previous NOAA studies, toxicity tests were selected to ensure different modes of contaminant exposure (i.e., bulk sediment, porewater, and chemical extracts of sediments) to a variety of test organisms (invertebrates, bacteria, and others) and to measure different assessment end-points (i.e., mortality, impaired reproduction, physiological stress, and enzyme induction).

The 1995 study results showed high levels of sediment contamination and severity of toxicity in several peripheral canals and tributaries, notably the lower Miami River. In terms of the areal extent, sediment toxicity as inferred from the amphipod mortality test was 13% of the total area, that inferred from the sea urchin fertilization test was about 47%, and that inferred from the MicrotoxTM test was 51%. In comparison, a compilation of results of NOAA's sediment toxicity from 23 different coastal areas in 1999 showed that 7% of the total studied area was classified as toxic based on the amphipod mortality tests, 39% based on sea urchin fertilization test, and 66% based on the MicrotoxTM test.

The 1995 data also showed an unexpectedly wide, but apparently sporadic, occurrence of sediment toxicity in southern Biscayne Bay. Although sediment toxicity was expected at sites located in or just outside Black Creek - Goulds Canal, Military Canal, and Mowry Canal, it was not expected in the open waters of the Bay extending eastward to Featherbed Banks and Elliott Key. Also, unlike other parts of the Bay, the observed toxicity in this area was not associated with high levels of contaminants; to the contrary, contaminant levels at those sites were generally very low, in some instances at or below method detection limits.

The 1999 NOAA follow-up study described here was intended to determine patterns of toxicity in southern Biscayne Bay and to define certain measures of environmental quality before major environmental restoration and mitigation activities are implemented in South Florida. Its initial objectives were to define the existence of toxicity associated with effluents from freshwater discharge canals in coastal waters of south Florida (including the C-111 canal), and to determine whether the pattern of sediment toxicity observed in southern Biscayne Bay was persistent. The study included a wider array of potential toxicants than before and a broader suite of toxicity tests, including tests for genotoxic effects. Samples were collected in November-December 1999 from 30 sites, most of which coincided with sites in the previous study conducted in 1995 and 1996.

2. METHODS

2.1. Sampling sites

Biscayne Bay is a shallow tropical saline lagoon located along the southeastern-most portion of the state of Florida (Figure 1). It is surrounded on the north by the growing urban areas of Dade County, which include Miami and Miami Beach, and on the south by the sparsely inhabited Homestead area and the northern Florida Keys. The eastern boundary of the Bay is composed of barrier islands which eventually become part of the Florida Keys. The western shore is the Florida mainland. Biscayne Bay can be divided into three major areas: North, Central and South. The southern portion of the Bay ranges from the Featherbed Bank to Card Bank. This section is undeveloped and fringed by mangrove wetlands. Benthic habitats are dense seagrass beds, large hard ground areas and algal communities. The main canals draining into the portion of the Bay are Black Creek, Princeton Canal, Military Canal, Mowry Canal and Model Land Canal. Ocean exchange is restricted to the tidal creeks between the islands of the northern portion of the Florida Keys. The southern portion of the Bay is connected to Card Sound, a small coastal lagoon. Restricted openings limit flushing and water exchange between Card Sound and Biscayne Bay. South of Card Sound is Barnes Sound, also a shallow lagoon with little water circulation. Manatee Bay is located off the western side of Barnes Sound. The C-111 Canal flows into this small coastal lagoon.

Figure 1. Sampling sites in Biscayne Bay and Manatee Bay, and land use pattern.

Thirty sites were sampled in southern Biscayne Bay and Manatee Bay in December 1999. Site locations are listed in Table 1 and shown in Figure 1. Additional sites were added for shrimp acetylcholinesterase assay tests. The type of analyses, number of samples and laboratory performing the collection and analyses are listed in Table 2. A schematic of the sample types and analyses is shown in Figure 2. Results are listed in Appendices I through V, and aerial photographs of the sampling area can be found in Appendix VI.

2.2. Sampling and processing methods

2.2.1. Seawater

The seawater samples were collected from a depth of one meter using a submersible marine pump. The pump was connected to a length of Teflon tubing connected to two in-line, stainless steel filter holders each housing a $1-\mu m$ pore size GF/F filter (Whatman no. 1825150). The particulate matter was discarded. The filtered water flowed into a pre-cleaned 20-L stainless steel canister and sealed with an airtight lid. A field blank was collected each sampling day by pumping 10 L of organic free water through the sampling and filtration system into a precleaned stainless steel container.

The water samples were shipped in coolers with dry ice to the testing laboratory in Beltsville, MD within 24 hr of collection. The samples were stored in a 4 $^{\circ}$ C cooler at the laboratory and extracted within 7 days of collection.

Water samples were collected concurrently with grass shrimp samples (Section 2.2.4.5).

2.2.2. Sediment

Sediment samples were collected at 30 Biscayne Bay and Manatee Bay sites using a Kynarcoated 0.04-m² Young-modified van Veen grab sampler deployed by hand. Only the upper $2 - 3$ cm of sediment were collected for chemical analyses and toxicity bioassays. Sediments were removed from the sampler with a plastic scoop and transferred to a lined, stainless steel container. Sediments were homogenized using a plastic paddle prior to distribution into individual containers for grain size, total organic carbon (TOC) and total inorganic carbon (TIC), chemistry, and bioassays.

2.2.2.1. Chemistry

The sample processing protocol is described in detail in Lauenstein and Cantillo (1993, 1998).

2.2.2.2. Bioassays

2.2.2.2.1. Microtox and Mutatox

Sediments were extracted using dichloromethane following the procedure in Long et al. (1998) by Columbia Analytical Services, Jacksonville, FL. This extract was used for Microtox™ and MutatoxTM assays.

2.2.3. Sediment pore water

Homogenized sediment samples were shipped chilled to USGS, Corpus Christi, TX, and received the following day. Samples were kept refrigerated and porewaters were extracted within 8 days of field sample collection and within 48 hours of arrival in Texas. The pore water was extracted using a pressurized pneumatic extraction device made of polyvinyl chloride and a

Table 1. Sampling site locations in Biscayne Bay and Manatee Bay.

Table 2. Number of samples and type of analyses performed.

 $5-\mu m$ polyester filter. The apparatus and procedure are described in USGS (2000). After extraction, the porewaters were centrifuged at 1200 x g for 20 min to remove suspended material, and stored frozen in polycarbonate bottles.

2.2.4. Specimens for assays

2.2.4.1. Amphipods

Specimens of Ampelisca abdita were purchased from Eastern Aquatic Bio Supply, Inc., and held in the laboratory in pre-sieved uncontaminanted sediment until use (Environmental Science and Engineering, Inc., 2000). The Biscayne Bay sediment samples were homogenized within the original sample containers after large objects such as stones, plant debris and organisms were removed by hand.

Following completion of the amphipod sediment bioassays, the remaining live amphipods (Ampelisca abdita) from each replicate were pooled, placed in 1 mL of ice-cold cryopreservative mix, and frozen on dry ice. Amphipods used to test sediments from sites 1 , 2, 3, 4, 5, 8, 9, 18, 21 and 23 were shipped frozen to the US Navy Space and Naval Warfare Systems Center (SSC-SD) Biomarker Lab, San Diego, CA, for DNA Damage analysis.

2.2.4.2. Clams

Specimens of the clam (Mercenaria mercenaria) were obtained from Sea Perfect, Charleston, NC, a hatchery located near the NOAA/NOS/NCCOS Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) facility.

Figure 2. Schematic of samples and analyses.

2.2.4.3. Oysters

Oysters (Crassostrea virginica) were pried off rock or mangrove root substrates at the mouth of drainage control canals corresponding to sampling sites 1, 2, 4, 5, canal C-111 and at a reference site in Little Card Sound, without damaging the animals' shell. Oysters were placed in ice chests in clean zip-loc bags filled with site water and transported by boat back to the Biscayne National Park headquarters laboratory for processing. Physical/chemical data were collected at each site at the time of collection. Ten oysters were collected at each site with the exceptions of site 2 where only 6 were found, and site 3 where no oysters were found.

The resident oyster populations were not plentiful at any site except along the breakwater at site 4, North Canal. In most cases the oysters from any one site were of many varying sizes. The site 4 collection was during low tide but only submerged individuals were collected. The collection at site 5 was also close to low tide, oysters were attached to rocks and mangrove roots above the waterline at the time of collection. Collection at sites 1 through 3 coincided with high tide, and all collected individuals were submerged. No oysters were found at site 3.

Oyster collection at C-111 occurred near low tide, but the gathered oysters were all collected from submerged rocks. Oysters collected from the Little Card Sound reference site were all attached to submerged rocks or mangrove roots.

2.2.4.4. Sea urchins

Specimens of the sea urchin Arbacia punctulata used in this study were obtained from Gulf Specimen Company, Inc., Panacea, FL.

2.2.4.5. Grass shrimp

Approximately 20 grass shrimp were collected live from seven sites in Biscayne Bay and three sites in Manatee Bay using a dip net. The shrimp were placed in plastic bags and frozen immediately using dry ice. The samples were transported to NOAA/NOS/NCCOS/CCEHBR, Charleston, SC, sorted on ice and identified to species. All shrimp, with the exception of those collected from site 3, were identified as Palaemonetes intermedius. The shrimp collected at site 3 were not analyzed because they were determined to be Palaemon floridanus. The shrimp from each site were separated into 2-animal samples, wrapped in aluminum foil, and stored in a -70 °C freezer until analysis. In addition to the field-collected P. intermedius, laboratoryreared P. intermedius were used as a control. Previous work on acetylcholinesterase (AChE) in Palaemonetes has concentrated on the species P. pugio. Because no previous AChE work on P . intermedius has been published, a laboratory-reared population of P. pugio was also sampled for AChE analysis for comparative purposes.

2.2.5. Benthos

A Young-modified Van Veen grab (area = 0.04 m²) was used to collect bottom samples at the 30 sites. Samples were prescreened through 0.5-mm mesh sieves in the field by NOAA personnel and fixed in a 10% formalin solution. The preserved sample fractions were transported to Barry A. Vittor & Associates, Inc. (BVA) laboratory in Mobile, AL.

2.3. Analyses and assays

2.3.1. Seawater analyses

2.3.1.1. Pesticides

Two 10-L aliquots of each seawater sample were measured into stainless steel canisters for duplicate processing. Field blanks were processed concurrently with the samples. Each sample canister was pressurized with high purity nitrogen forcing the water sample through a certified solid phase extraction (SPE) cartridge containing hyper-cross-linked styrene-divinylbenzene copolymer, ENV+ (Jones Chromatography) extraction resin. After extraction, the ENV+ cartridge was dried with nitrogen and eluted with certified high purity solvents (6 mL dichloromethane followed by 9 mL of 3:1 acetone:acetonitrile). This 15-mL extract was concentrated to a final volume of 0.5 mL under nitrogen and analyzed by two gas chromatograph-mass spectrometers.

2.3.1.2. Alkyl phenols

One liter of non-filtered seawater was extracted for each site. The liter of seawater was placed in a separatory funnel, and 100 mL dichloromethane and 40 g NaCl were added. The funnel was shaken for 3 minutes. The organic (dichloromethane) phase was separated and retained. Another aliquot of 100 mL of dichloromethane was added and the funnel again shaken and the organic phase added to the previous one. The collected organic phase was passed through a Na_2SO_4 column to remove any water present. The organic phase was placed in a Rotoevap and the solvent exchanged to hexane. The resulting solution was evaporated to 1 mL.

Dichloromethane extracts were prepared for GC/MS analysis by adding pentafluorobenzoyl chloride according to the method of Wahlberg et al. (1990). Briefly, the extracts were reduced in volume to approximately 0.2 mL, and diluted to 2 mL using toluene. To perform the derivatization, 10 μ L of pentafluorobenzolyl chloride (Aldrich Chemical Co.), and 5 μ L of lowwater containing pyridine were mixed, heated to 60 °C, and maintained at 60 °C for 15 min. A basic solution, 10 mL of NaOH solution (4 g/100 mL), was added to neutralize excess acid. The mixture was placed in a 4"°C refrigerator overnight. The organic phase was removed and analyzed using negative chemical ionization gas chromatographic mass spectrometry. Prior to injection in the gas chromatograph (GC), the samples were passed through $Na₂SO₄$ cartridges to remove excess water and particulates.

The GC column used was a 30 m long DB-17MS column, 0.25 mm ID, and 0.25 μ m support. Column flow was 1.13 mL/min of helium gas. The temperature program was: 130 °C for 4 minutes, up to 170 °C ramped at 20 °C/min, up to 250 °C at ramped $\frac{7}{3}$ °C/min, up to 300 °C at ramped 10 °C/min, and ending with a 20-min hold, for a total run time of 42.43 min.

The mass spectrometer was a 5890A Hewlett Packard GC/MS operated in electron capture negative ionization mode. The reagent gas was methane at 2.0 torr and the source operated at 250 °C. The other heated zones were the injector at 250 °C and the transfer line, 280 °C. The halogenated derivative was selectively determined using electron capture negative chemical ionization (NCI) detection methods. The standard for the octylphenol analysis were provided by Aldrich Chemical Company as tetramethylbutylphenol. The other standards were combined as a mixture in POE(3) [same as Triton X-100] (Chem Services), which was analyzed and determined to have the following composition of octylphenol and octylphenol ethoxylates: 0.9% octylphenol, 24.5% octylphenolmonoethoxylate, 38.7% octylphenoldiethoxylate, 29.4% octylphenoltriethoxylate, 5.8% octyltetraethoxylate, 0.7% octylpentaethoxylate. This composition was determined by fluorescence after separation by HPLC using a Hypersil column. GC analysis confirmed this composition determination. However certain impurities, approximately 11%, were present and reduced the octylphenol ethoxylate composition slightly. The mass spectrometer was operated in SIM mode dwelling on the following ions: 400 octylphenol, 444 - octylphenolmonoethoxylate (isomeric mixture), 488 octylphenoldiethoxylate (isomeric mixture), 532 - octylphenoltriethoxylate (isomeric mixture), 576 - octylphenoltetraethoxylate (isomeric mixture) and 620 octylphenoltriethoxylate (isomeric mixture). The retention time window was set into the quantitation program for the report generator for the Hewlett Packard system and the appropriate ions searched. Standard concentrations ranged from 0.005 to 1.3 μ g/mL. Linearity was maintained over the low end for standards, the highest range being 1 μ g/mL to 0.25 μ g/mL. To quantitate the nonylphenol and nonylphenol ethoxylates, the instrument was calibrated using purified standards of the ethoxylates 1 through 5, and a standard of nonylphenol obtained from Schenectady International. The derivatives of these compounds form stable substitutions of 194 mass units with no apparent fragmentation, thus providing maximum sensitivity. These mixed standards each yield about 8 to 11 peaks on chromatography which are summed across each analyte group, i.e. nonylphenol $414 \, \text{m/s}$, $[4-n-1]$ nonylphenolmonoethoxylate (isomeric mixture)] (np1eo) 458 m/z, [4-nnonylphenoldiethoxylate (isomeric mixture)] (np2eo) 502 m/z, [4-n-nonylphenoltriethoxylate (isomeric mixture)] (np3eo) 546 m/z, [4-n-nonylphenoltetraethoxylate (isomeric mixture)] (np4eo) 590 m/z and [4-n-nonylphenolpentaethoxylate (isomeric mixture)] (np5eo) 620 m/z. Each retains a characteristic pattern similar to the standard. However pattern variations do appear to occur in the field collected samples. These pattern shifts are also available for interpretation.

The recovery of the spiked sample was adequate: 120% nonylphenol, 149% np1eo, 169% np2eo, 159% np3eo, 97% np4eo and 43% np5eo. The precision was excellent, notice Princeton Canal Mouth samples A and B show an average relative percent difference of 20% (ranging from 3.2 to 45%). The percent differences were greatest with the 4 and 5 nonylphenolethoxylates, which are the more difficult to quantitate because the GC/MS loses sensitivity as the ethoxy substitution increases.

Results of analyses are listed in Tables I.1 and I.2.

2.3.2. Whole sediment

2.3.2.1. Chemical analyses

The analytical protocols for the determination of carbon content, solids, and particle size distribution, trace organic contaminants, and element analyses are described in detail in Lauenstein and Cantillo (1993, 1998). Results are listed in Appendix II: carbon content, solids, and particle size distribution (Table II.1); polycyclic aromatic hydrocarbons (PAHs) (Table II.2); pesticides (Table II.3); polychlorinated biphenyl congeners (PCBs) (Table II.4); major and trace elements, and acid volatile sulfides (AVS) (Table II.5); and tributyltins (TBTs) $(Table" II.6)$.

2.3.2.2. Juvenile clam assay

Sediments for the clam (Mercenaria mercenaria) bioassays were warmed to room temperature and press-sieved through a 212-µm mesh screen. Bioassays were done in pre-cleaned 16-oz glass jars containing 60 mL of sediment and 180 mL of 20 μ m filtered seawater. There were five replicates for each sediment sample. Following the addition of the seawater, sediments were allowed to settle under active aeration in the bioassay beakers for 24-hr before the addition of the clams. After settling, thirty (212 to 350 μ m in length) clams were added to each beaker. The bioassays were run at 30 ppt salinity, 20° C, and a 12-hr light: 12-hr dark cycle in environmental chambers. Clams in each beaker were fed 5 mL of the flagellate Isochrysis galbana every 48 hr. Temperature, dissolved oxygen, salinity, pH and ammonia were monitored during all bioassays. At the end of ten days, clams were retrieved by resieving the sediment through a 212- μ m mesh sieve. Clam mortality in each replicate was determined using an Olympus SZH10 microscope under 7.0x magnification. Site-specific mortality was evaluated in comparison to a reference site (Folly River, SC) using ANOVA and Dunnett's Test (arcsin transformed percent mortality data). Due to the large number of sediment samples to be evaluated, sediments were tested in three separate 10-day assays. A reference sediment (Folly River, SC) sample was included in each of the assays. Results are listed in Table III.1.

2.3.2.3. Amphipod survival

Amphipods, Ampelisca abdita, were exposed to the test sediments for 10 days under static conditions following American Society for Testing and Materials (ASTM) procedures (ASTM, 1995). Two hundred mL of homogenized sediment were added to each of five replicate chambers per sample, and 800 mL of filtered seawater was added. The sediment and seawater were allowed to equilibrate for one day prior to introduction of the amphipods. The Ampelisca specimens were sieved from the holding chambers, and rinsed with seawater. Twenty sub-adult amphipods were randomly distributed into plastic weigh boats to determine average weight per specimen. Condition and number of organisms was noted prior to loading of the test chambers. Organism loading of 20 amphipods per test chamber was performed in random order. After one hour, the test chambers were examined for any amphipods that did not burrow into the

sediment. The chambers were placed in random order within a water bath. Oil-free air was delivered into the water column of each test chamber to maintain acceptable oxygen levels. Ambient laboratory lighting was continuous to promote tube-dwelling activity by the amphipods. The amphipods were not fed during the test. The test chambers were examined daily and the number of animals found on the sediment surface, water column or water surface was recorded. Dead amphipods were removed and noted. Live amphipods trapped in the water surface were gently prodded with a stream of overlying water applied with a plastic pipette and allowed to descend and reburrow. Water quality parameters measured included temperature, salinity, dissolved oxygen, pH and ammonia.

The tests were terminated after 10 days. The sediments were washed with seawater and sieved. The material remaining on the screen was rinsed into a dish and labeled. The living organisms were removed to a plastic weigh boat, counted and weighed. All samples for which greater than 10% (2 out of 20) of the original organisms were unaccounted for were reexamined. Amphipods not accounted for at test termination were assumed dead and recorded as such. Results are listed in Table III.2.

Individuals from sites 1, 2, 3, 4, 5, 8, 9, 18, 21 and 23 were hand-picked and placed into glass vials, preserved with a cryopreservative and shipped on dry ice to another laboratory for genetic testing.

2.3.3. Bioassays using sediment extracts

2.3.3.1. HRGS P450 bioassay on sediment extracts

Sediment samples were extracted using EPA Method 3550 (Anderson and McCoy, 2000). Briefly, approximately 20 g of sediment were extracted with dichloromethane to yield 1 mL of extract. A separate sediment sample was used to determine percent solids. Extracts were exchanged into a 2:1:1:1 dimethyl sulfoxide (DMSO), toluene and isopropyl alcohol solution. The final extract volume was 2 mL. Two 1-mL vials were prepared from each sample. One was shipped to Columbia Analytical Services in Vista, CA, for P450 Human Reporter Gene System (HRGS) analysis (EPA Method 4425), and the other to CCEHBR in Charleston, NC.

For Tier I testing, 5 μ L extract samples were applied to three replicate sample wells and incubated for 16 hours. Cells were then washed, lysed, and the solution centrifuged. Fifty μL were used if the supernatant was applied to a 96-well plate, followed by 100 μ L of a co-factor solution and 100 μ L of the enzyme substrate luciferin. Luminescence was measured as relative light units (RLU) using a ML 2250 Luminometer. A solvent blank and reference inducers {2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and benzo[a]pyrene (B[a]P)} were also included for each sample test run.

Benzo[a]pyrene equivalents (B[a]PEq) were calculated for all sample extracts and duplicate extracts. The B[a]PEq is a measure of the CYP1A1-inducing PAHs, plus any coplanar PCBs, dioxins or furans that may be present in the sample and are calculated as follows:

$$
B[a]PEq (\mu g/g) = \frac{fold'' \text{ induction}}{60} \times \frac{\text{volume'' factor}}{\text{dry'' weight}} \times \text{df}.
$$

Fold induction is calculated as the mean relative light units (RLU) produced by the sample divided by the mean RLU produced by the solvent blank. The factor of 60 represents the approximate fold induction produced by 1.0 μ g of B[a]PEq/mL. The volume factor (400) represents the total extract volume (2 mL) divided by the volume extract applied to the cells (5 μ L). Dividing by the dry weight of each sample, calculated using percent solids of the 20 g samples, yields B[a]PEq in μ g/g dry weight. If a dilution is used, the B[a]PEq value is multiplied by the dilution factor (df).

A standard curve for dioxin/furan mixture demonstrated that fold induction per mL is equal to the dioxin Toxic Equivalents (TEQ_{HRGS}) in pg/g dry weight. Therefore, the equation to express the data as only chlorinated inducers (in ng/g) is as follows:

 TEQ_{HRGS} = fold induction x $\frac{\text{volume}^{\text{v}}}{1000^{\text{v}}}$ x" dry" weight $\,$ x df.

Tier II testing was conducted on the three sample extracts producing the highest level of induction at 16 hours of exposure. Selected sample extracts (sites 2, 4 and 5) were used in the HRGS assay at 6 and 16 hours of exposure to evaluate the contribution from rapid-acting PAHs and the chlorinated inducing compounds (dioxins, furans, coplanar PCBs) which require 16 hours for maximum response.

The results are listed in Tables III.3 and III.4.

2.3.3.2. Microtox[™]

The MicrotoxTM assay was performed using dichloromethane extracts of sediments provided to NOAA/NOS/NCCOS/CCEHBR by Columbia Analytical Services.

A suspension of luminescent bacteria, Vibrio fischeri, was thawed and reconstituted with deionized water, covered and stored in a 4° C well on the MicrotoxTM analyzer. To assess toxicity, each sample was diluted into four test concentrations. A total of three replicate analyses were performed for each sediment sample. The percent decrease in luminescence in each concentration relative to the reagent blank was then calculated and used to calculate an EC50 (the sediment concentration causing a 50% reduction in luminescence). EC50 results are reported as mg/ml (corrected for dry wt.). Site-specific toxicity was evaluated by comparing to a reference site (North Inlet, SC) using ANOVA and Multiple Comparison Tests as well as a nonparametric Distribution Free approach. Results are listed in Table III.5

$2.3.3.3.$ MutatoxTM

The MutatoxTM genotoxicity bioassay was performed by NOAA/NOS/NCCOS/CCEHBR as described in Microbics Corporation's Mutatox[™] manual using the same solvent extracts prepared for the MicrotoxTM organic extract assay (Microbics Corporation, 1993).

Two assay protocols were utilized. The first, the S-9 assay, utilizes media which contain mammalian hepatic enzymes which metabolize promutagenic compounds and thus can be used to screen sediments for mutagens which require metabolic activation. The second assay, the direct assay, uses media which contains no mammalian enzymes and thus can be used to screen for mutagens which do not require activation. The mutagenic potential of samples was evaluated using the criteria described in the Microbics Corporations' MutatoxTM Manual (Microbics Corporation, 1993). A total of three replicate analyses were performed for each sediment sample. A sediment was considered to be mutagenic only if all three replicates met the criteria for mutagenicity. Results are listed in Table III.6

2.3.4. Bioassay using sediment porewaters

2.3.4.1. Sea urchin fertilization

Sea urchin (Arbacia punctulata) fertilization pore-water toxicity tests were performed at the US Geological Survey National Biological Service (NBS), Texas Gulf Coast Field Station, Corpus Christi, TX.

Urchin sperm was exposed for 30 min to 100%, 50% and 25% dilutions of sediment porewater using 0.45 μ m filtered seawater. The reference porewater sample used was collected from Redfish Bay, TX. Salinity of the porewaters was adjusted as needed using a brine prepared with Milli-Q deionized water. Subsamples of sperm exposed to porewater were removed for DNA damage assessment (see Section 2.3.6.3).

2.3.4.2. Sea urchin embryological development

After 30 min exposure, sea urchin eggs were added to the sperm to determine fertilization. The number of embroys is determined.

2.3.5. Grass shrimp acetylcholinesterase activity

Organophosphate and carbamate insecticides produce toxicity in vertebrates and invertebrates by inhibiting the nervous system enzyme acetylcholinesterase, AChE. The inhibition of this enzyme can be used as a biomarker of exposure and/or effects due to these classes of pesticides. The use of this biomarker offers several advantages over chemical contaminant monitoring alone. First, this indicator will respond to any chemical which produces toxicity through this mechanism. Additionally, the inhibition produced by many of these compounds persists long after waterborne chemical concentrations have decreased to nondetectable levels.

Previous work on whole body AChE activity was performed on *Palaemonetes pugio* to determine the presence of AChE (Key et al., 1998). Each sample analyzed consisted of two adult shrimp. Depending on the number of shrimp that were collected from each of the sites, the number of samples ranged from 6 to 10. Each sample was homogenized (Pro Scientific model Pro 200 motor with a 20 mm x 150 mm stainless steel generator) on ice in 50 mM Tris-HCl buffer (pH $= 8.1$) at 20 mg/mL for 45 seconds. Next, 75 μ L of each homogenate was added to a test tube containing 1.425 mL of Tris HCl buffer. After a 15 min incubation period at 30 $^{\circ}$ C, 967 μ L of the dilute homogenate was added to a cuvette containing 33 μ L of 0.87% 5.5'-dithiobis-(2nitrobenzoic acid), the color reagent. Finally, 10 μ L of 75 mM acetylthiocholine, the substrate, was added to the cuvette then covered with parafilm, inverted to mix, and placed in a spectrophotometer to read continuously for 1 min at a wavelength of 412 nm. For each homogenate sample, three subsamples was assayed. A fourth subsample was incubated with 15 μ L of 10 μ M eserine to account for nonenzymatic hydrolysis of the substrate. The protein content of the homogenate was determined using the Sigma Assay Procedure, a modification of the original Lowry method (Lowry et al., 1951). Whole body AChE activity was reported as nmol product formed/mg protein/min.

Statistical analysis of the results from the AChE analysis was evaluated using ANOVA and Dunnett's Multiple Comparison Test. All statistical analyses used the lab-reared P. intermedius as the control group. Results are listed in Table III.9.

2.3.6. DNA damage

Increased or higher incidence of DNA damage in fish or mussel tissue has previously been found to be correlated with contamination. In this study, testing with oysters and sea urchin sperm were carried out on an exploratory basis to further evaluate the applicability of the procedure as a biomarker.

2.3.6.1. Oysters

In the laboratory, a notch was filed in each oyster to allow the passage of a 25-gauge syringe needle that was inserted into the adductor muscle and 100 μ L of hemolymph withdrawn. The hemolymph was placed in a 1.5 mL microcentrifuge tube and spun at 600 x g for 2 minutes to pellet hemocytes. The supernatant was discarded and cell pellets resuspended in 1 mL icecold cryopreservation solution, gently mixed, and frozen on dry ice. The samples were kept frozen, and shipped to the US Navy Space and Naval Warfare Systems Center (SSC-SD), San Diego, CA, Biomarker Lab for DNA damage analysis.

For SCG electrophoresis or Comet assay, frozen samples were thawed on ice, 200 uL of the sample were transferred to a fresh microcentrifuge tube on ice, and the cells pelleted by spinning at 600 x g for 2 min. Depending on the size of the pellet, which is proportional to the number of cells in the pellet, the pellet was re-suspended in anywhere from 50 to 200 μ L of LMA/Kenny's solution (0.65% low melting point agarose in Kenny's salt solution, 0.4 M NaCl, 9 mM KCl, 0.7 mM K₂HPO₄, and 2 mM NaNCO₃, pH 7.5) at 30 °C, and 50 μ L of the suspension was coated on a SCG/Comet slide. Results are listed in Table IV.2.

2.3.6.2. Amphipods

For SCG/Comet analysis frozen samples were thawed on ice and 3 - 4 amphipods (Ampelisca abdita) from each tube were transferred into a fresh microcentrifuge tube. All accompanying cryopreservative medium was discarded and the organisms suspended in 200 μ L ice cold Kenny's salt solution. The organisms were homogenized briefly in the tube using a mini-pestle and 200 μ L of the suspended cells transferred to a fresh microcentrifuge tube on ice. The cells were pelleted by spinning at $600 \times g$ for 2 min and depending on the size of the pellet was re-suspended in anywhere from 100 to 200 μ L LMA/Kenny's agarose at 30 °C. Fifty microliters were withdrawn and coated on a SCG/Comet slide. Results are listed in Table $IV.3.$

2.3.6.3. Sea urchin sperm

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The LMA/Kenny's resuspended cells (mentioned in Section 2.3.4.1) were applied to slides previously coated with 0.65% agarose [Fisher Biotech, low electroendosmosis (EEO)^{*} agarose] in 40 mM tris-acetate and 1 mM EDTA, at pH 7.5 (TAE buffer)^{Δ}, or in the case of the urchin sperm samples applied to a GelBond sheet. A slide cover was placed over the sample which was then allowed to gel on an ice chilled stainless steel tray for 3 min. A top-coat of 50 μ L agarose was applied over the sample, the coverslip replaced, and the gelling step repeated. After gelling, the coverslip was removed and the slides placed in a lysing solution of 2.5 M NaCl, 10

^{*} Electroendosmosis (EEO) is a functional measure of the number of sulfate and pyruvate residues present on the agarose polysaccharide. This phenomenon occurs during electrophoresis when the anticonvective medium (the agarose) has a fixed negative charge. In an electric field, the hydrated positive ions associated with the fixed anionic groups in the agarose gel migrate toward the cathode. Water is thus pulled along with the positive ions, and migration of the negative molecules such as DNA is retarded.

 Δ TAE buffer is composed of Tris, EDTA-Na₂-salt and acetic acid.

mM Tris [tris(hydroxymethyl)aminomethane], 0.1 M EDTA, 1% Triton X-100[◇], and 10% DMSO at pH 10.0 in a glass screw-top Coplin jar and incubated at 4 °C for at least 1 hr.

Between-batch variability of SCG/Comet slides was monitored by running laboratory standards prepared from bird blood cells of known damage levels.

Prior to unwinding and electrophoresis, the lysing solution was rinsed from the slides with three 2-min rinses of distilled water. The rinsed slides were placed in a submarine gel electrophoresis chamber filled with 300 mM NaOH and 1 mM EDTA, and the DNA allowed to unwind under alkaline conditions for 15 min. After unwinding, electrophoresis was performed at 300 mA, 25 V for 10 min. The slides were transferred to Coplin jars and neutralized with three 2-min rinses in 0.4 M Tris at pH 7.5. Excess solution was blotted away, and the neutralized slides fixed in ice cold ethanol for 5 minutes. The fixed slides were dried in an oven at 37 °C for 20 minutes and transferred to slide boxes for storage.

To determine the levels of DNA damage, the slides were stained with 35 μ L of a 20 μ g/mL solution of ethidium bromide^{*} in distilled water (EtBr), and covered with a coverslip. Stained slides were analyzed by viewing at 200x with an epifluorescent microscope (excitation filter 510-560 nm green light, barrier filter 590 nm) with an attached CCD camera and image analysis software (Komet image analysis system, Kinetic Imaging, Ltd., UK).

For all Comet assays, the fluorescent "head" or nucleus diameter and the length (μm) of any accompanying trailing DNA "tails" resulting from strand breakage are measured for each nucleus analyzed. Measurements were made in five sectors on each slide, counting 5 nuclei in each sector randomly positioning the lens above each sector and counting left to right from the upper left-hand corner of the field of view. Overlapping nuclei or tails were not counted. For the oyster samples 25 nuclei from each individual were scored, 25 nuclei from each replicate of amphipods, and 3 subsamples were prepared and separately scored from each urchin sperm sample. The image system calculated a large number of quantitative parameters for each nuclei, the most important being the total intensity of each comet (comet optical intensity), the percentage of damaged DNA in the tail, and the tail moment (TM) which is the product of the percentage of DNA in the tail times the tail length divided by 100. Data was analyzed by ANOVA using InStat statistical software (GraphPad). Results are listed in Table IV.4.

2.3.7. Benthos

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At the Barry A. Vittor & Associates, Inc. (BVA) laboratory, benthic sediment samples were inventoried, rinsed gently with tap water through a 0.5 mm mesh sieve to remove preservatives and sediment, stained with Rose Bengal, and stored in 70% isopropanol solution until processing. Sample material (sediment, detritus, organisms) was placed in white enamel trays for sorting under Wild M-5A dissecting microscopes. All macroinvertebrates were carefully removed with forceps and placed in labeled glass vials containing 70% isopropanol. Each vial represented a major taxonomic group (e.g. Polychaeta, Mollusca, Arthropoda). All sorted macroinvertebrates were identified to the lowest practical identification level (LPIL), which in most cases was to species level unless the specimen was a juvenile, damaged, or otherwise unidentifiable. The number of individuals of each taxon, excluding fragments, was recorded. A voucher collection was prepared, composed of representative individuals of each species not previously encountered in samples from the region.

 \diamond Detergent, octylphenol ethylene oxide condensate.

^{*} 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide, CAS number 1239-45-8.

All data generated as a result of laboratory analysis of macroinfaunal samples were first coded on data sheets. Enumeration data were entered for each species according to site and replicate. These data were reduced to a data summary report for each site, which included a taxonomic species list and benthic community parameters information. Archive data files of species identification and enumeration were prepared. The data and quality assurance/quality control (QA/QC) reports for the Biscayne and Manatee Bay samples are given in Appendix V. Quality control comments for common LPIL taxa are annotated in data tables. Summary of results are in Table V.6.

3. RESULTS

3.1. Seawater

3.1.1. Pesticides

The results of analyses of pesticides are listed in Table I.1. Eight of the 52 pesticides analyzed for were found in the seawater samples. These were two herbicides (atrazine and metolochor) and two herbicide metabolites (CEAT and CIAT), three organophosphate insecticides (chlorpyrifos, diazinon and malathion), and a DDT metabolite (4,4'-DDE) (Table I.1). The herbicides were the most prevalent compounds with metolochor present at all sites sampled. Organophosphates were detected at three sites including Military Canal and North Canal. Higher levels were found at the upstream sites than at the mouth of the canals.

3.1.2. Alkyl phenols

Most concentrations were below those of the blanks for these samples. This suggests that there was a background problem with the sample containers. Use of a larger volume of water would have lowered the limit of detection. In spite of the moderate blank levels, two sites did stand out as having moderate levels of the ethoxylates, especially nonylphenolethoxylate. The Florida City Mouth sample had moderate levels of nonylphenols and the Princeton Canal Mouth had rather high levels of the 3- and 4-ethoxylates and moderate levels of the 3- and 4 octylphenolethoxylates. These octylethoxylate compounds were unique because usually the tetramethylbutylphenol is the only alkyl chain component present, but in these samples there appears to be significant levels of the branched chain octylphenol forms which would easily double the amount at the Princeton Canal Mouth. Of the two, nonylphenol versus octylphenol, it is the octyl form which is the more potent endocrine disruptor. Therefore these results may signify problems for this area. It appears likely that there is some sewage treatment discharge at this site or perhaps an industrial discharge that may account for the presence of these compounds.

The results of water analyses for nonylphenols, nonylphenol ethoxylates, octylphenol, and octylphenol ethoxylates are listed in Table I.2. Concentrations were generally lower than the highest values measured in effluent-dominated rivers in the upper Midwest (Barber et al., 1999; Snyder et al., 1999). In the Des Plains, Illinois and Detroit Rivers, the concentrations of nonylphenol were about 0.5 $\mu q/L$ and the total amount of ethoxy nonylphenols were often higher. None of these rivers had 3-, 4- and 5-ethoxy substituted nonylphenol concentrations as high as found in these samples. The octylphenols and ethoxylates concentrations in the rivers were comparable to those measured here.

3.2. Sediment

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3.2.1. Chemistry

NOAA's National Status and Trends Program (NS&T) determines the status of, and detects changes in, the environmental quality of the nation's coastal waters. This program monitors levels of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs) congeners, several pesticides, butyltins, and selected trace elements in sediment and mollusk samples from U.S. coastal waters. Sediments collected at the 30 sites in Biscayne Bay were analyzed for the NS&T "suite" of analytes. Results of sediment analyses are listed in Tables II.1 (carbon content, percent moisture and grain size distribution), II.2 (PAHs), II.3 (pesticides, herbicides), II.4 (PCBs), II.5 (major and trace elements), and II.6 (tributyltins) in Appendix II. Results were compared to the nationwide NS&T median and 85th percentile values for sediment (Table II.7). Concentrations above the 85th percentile are in the highest 15% of the data set and are used to indicate "high" concentrations. Distribution of NS&T "high" and "median" concentrations at the sites sampled in this study is shown in Figure"2.

In general, mean analyte concentrations in sediment were below the NS&T "median" with the exception of sediment collected in or at the mouth of the canals. High levels of many NS&T analytes and aggregate∆ data in sediments have been found at Mussel Watch sites near high human population densities and in sediments with a high percentage of clay- and silt-sized particles nationwide. Sites 1 through 5 are south of Miami and drain urban and agricultural areas. Sites 22 through 23 are influenced by the C-111 canal which drains agricultural areas and portions of the Everglades National Park. Sediments from sites 2, 5, 22, 23 and 24 are composed of more than 60% clay- and silt-sized particles.

Shown graphically in Figures 3 and 4, are the results for the sum of concentrations of Cu, Zn, Ni, Pb, Cd, Hg, and Ag (TotTM) as measured by NS&T via hydrofluoric acid extraction. These are the metals whose toxicity is mitigated if the concentration of AVS (S volatized by 1N HCl) exceeds the sum of concentrations of the Simultaneously Extracted Metal (SEM) in 1N HCl. Since TotTM is greater than SEM, if AVS is greater than TotTM, then AVS is also greater than SEM and the metals cannot be toxic (assuming that the assumptions of the AVS/SEM guideline are acceptable) (O'Connor, 1993),^{*} As shown in Figures 3 and 4, the five sites at the mouth of the canals (sites 1 - 5), have high TotTM relative to AVS concentrations, indicating that the sediments may be toxic. Sites 22 - 30 (Manatee) Bay have high AVS concentrations and low TotTM indicating that these sediments are probably not toxic. Site 23 located at the mouth of the C-111 canal has an AVS of 24 μ g/g and a TotTM of 57 μ g/g thus being potentially toxic. Curiously, site 22 located in the C-111 canal itself has slightly higher AVS, 54 μ g/g, and lower TotTM, 41 μ g/g. The salinity at site 22 is slightly lower than that at site 23 (Figure 5). The area between sites 22 and 23 may be a mixing zone where freshwater from the canal mixes with more saline water, resulting in possible deposition of sediment. Such depositional material is often high in Fe and Mn oxyhydroxides, and clay. The concentrations of Fe at sites 22 and 23 are 6360 and 9480 μ g/g, and for Mn, 67 and 91 μ g/g respectively, showing an increase between the two sites. The levels of Al, an indicator of the presence of clays, were 2310 and 6560 μ g/g respectively. Sites 7 and 16 have high TotTM to AVS ratios but the TotTM and AVS values are low. Sediment from these sites have high percentages of sand-sized particles.

 Δ Aggregates are sums of the concentrations of similar chemical compounds such as DDT and its metabolites. The aggregate definitions are found in Table II.7, Appendix II.

 * AVS > TotM > SEM. Therefore if (totM/AVS) < 1, then metals are not considered toxic.

Figure 3. NS&T "median" and "high" concentrations in sediment collected in Biscayne Bay and Manatee Bay.

Figure 4. Sum of the concentrations of trace metals (TotTM = $[Cu] + [Zn] + [Ni] + [Pb] + [Cd] +$ [Hg] + [Ag]) versus AVS concentration (μ g/g dry wt.).

Figure 5. Spatial distribution of bottom salinity (ppt).

3.2.2. Bioassays

3.2.2.1. Juvenile clam assay

The results of the juvenile clam bioassay are shown in Figure 6 and the spatial distribution in Figure 7. Four sites in Biscayne Bay (sites 8, 9, 12, 20) and one site (site 30) in Manatee Bay had >15% mortality and were significantly different from the Folly River reference site. Highest mortality was observed in sediments from site 9 (47%) and site 30 (39%). Sediments from 11 sites (sites 2, 4, 10, 13, 15, 19, 21, 22, 23, 25, 28) were associated with less than 15% mortality, but had survival significantly depressed relative to reference site sediments. Sediments from two of the sites (sites 9 and 30) with the highest mortality also had elevated ammonia levels in the overlying water during the laboratory test. However, two other sites with high mortality (sites 4 and 20) had ammonia levels in the same range as the Folly River reference site, where the sediment bioassay resulted in no mortality. The role that ammonia may have played in the observed toxicity is unclear and should be considered along with other contaminant information.

3.2.2.3. Amphipod survival

Results of the amphipod survival assays are presented graphically in Figures 8 and 9. Results significantly different than controls using Dunnett's one-tailed t-test were found for sites 8, 12, 13, 14, 15, 20, 23 and 30. Site 20, which has the lowest percent survival (27%), was composed of approximately 81% sand. Amphipods do not thrive in sandy sediments. It can be seen in Figure 9 that the sites with low amphipod percent survival had high percentages of sand-sized particles. The sites showing significant amphipod mortality and high percent sand are located in the center of the Bay at some distance from known contamination sources. However, the area where site 20 is located is in a well-known boating recreation area (J. Craynock, NOAA/AOML, personal communication). Aerial reconnaissance of the site from a helicopter and observations from a sampling vessel were performed by NOAA/AOML/Ocean Chemistry Division (Appendix VI). No apparent contamination source, however, was found. No other apparent anthropogenic activity takes place at the sampling site, located west of Elliott Key, a mostly uninhabited key located between Biscayne Bay and the Atlantic Ocean.

3.2.2.4. HRGS P450 analysis

There appeared to be low levels of CYP1A1 inducing compounds in the sediment samples. The three sites (2, 4 and 5) that produced the highest responses tested at two time intervals, appear to contain mostly PAHs. Comparison of the Biscayne Bay results with those of other areas indicate that the sediment samples contain lower amounts of PAHs, coplanar PCBs, dioxins and furans than most previously studied areas. The 3.6 μ g B[a]PEq/g mean and 6.1 upper 99% confidence interval observed in this study are the lowest of any region investigated by NOAA at the time of analysis using the P450 HRGS assay. The earlier Biscayne Bay study produced a mean and upper 99% confidence interval of 8.2 and 10.2 respectively. The two highest values observed in this study were at sites 2 and 4 and were above the 11 μ g B[a]PEq/g that appears to be the level below which effects on the biota would not be expected (Figure 10). Only four sites exhibited concentrations above the upper 99% confidence interval (sites 2, 3, 4 and 5), but none reached the concentration of 32 μ g B[a]PEq/g indicative of potential biological effects. Tier II testing of the samples from sites 2, 4 and 5 showed increases in response from 6 to 16 hours of exposure indicating that the only inducing compounds present in the sediment samples were likely rapid-acting high molecular weight PAHs.

Figure 6. Mortality of juvenile Mercenaria mercenaria clams exposed to sediment. [Red bars indicate results significantly different from reference site ($\alpha = 0.05$) and higher than 15% mortality.]

Figure 7. Spatial distribution of juvenile Mercenaria mercenaria clam survival assay of Biscayne Bay and Manatee Bay sediments.

Figure 8. Survival of Ampelisca abdita exposed to whole sediment from Biscayne Bay during a 10-day toxicity test (percent survival).

Figure 9. Survival of Ampelisca abdita and percent silt- and clay-sized particles in Biscayne Bay and Manatee Bay.

Figure 10. Distribution of HRGS P450 in benzo[a]pyrene equivalent units in Biscayne Bay sediments (μ g B[a]PEq/g).

3.2.2.5. Microtox[™]

The MicrotoxTM results for the 5-min and 15-min assays were considered similar. For the 5min assay, sites 1 - 15 were considered non toxic. For the 15-min awway, sites 1 - 18 were considered non toxic. These sites were all considered non-toxic and were not subjected to further statistical analysis. The toxicities of the remaining sites were compared to that in North Inlet sediments using both nonparametric (Distribution Free) and parametric (ANOVA; Dunnets) procedures. None of the sites were found to be significantly more toxic than North Inlet reference site using the nonparametric approach. Sites 21 - 30 were significantly more toxic than North Inlet sediments at both 5 and 15 minutes using the parametric procedures.

3.2.2.6. Mutatox[™]

Results of the Mutatox[™] assay are listed in Table III.6. Only two of the sediment samples (sites 6 and 11) met the criteria for mutagenicity established in the MutatoxTM Manual for all replicates. The levels of chemical contaminants determined in the sediments from sites 6 and 11 were below the NS&T 85th percentile concentrations (Figure"2).

Figure 11. DNA damage in sea urchin sperm (mean tail moment). Error bar is the standard error of the mean.

3.2.2.7. Sea urchin sperm

DNA damage in sea urchin sperm after exposure to sediment porewater at 100%, 50% and 25% dilution was determined to be statistically significant at sites 5, 11, 21, 23 and 28 at 50% dilution, and significant only at site 23 at 25% dilution. In addition, sea urchin sperm was exposed to control seawater, control sediment porewater, and sediment porewater from sites 1, 2, 3, 4, 5, 8, 9, 18, 21 and 23. Results are presented in Figure 11. Site 23 (C-111 Canal) had damage levels so high that nuclei could not be identified by the image analysis software in 2 of the 3 replicates. A Dunnett's test comparison of all samples (except 23) to the control sediment porewater identified two sites, 2 and 8, as having statistically higher DNA damage than the control. Site 23 is considered to be significant since the damage to nuclei in those samples was many times greater than that observed in samples 2 and 8.

3.2.2.8. Grass shrimp

The results of the grass shrimp AChE assays are provided in Figures 12 and 13. Grass shrimp (P. intermedius) from three of the sites (site 4 [mouth of North Canal], site 33 [North Canal upstream] and site 2 [Military Canal]) had significantly reduced levels of AChE in comparison to a laboratory control population. AChE inhibition is often used as a biomarker of exposure to organophosphate and carbamate insecticides, however, other compounds such as cadmium, mercury and lead have been found to cause decreased levels of AChE activity in crustaceans (Reddy and Venugopal, 1993; Devi and Fingerman, 1995). Surface water analysis revealed two herbicides (atrazine and metolochor) and two atrazine metabolites 2-chloro-4-ethylamino-6 amino-s-triazine (CEAT) and 6-amino-2-chloro-4-isopropylamino-s-triazine (CIAT), three organophosphate insecticides (chlorpyrifos, diazonin and malathion), and an organochlorine metabolite (4,4'-DDE) in seawater collected at these sites (Table I.1).

Figure 12. Statistical significance of grass shrimp AChE assay of Biscayne Bay and Manatee Bay sediment.

Whole body AChE activity in grass shrimp (P. intermedius and P. pugio). [$*$ " Significantly different from P. intermedius control.]

Figure 14. DNA damage in Biscayne Bay oysters (mean tail moment). Error bar is the standard error of the mean. [* Significantly different from control.]

3.3. DNA damage

3.3.1. Oysters

Oyster populations at the sampling sites were not large except at site 4. The oysters collected were of varying size and were found at different tidal exposure areas. The SCG/Comet results of DNA damage in the collected oysters are presented graphically in Figure 14. The variability in individual values in most cases can be attributed to a single high or low value outlier. Though normally distributed, the standard deviations were different enough to warrant using the nonparametric Kruskal-Wallis test. This comparison indicated that only the DNA damage from oysters collected at site 4 was significantly higher than those from the reference site. Though not of statistical significance, the mean TM values at all sites were higher than the reference site. Omission of the highest and lowest data points in each data set resulted in equal standard deviations which allowed parametric analysis using Dunnett's test comparing all sites to the reference value. All sites were identified as having mean TMs significantly higher than the reference even if the outliers in the reference data set were included.

3.3.2. Amphipod survival

The amphipods that survived exposure to Biscayne Bay sediments from sites 1, 2, 3, 4, 5, 8 , 9, 18, 21 and 23 (see Section 2.3.2.3) were examined for DNA damage. The results are shown in Figure 15. No control samples were examined so statistical analysis was limited. The largest TM values were found at sites 1, 2, 3 and 23.

3.3.3. Sea urchin sperm

The sea urchin sperm exposed to control seawater, control porewater and sediment porewater from sites 1, 2, 3, 4, 5, 8, 9, 18, 21 and 23 (see Section 2.3.4.1) were examined for DNA damage. The results are shown in Figure 11. Damage to nuclei of sperm exposed to porewater

Figure 15. DNA damage in Ampelisca abdita exposed to Biscayne Bay and Manatee Bay sediments. [* Significantly different from control.]

from site 23 was so high that the image analysis software was unable to quantify the results. A Dunnett's test comparison of all the results except for those of site 23 to the control sediment porewater sample identified the results of samples from sites 2 and 8 and being statistically significant.

3.4. Benthos

3.4.1. Assemblage structure

Several numerical indices were chosen for analysis and interpretation of the macroinfaunal data. Infaunal abundance is reported as the total number of individuals per site and the total number of individuals per square meter (= density). Taxa richness is reported as the total number of taxa represented in a given site collection.

Taxa diversity, which is often related to the ecological stability and environmental "quality" of the benthos, was estimated by the Shannon-Wiener Index (Pielou, 1966), according to the following formula:

$$
H' = - \frac{s}{i} p_i \left(\ln p_i \right)
$$

where, s is the number of taxa in the sample, i is the i'th taxon in the sample, and p_i is the number of individuals of the i'th taxon divided by the total number of individuals in the sample.

Figure 16. Bottom temperature (°C) in Biscayne Bay and Manatee Bay.

Figure 17. Bottom dissolved oxygen (mg/L) in Biscayne Bay and Manatee Bay.

3.4.2. Data analysis

Taxa diversity within a given community is dependent upon the number of taxa present (taxa richness) and the distribution of all individuals among those taxa (equitability or evenness). In order to quantify and compare faunal equitability to taxa diversity for a given area, Pielou's Evenness Index J' (Pielou, 1966) was calculated as J' = H'/ln S, where $\ln S$ = H' max, or the maximum possible diversity, when all taxa are represented by the same number of individuals; thus, $J' = H'/H'$ max.

3.4.3. Habitat characteristics

Water quality data for the 30 sites are presented in Table V.1 and Figures 5, 16, and 17. Highest bottom water temperatures were found in Manatee Bay and at sites 4, 9, 20 and 21. Bottom salinity ranged from 7 ppt to 21 ppt for the shoreline sites 1 - 5 and between 12 ppt and 35 ppt for the remaining sites in Biscayne Bay. Salinity in Manatee Bay was 20 ppt or less for all sites. Bottom dissolved oxygen in Biscayne and Manatee Bay was below 7 mg/L at the sites close to the canals and at mid Bay. Higher dissolved oxygen levels were observed in a zone offshore from the canals and in Manatee Bay.

Particle clay- and silt-sized particle distribution is shown in Figure 18. Sediments with high percentages of fine particles were found in Manatee Bay, the canals and site 21. The highest percentages of sand-sized particles were found mid Bay.

3.4.4. Benthic community characterization

The complete phylogenetic listing for the Biscayne Bay and Manatee Bay sites as well as data on taxa abundance and strata occurrence is listed in Table V.2. A total of 14,051 organisms, representing 392 taxa, were identified from the 30 sites (Table V.3). The lowest numbers of

Figure 18. Clay- and silt-sized particles (percent) in sediments collected in Biscayne Bay and Manatee Bay.

Figure 19. Number of taxa found in sediments collected in Biscayne Bay and Manatee Bay.

taxa were found in sites 1 - 5, 22 and 23 (Figure 19). These sites correspond to those with large percentages of clay- and silt-sized particles in sediment except for site 21. Polychaetes were the most numerous organisms present representing 41.3% of the total assemblage, followed in abundance by malacostracans (23.2%), gastropods (15.6%), and bivalves (11%). Polychaetes represented 31.8% of the total number of taxa followed by bivalves (21.4%), malacostracans (21.1%) and gastropods (15.2%) (Table V.3). The percentage abundance of the major taxa by site is given in Table V.4 and Figures 20 and 21.

The dominant taxa collected from the 21 Biscayne Bay sites were the gastropod, Caecum pulchellum, the malacostracan, Hargeria rapax and the polychaetes, Exogone rolani and Fabricinuda trilobata, representing 14.8%, 14.2%, 9.1%, and 5.3% of the total number of individuals, respectively (Table V.2). Hargeria rapa and the annelid family, Tubificidae (LPIL) were the most widely distributed taxa being found at 95% of the sites. The distribution of taxa representing less than 10% of the total assemblage at each site is given in Table V.5. Nearshore sites 1 - 5 in Biscayne Bay were dominated by a more estuarine fauna (Table V.5).

The dominant taxon collected from the nine Manatee Bay sites was the bivalve, Brachidontes exustus, representing 46.2% of the total number of individuals (Table V.2). Other common taxa included the gastropod, Caecum pulchellum, the arthropod, Grandidierella bonnieroides, and the annelid family, Tubificidae (LPIL), representing 7.6%, 5.3%, and 5.2% of the total number of individuals, respectively. Tubificids were the most widely distributed taxon being found at 100% of the sites. The sites in Manatee Bay were dominated by a more estuarine fauna than all but the most near shore sites in Biscayne Bay (Tables V.2 and V.5). For example, tubificid oligochaetes were the dominant taxa at 4 of the 9 sites, while the chironomid, Clunio (LPIL) was abundant at two of the nine sites in Manatee Bay.

Figure 20. Percent abundance of major taxonomic groups for the Biscayne Bay sites.

Figure 21. Spatial distribution of major taxonomic groups for the Manatee Bay sites.

Figure 22. Spatial distribution of macroinvertebrate density in Biscayne Bay and Manatee Bay (number of individuals per square meter).

Site abundance and taxa data are summarized for the Biscayne Bay and Manatee Bay sites in Table V.6. In Biscayne Bay the number of taxa per site ranged from 13 at site 2 to 96 at site 7 (Table V.6; Figure 19). Near shore sites 1 through 5 had considerably lower taxa richness than the remaining sites in Biscayne Bay. In Manatee Bay the number of taxa per site ranged from 2 at site 23 to 74 at site 29.

Density per site in Biscayne Bay ranged from 1,075 organisms per square meter at site 2 to 24,725 organisms per square meter at site 7 (Table V.6; Figure 22). Densities were generally lower at the near shore sites 1 through 5. Density per site in Manatee Bay ranged from 150 organisms per square meter at site 23 to 74,050 organisms per square meter at site 25.

Taxa diversity (H') and evenness (J') for the Biscayne Bay and Manatee Bay sites are given in Table V.6 and Figures 23 and 24. Taxa diversity (H') in Biscayne Bay varied considerably and ranged from 1.62 at site 1 to 3.65 at site 20. Diversity was lowest at the near shore sites 1 through 5. Taxa evenness (J') in Biscayne Bay also exhibited considerable variation and ranged from 0.56 at site 15 to 0.88 at site 16. Taxa diversity (H') in Manatee Bay varied considerably and ranged from 0.64 at site 23 to 3.53 at site 29. Taxa evenness (J') in Manatee Bay exhibited variation and ranged from 0.24 at site 25 to 0.92 at site 23.

Figure 23. Taxa diversity, H', for Biscayne Bay and Manatee Bay.

Figure 24. Taxa evenness, J', for Biscayne Bay and Manatee Bay.

4. SUMMARY OF RESULTS

A graphical summary of the assay tests responses for the sites evaluated in Biscayne Bay and Manatee Bay is shown in Figure 25. Not all tests were performed at each site so statistical analysis or calculation of toxicity indices is not warranted. In addition, the ecosystems of Biscayne Bay and Manatee Bay are different and insufficient numbers of samples were collected in Biscayne Bay to allow full characterization of its ecosystem.

- Benthic assessment results are summarized in Figure 26. The benthic summary indicates that the sites located near the canals had fewer species and larger number of individuals, i.e., diversity was low and evenness high, an indication of poor ecological conditions.
- Eight of the 52 pesticides analyzed for were found in the seawater samples. The herbicides were the most prevalent compounds with metolochor present at all sites sampled. Organophosphates were detected at three sites including Military Canal and North Canal. Higher levels were found at the upstream sites than at the mouth of the canals. Concentrations of alkyl phenols in seawater were generally low.
- Mean contaminant concentrations in sediment were below the NS&T "medians" with the exception of sediment collected in or at the mouth of the canals (sites 1 - 5), and at site 16. Sites 1 - 5 have high TotTM relative to AVS concentrations, indicating that the sediments may be toxic. Sites 22 - 30 (Manatee) Bay have high AVS concentrations and low TotTM indicating that these sediments are probably not toxic. Sediment from site 23 located at the mouth of the C-111 canal may be toxic. Site 22 located in the C-111 Canal itself has slightly higher AVS and lower TotTM than site 22. The salinity at site 22 is slightly lower than that at site 23 and the area between the two sampling sites may be the mixing zone.

Figure 25. Summary of assay tests in Biscayne Bay and Manatee Bay.

Figure 26. Summary of benthic assessment of Biscayne Bay and Manatee Bay. (In general, the lighter the colors, the lower the number of taxa, density, diversity and evenness.)

- Three sites had clam mortalities higher than 20%: sites 9, 20 and 30. Low contaminant concentrations were found when sediment chemistry analyses were performed.
- Results of the amphipod Ampelisca survival tests were significantly different than controls for sites 8, 12, 13, 14, 15, 20, 23 and 30. Site 20 has the lowest percent survival, 27%, and the sediment is approximately 81% sand. There are no marinas, canals or other sources of contaminants other than those from recreational boating activities. Ampelisca is known to be sensitive to sediment particle size and these results may reflect that.
- The two highest values observed for HRGS P450 in this study were at sites 2 and 4 and were above the level at which effects on the biota are detected.
- MicrotoxTM tests were of limited use since only one sample from Biscayne Bay and nine samples from Manatee Bay were assayed and all were considered significantly more toxic than the sediment control. Samples from some of the sites were not expected to be statistically significant but all were found to be so.
- Only two of the sediment samples (sites 6 and 11) met the criteria for mutagenicity established in the MutatoxTM Manual. These sites have low sediment contaminant concentrations.
- Sea urchin sperm tests identified three sites, sites 2, 8 and 23, as having statistically higher DNA damage than the control. Site 23 is considered to be significant since the damage to nuclei in those samples was many times greater than that observed in samples 2 and 8.
- Grass shrimp samples from only five of the 30 sites plus four additional sites higher up in the canals were assayed for AChE activity. Three of the sites (sites 2, 4 and 33) had reduced levels of AChE in comparison to a laboratory control population.
- Oyster DNA damage analyses was not performed on all samples and statistically significant damage was detected only in oysters collected at site 4.
- No control samples were examined so statistical analysis of the amphipod DNA assay was limited. The largest values were found at sites 1, 2, 3 and 23.
- Damage to nuclei of sea urchin sperm exposed to porewater from site 23 was so high that the image analysis software was unable to quantify the results. In addition, results of samples from sites 2 and 8 were found to be statistically significant.

In summary, consistent statistically and environmentally significant chemical analysis and assay responses were found at only a few sites: 1, 2, 3, 4, 5, and 23. This is not an unexpected result since the sites are located at the mouth of canals that are known to be contaminated.

5. CONCLUSIONS

This Biscayne bioeffects study used the triad approach to document the environmental health of the ecosystem. The legs of the triad consist of sediment chemistry, species numbers and richness, and bioassays. Viewing the results of the three legs, using the preponderance of evidence approach generally makes it possible to determine where the estuarine/coastal environment is degraded. Work by I. Hartwell and L. Claflin (NOAA/NOS/NCCOS, personal communication, 2003) indicate that the physical parameters of salinity and grain size are also important factors when determining species diversity and richness. For Biscayne Bay, the monitoring sites along the shore (sites 1-5) have the lowest salinity, the greatest amount of fine-sized particles (silt and clay fractions), the greatest number of elevated trace element and organic contaminant concentrations, the lowest number of different taxa, and the lowest species density. It is expected that sites 1-5 would have the highest amount of contamination because these are the Biscayne Bay sites closest to the urban centers and because high contaminants levels are commonly found in sediment with high percentages of fine-sized particles. If this bioeffects study were considered to consist of four components (physical parameters, chemistry, species information, and bioeffects results), the preponderance of evidence for the first three categories of derived information all indicate that shoreline sites (1-5) in Biscayne Bay, and to a lesser extent site 23 in Manatee Bay, are all degraded.

Because Biscayne and Manatee Bays were assessed in 1995, the expanded suite of bioeffects tests (Grass shrimp AchE; and DNA damage in oysters, amphipods and urchins) were only performed using sediments from sites that had previously indicated a potential environmental concern. For the most part, the expanded suite of bioassays bears out those concerns for sites 1-5 and 23. The open water Biscayne Bay site 18 exhibited low sediment contamination, no adverse effects on any of the seven bioeffects assays performed, no unusual results for the physical parameters (i.e. salinity or grain size) and good species density and diversity. While site 21 did exhibit some significant bioassay effects, like those found in the 1995 study (Long et al., 1999) the current conclusion for the open water sites confirm the earlier conclusions: contaminant levels at those sites are generally low with no apparent reason for the few anomalous bioassay results apparent.

In conclusion:

- (1) Sediment sites (1, 2, 3, 4, 5 and 23) near the mouth of canals show evidence of contamination ;
- (2) Contaminant plumes and associated toxicity do not appear to appreciately extend seaward of the mouth of the canals;
- (3) Concentrations of contaminants in the sediments in open areas of Biscayne and Manatee Bays are generally low.

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8. APPENDIX I. Seawater

Table I.1. Pesticides in seawater samples collected in South Florida canals. (Zeros indicate concentrations below instrumental limit of dectection.)

Table I.1 Pesticides in seawater samples collected in South Florida canals. (Zeros indicate concentrations below instrumental limit of dectection.) (cont.)

Table I.1 Pesticides in seawater samples collected in South Florida canals. (Zeros indicate concentrations below instrumental limit of dectection.) (cont.)

Table I.1 Pesticides in seawater samples collected in South Florida canals. (Zeros indicate concentrations below instrumental limit of dectection.) (cont.)

Table I.2. Alkylphenol ethoxylates in seawater samples collected in South Florida canals. (Zeros indicate concentrations below the blank.)

9. APPENDIX II. Sediment chemistry

Site	TOC	TIC	Solids	Sand	Silt	Clay	Fines $(Silt + Clay)$
1	1.75	8.08	39	63.4	25.1	11.5	37
\overline{c}	4.47	6.95	12	17.6	53.0	29.4	82
3	2.12	8.83	30	53.4	34.5	12.1	47
$\overline{4}$	2.84	8.91	27	67.4	25.1	7.5	33
5	3.25	9.97	26	33.8	51.5	14.7	66
$\,$ 6	0.30	10.42	59	87.4	3.9	8.7	13
$\overline{7}$	0.48	10.51	59	89.8	4.1	6.1	10
$\,8\,$	0.59	9.12	59	79.4	9.2	11.5	21
9	0.39	7.67	61	89.0	4.4	6.6	11
10	0.42	10.66	61	90.0	4.2	5.7	10
11	0.36	7.08	69	90.4	3.1	6.5	10
12	0.27	5.35	68	92.5	1.3	6.2	$\,8\,$
13	0.43	10.43	60	91.5	6.1	2.3	8
14	0.26	7.88	66	93.0	4.6	2.3	$\overline{7}$
15	0.25	3.74	72	94.3	2.5	3.3	6
16	0.14	1.22	74	96.8	1.9	1.3	3
17	0.19	2.47	73	95.6	2.6	1.8	4
18	0.15	1.09	75	97.4	2.0	0.6	3
19	0.22	1.56	72	95.3	3.4	1.3	5
20	1.01	5.45	54	81.1	10.2	8.8	19
21	2.04	8.62	33	59.1	20.8	20.1	41
22	2.45	7.92	35	6.1	48.5	45.5	94
23	2.47	6.93	27	5.1	64.2	30.8	95
24	1.62	7.74	31	19.9	31.8	48.4	80
25	0.89	9.53	41	61.3	17.2	21.5	39
26	2.25	8.64	35	57.3	25.0	17.7	43
27	1.15	8.34	44	75.2	11.2	13.7	25
28	1.33	9.01	34	49.1	30.0	21.0	51
29	2.60	6.03	31	42.8	30.6	26.6	57
30	1.26	8.25	30	73.4	13.0	13.7	27

Table II.1. Carbon content, solids, and particle size distribution in Biscayne Bay sediments (percent).

* Total PAHs: The sum of concentrations of the PAH compounds determined.

∆ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

* Total PAHs: The sum of concentrations of the PAH compounds determined.

 Δ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

* Total PAHs: The sum of concentrations of the PAH compounds determined.

 Δ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

* Total PAHs: The sum of concentrations of the PAH compounds determined.

 Δ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

* Total PAHs: The sum of concentrations of the PAH compounds determined.

 Δ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

Site	26	27	28	29	30
Total PAHs*	89.1	66.8	126.0	105.5	55.2
Total NS&T PAHs [∆]	27.2	22.3	56.7	35.4	18.9
Naphthalene	3.2 J	J 2.8	17.2	4.3 J	2.6 J
C1-Naphthalenes	3.6 J	3.5 J	17.5	5.5 J	3.1 J
C2-Naphthalenes	4.0 J	4.2 J	7.3 J	6.9 J	3.8 J
C3-Naphthalenes	3.3 J	2.8 J	3.2 J	4.1 J	2.8 J
C4-Naphthalenes	1.8 J	1.5 J	2.0 J	2.0 J	1.5 J
Biphenyl	0.9 J	0.9 J	1.1 J	1.2 J	0.6 J
Acenaphthylene	0.3 J	0.2 J	0.2 J	0.9 J	0.2 J
Acenaphthene	0.9	0.7	0.8	1.3	0.6 J
Fluorene	0.7 J	0.5 J	0.7 J	J 0.8	0.4 J
C1-Fluorenes	2.1 J	1.8 J	1.9 J	2.9 J	2.1
C2-Fluorenes	4.3	2.4	4.8	6.4	2.3
C3-Fluorenes	8.9	6.5	6.9	3.5	3.7
Phenanthrene	2.0 J	1.2 J	1.5 J	2.1 J	1.3 J
Anthracene	1.0 J	0.5 J	0.8 J	1.8	0.6 J
C1-Phenanthrenes/Anthracenes	2.5	1.4 J	2.1 J	2.5 J	1.4 \Box
C2-Phenanthrenes/Anthracenes	3.2	1.8 J	4.6	2.9	2.3
C3-Phenanthrenes/Anthracenes	4.5	2.3	5.9	4.0	2.6
C4-Phenanthrenes/Anthracenes	3.0	2.6	5.1	3.4	1.6 J
Dibenzothiophene	0.3 J 0.9	0.2 J 0.6 J	0.3 J 1.1	0.4 J	0.2 IJ 0.6 J
C1-Dibenzothiophenes	J 1.5 J	0.9 J	J 2.7	0.8 J 1.7 J	0.9 J
C2-Dibenzothiophenes C3-Dibenzothiophenes	1.8 J	1.0 J	4.4	2.7 J	J 1.4
Fluoranthene	2.8	2.0	2.4	3.3	1.6
Pyrene	2.1 J	1.7 J	2.0 J	2.5 J	1.3 J
C1-Fluoranthenes/Pyrenes	3.0 J	2.8 J	4.2 J	5.0 J	1.4 \cdot
Benzo[a]anthracene	0.8 J	0.7 J	0.5 J	1.0 _J	0.6 IJ
Chrysene	1.9	1.3	1.9	2.0	1.1
C1-Chrysenes	1.3	1.1	1.3	1.9	0.6 \Box
C2-Chrysenes	4.5	2.7	4.6	2.9	0.9 J
C3-Chrysenes	2.0	2.2	2.2	3.7	1.2
C4-Chrysenes	1.7	J 0.9	1.0 J	$1.0\,$ J	0.6 J
Benzo[b]fluoranthene	3.3	2.6	2.7	4.8	2.3
Benzo[k]fluoranthene	1.1	0.7	0.7 J	1.3	0.6 J
Benzo[e]pyrene	1.4 J	1.3 J	1.2 J	2.3 J	1.0 \Box
Benzo[a]pyrene	1.5 J	1.2 J	1.1 J	2.2 J	1.0 J
Perylene	1.2 J	1.4 \cdot	3.3 J	1.8 J	1.0 _J
Indeno[1,2,3- c ,d]pyrene	2.8	1.8	2.2	3.5	1.6
Dibenzo[a,h]anthracene	0.2 J	0.2 J	0.2 J	J 0.4	0.2J
Benzo[g,h,i]perylene	2.8 J	1.9 J	2.3 J	4.0 J	1.7 J
2-Methylnaphthalene	2.4 J	2.3 J	11.4	3.7 J	2.0 J
1-Methylnaphthalene	1.3 J	1.2 J	6.1	1.9 J	1.1 J
2,6-Dimethylnaphthalene	2.5 J	1.9 J	3.9	2.5 J	1.6 J
1,6,7-Trimethylnaphthalene	0.8 J	0.6 J	0.8 J	0.9 J	0.5 J
1-Methylphenanthrene	0.5 J	0.4 J	0.7 J	0.7 J	0.4 J

Table II.2. Polycyclic aromatic hydrocarbons (PAHs) in Biscayne Bay sediments (ng/g dry weight) (cont.).

* Total PAHs: The sum of concentrations of the PAH compounds determined.

 Δ Total NS&T PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

J - Value below the limit of detection.

Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.

Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight) (cont.).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.

Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight) (cont.).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.
Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight) (cont.).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.

Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight) (cont.).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.

Table II.3. Pesticides in Biscayne Bay sediments (ng/g dry weight) (cont.).

B - Blank contamination greater than three times the limit of detection.

I - Interference.

J - Value below the limit of detection.

Table II.4. PCBs in Biscayne Bay sediments (ng/g dry weight).

ND - Not detected.

J - Value below the defined limit of detection.

Table II.4. PCBs in Biscayne Bay sediments (ng/g dry weight) (cont.).

ND - Not detected.

J - Value below the defined limit of detection.

Table II.4. PCBs in Biscayne Bay sediments (ng/g dry weight) (cont.).

ND - Not detected.

J - Value below the defined limit of detection.

* TotTM = Sum of concentrations of Cu, Zn, Ni, Pb, Cd, Hg and Ag.

Table II.6. TBTs in Biscayne Bay sediments (ng Sn/g dry weight).

ND - Not detected.

J - Value below the limit of detection.

Table II.7. NS&T Mussel Watch sediment data medians and 85th percentile values (1986 - 1993). (Medians and percentiles were determined using the average at each site across all sampled years. Element data in μ g/g dry wt. unless noted, and organic data in ng/g dry wt.).

∑DDTs: The sum of concentrations of DDTs and its metabolites, DDEs and DDDs.

∑PCBs: The sum of the concentrations of homologs, which is approximately twice the sum of the 18 congeners.

∑PAHs: The sum of concentrations of the 18 PAH compounds determined on a long term basis as part of the NS&T Program.

∑Cdane: The sum of cis-chlordane, trans-nonachlor, heptachlor and heptachlorepoxide.

∑Dieldrin: The sum of dieldrin and aldrin.

∑BTs: The sum of the concentrations of tributyltin and its breakdown products dibutyltin and monobutyltin (as ng Sn/g dry wt.). n: Number of data points (roughly equivalent to the number of sampling sites).

10. APPENDIX III. Sediment bioassay

Table III.1. Survival of Mercenaria mercenaria exposed to whole sediment from Biscayne Bay during a 10-day toxicity test.

ns - Not significant.

 * - results significantly different than controls, α < 0.05.

Table III.2. Survival of Ampelisca abdita exposed to whole sediment from Biscayne Bay during a 10-day toxicity test.

 \circ - Percent survival based on 100 organisms per sample except for site 21 which is based on 80 organisms per sample.

ns - Not significant.

** - Results significantly different than controls using Dunnett's one-tailed t-test and differences exceed minimum detectable significance, α < 0.01.

Table III.3. HRGS P450 and toxic equivalent results for Biscayne Bay sediments.

* NIOL. North Oyster Inlet Landing reference sample.

Table III.4. HRGS P450 of Tier II testing of selected Biscayne Bay sediments.

Table III.5. Microtox™ tests using dichloromethane extracts of Biscayne Bay sediments.

< - Significantly less toxic than North Inlet (ANOVA; Dunnet's test).

> - Significantly more toxic than North Inlet (ANOVA; Dunnet's test).

NC - Not calculated.

NS - Not significantly different from North Inlet (ANOVA; Dunnet's test).

Table III.6. Mutatox™ tests using dichloromethane extracts of Biscayne Bay sediments.

nm - Sample did not meet criteria for mutagenicity.

m - Sample met criteria for mutagenicity.

Table III.7. Sea urchin fertilization bioassay data for Biscayne Bay sediments.

Table III.7. Sea urchin fertilization bioassay data for Biscayne Bay sediments (cont.).

ns - Not significant.

** - Results significantly different than controls using Dunnett's one-tailed t-test and differences exceed minimum detectable significance, α < 0.01.

++ - Results significantly different than controls using Dunnett's one-tailed t-test, α < 0.01.

+ - Results significantly different than controls using Dunnett's one-tailed t-test, α < 0.05.

Table III.8. Urchin development bioassay data for Biscayne Bay sediments.

Urchin Development at 25%

Table III.8. Urchin development bioassay data for Biscayne Bay sediments (cont.).

ns - Not significant.

** - Results significantly different than controls using Dunnett's one-tailed t-test and differences exceed minimum detectable significance, α < 0.01.

++ - Results significantly different than controls using Dunnett's one-tailed t-test, α < 0.01.

+ - Results significantly different than controls using Dunnett's one-tailed t-test, α < 0.05.

Site	Count	Mean	Std. Dev.	Std. Err.	
	7	57.5	14.7	5.56	
2	12	47.9	7.19	2.07	Significant
4	10	45.0	9.6	3.04	Significant
5	10	49.2	8.24	2.61	
23	10	60.9	10.2	3.22	
$31*$	6	59.9	10.2	4.16	
$32*$	6	64.4	8.11	3.31	
$33*$	12	40.9	4.11	1.19	Significant
$34*$	10	58.9	7.05	2.23	
Control P. intermedius	10	59.2	11.2	3.55	
Control P. pugio	10	56.5	9.04	2.86	

Table III.9. Grass shrimp acetylcholinesterase activity (nmol/mg P/min).

* Sampled only for shrimp AChE assay.

11. APPENDIX IV. DNA damage

Table IV.1. Physical and chemical data collected December 1, 1999 during oyster field sampling.

* Samples collected December 2, 1999.

Table IV.2. Oyster DNA damage results.

* Reference sample collected in Little Card Sound.

Table IV.3. Amphipod DNA damage results.

Table IV.4. Sea urchin sperm DNA damage results.

12. APPENDIX V. Benthos

Table V.1. Summary of site location and water quality data for the Biscayne Bay and Manatee Bay sites.

Table V.1. Summary of site location and water quality data for the Biscayne Bay and Manatee Bay sites (cont.).

Table V.2. Abundance and distribution of taxa for the Biscayne Bay and Manatee Bay sites.

Table V.2. Abundance and distribution of taxa for the Biscayne Bay and Manatee Bay sites (cont.).

Biscayne Bay:

Table V.2. Abundance and distribution of taxa for the Biscayne Bay and Manatee Bay sites (cont.).

Biscayne Bay:

Table V.2. Abundance and distribution of taxa for the Biscayne Bay and Manatee Bay sites (cont.).

Biscayne Bay:

L,

L,

Manatee Bay:

Manatee Bay:

Manatee Bay:

Manatee Bay:

Taxa Key

Phoronida Platyhelminthes rb = Turbellaria
Porifera Rhynchocoela nop = Anopla Sipuncula[']

Table V.5. Percentage abundance of dominant benthic macroinfaunal taxa (>10% of the total) for the Biscayne Bay and Manatee Bay sites.

Table V.5. Percentage abundance of dominant benthic macroinfaunal taxa (>10% of the total) for the Biscayne Bay and Manatee Bay sites (cont.).

Table V.5. Percentage abundance of dominant benthic macroinfaunal taxa (>10% of the total) for the Biscayne Bay and Manatee Bay sites (cont.).

Table V.6. Summary of benthic macroinfaunal data for the Biscayne Bay and Manatee Bay sites.

13. APPENDIX VI. Aerial photography

Location of aerial images in the NOAA/NOS Coastal Photography website.

Plate VI.1. Princeton Canal.

[5WPA1398. 1999. Scale 1:40000; azimuth 211.4; 25.526° N, 80.320° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts.nos.noaa.gov/images/photos/5wpa 1398.gif>.]

Plate VI.2. Military, Mowry Canals, North and Florida City Canals.

[5WPA1400. 1999. Scale 1:40000; azimuth 211.2; 25.472° N, 80.356° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http:// mfproducts.nos.noaa.gov/images/photos/5wpa1400.gif>.]

Plate VI.3. Turkey Point.

[5WPA1376. 1999. Scale 1:40000; azimuth 32.9; 25.411° N, 80.313° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts. nos.noaa.gov/images/photos/5wpa 1376.gif>.]

Plate VI.4. Elliott Key, Caesar's Creek and Old Rhodes Key.

[5WPA1356. 1999. Scale 1:40000; azimuth 212.4; 25.412° N, 80.225° W. (Coastal Aerial Photography, NOAA/ National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts.nos. noaa.gov/images/photos/5wpa1356.gif>.]

Plate VI.5. Sands Key and Elliott Key.

[5WPA1533. 1999. Scale 1:40000; azimuth 186.5; 25.478° N, 80.194° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts.nos.noaa.gov/images/photos/5wpa 1533.gif>.]

Plate VI.6. Sands Key and Ragged Keys.

[5WPA1531. 1999. Scale 1:40000; azimuth 186.2; 25.533° N, 80.187° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts.nos.noaa.gov/images/photos/ 5wpa1531.gif>.]

Plate VI.7. Manatee Bay and the C-111 Canal.

[5WPA1370. 1999. Scale 1:40000; azimuth 30.2; 25.258° N, 80.419° W. (Coastal Aerial Photography, NOAA/National Ocean Service, <http://mapfinder.nos.noaa.gov:80/>, <http://mfproducts.nos.noaa.gov/images/photos/5wpa 1370.gif>.]

Plate VI.8. Recreational boat mooring site east of Elliott Key. [Photographed by J. Craynock (NOAA/AOML), on December 19, 2001 from an R-22 Helicopter (Wilderness Air and Land, Miami, FL) at an altitude of 600 ft.]

Plate VI.9. Recreational boat mooring site east of Elliott Key. [Photographed by J. Craynock (NOAA/AOML), on December 19, 2001 from an R-22 Helicopter (Wilderness Air and Land, Miami, FL) at an altitude of 600 ft.]

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