Status of Contaminant Levels in Biota and Sediments of the St. Lucie Estuary

NOAA Technical Memorandum NOS NCCOS 32

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Cover photograph of Boat in the St. Lucie Estuary

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Sediment Quality Triad

1.0 Introduction

1.1 Background

Largely in response to increasing human population along the coast and typical of south Florida's estuaries and coastal bays, St. Lucie Estuary and its watershed have experienced considerable alterations due to increased agricultural and urban development. Most notable in this regard is a freshwater management system consisting of a system of canals, drainage ditches and water control structures. In addition to affecting freshwater supply to the estuary, the system has also increased the drainage area of the estuary, now estimated at 2,000 sq km. Further, it transports fungi and other pathogens, contaminants, sediment and organic matter into the estuary. The C-44 canal, one of several drainage canals, conveys flood control discharges from Lake Okeechobee to the South Fork of the estuary. Large quantities of freshwater release from Lake Okeechobee after heavy rainfall associated with an El Nino-Southern Oscillation (ENSO) reportedly produced unusually high incidences of fish disease and mortality, promoted toxic dinoflagellate blooms, and reduced overall biodiversity of estuarine and freshwater fish communities. Prompted by widespread concern about the water quality and biological resources of the estuary, the St. Lucie River Issue Team (SLRIT), established under the South Florida Ecosystem Restoration Working Group, identified and recommended funding of several research project to address water quality related resource management issues in the estuary and its watershed. This study, performed under a Joint Project Agreement between NOAA and State of Florida was designed to characterize the estuary in terms of chemical contamination and its associated adverse biological effects.

This NOAA study was one of the eight selected for funding by the St. Lucie River Issue Team (SLRIT) in the Year 2000. The study purpose was to characterize St. Lucie Estuary in terms of environmental toxicity and to describe the extent and severity of habitat degradation using the sediment quality triad approach. The study was specifically designed to monitor and assess the concentrations, distributions, and biological effects (including potentially multi-generational genotoxic damage) of multiple stressors in the St. Lucie Estuary and its environs. The study addressed various aspects of key environmental issues that have been defined for the estuary, including possible impacts from agricultural runoff (i.e., those from citrus groves and row crops), urbanization and probable relationships between prevalence of fish abnormalities and severity of contamination.

The St. Lucie Estuary (SLE) in southeast Florida, discharges to the Atlantic Ocean thought the St. Lucie Inlet by way of the southern portion of the Indian River Lagoon. Extensive urban and agricultural drainage projects in the SLE watershed have increased the drainage basin to almost 775 square miles (Haunert et al. 1994). Canals in the watershed include C-23, C-24, part of the Central and South Florida Flood Control Project, and the St. Lucie Canal (C-44), which provides both navigation and the release of water from Lake Okeechobee. These changes have altered the timing (excess wet season flows, insufficient dry season flows), distribution, quality, and volume of

freshwater entering the estuary (Haunert et al. 1994). The St. Lucie Estuary is hydrologically connected to the Everglades, its study is also part of the larger ecosystem restoration and freshwater management efforts in South Florida.

Heavy freshwater discharge such as the one following the 1998 El Nino event was associated with observations of unusually large incidences of fish with lesions and other deformities, and oceanic plumes of colored water and suspended particulates extending to the near-shore Atlantic Ocean reefs. Water releases transport excess of sediment, which contribute to deposits of muck in the estuary (Shrader 1984, Gunter and Hall 1963, Pitt 1972). Muck sediment accumulation decreases the quality and quantity of habitat for benthic infauna including ecologically and economically important shellfish and finfish.

On September 26, 2004 Hurricane Jeanne (Category 3) made landfall at the south end of Hutchinson Island in Martin County, within two miles of where Hurricane Francis (Category 3) came ashore exactly three weeks earlier. Not since 1886 had a state (Texas) experienced four hurricanes in a single season. Jeanne was the fourth hurricane to hit Florida in six weeks.

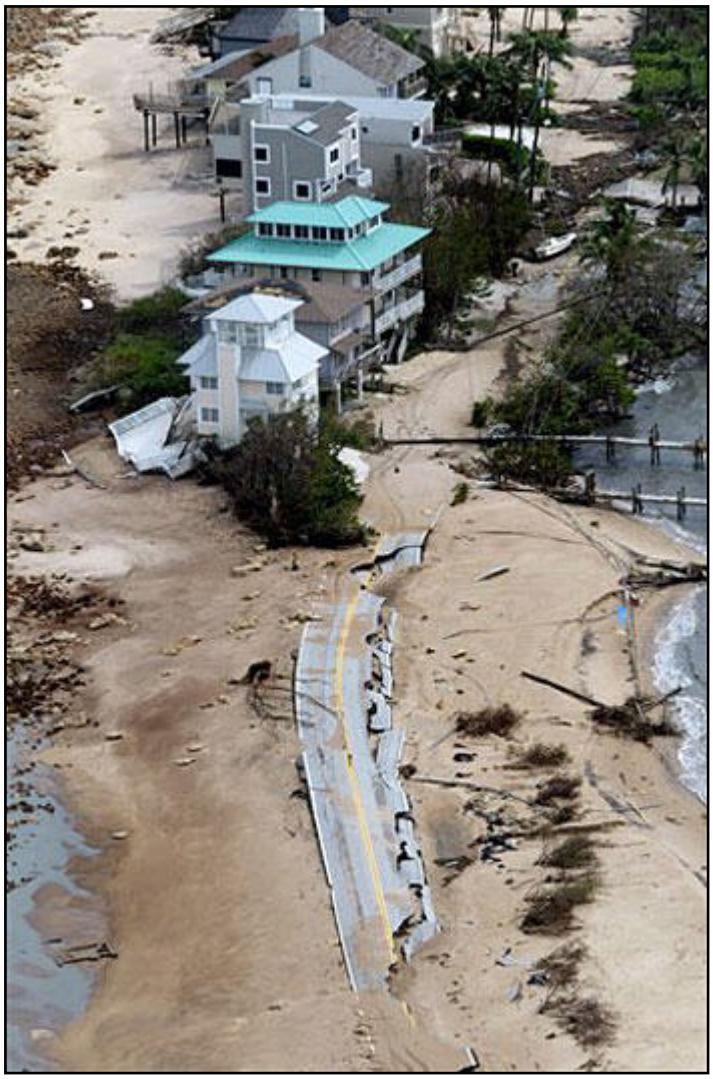

Figure1-1. Storm Surge damage Hutchinson Island after Hurricane Jeanne.

1.2 Approach

The study began in the Year 2001 and progressed through a series of phased activities. The foundation of this study was based on a sediment quality triad (SQT) approach with a probabilistic sampling design, which characterized the estuary in terms of chemical contamination in sediment, sediment toxicity (MicroTox, amphipod assays; sea urchin assay; and P450 HRGS) and benthic infauna community structure. Where possible, published guidelines were used to compare observed values with sediment quality guidelines for chemical contaminants for example Threshold Effects Level (TEL) and Probable Effects Level (PEL) values of MacDonald (1994, MacDonald et al. 1996).

Concurrent and subsequent investigations were conducted to further elucidation of cause-effect relationships and are reported as ancillary studies in this report or sited elsewhere. These additional investigations included 1) assessment of chemical contaminant burdens in fish tissue; Comet assay; acetyl cholinesterase activity in fish and shrimp; endocrine disruption in fish; characterization of contaminants in the nepheloid layer and sediment traps; survey for emerging contaminants of concern (alkyl phenols, polybrominated diphenylethers, perfluoro compounds and pesticides) in sediment and water; assessment of oyster biomarkers to describe the nature and severity of the copper problem. Finally, a thematic website and data portal was developed to manage and disseminate nation-wide data on contamination, toxicity, benthic faunal distribution, and fish histopathology (St. Lucie Estuary data were used as a prototype). This is an ongoing effort to provide ready access to data. The web site address is: http://NSandT.noaa.gov

2.0 Methods

2.1 Sampling sites

A probabilistic sampling design was used to determine sites that were sampled using the sediment quality triad approach. The study area was divided into five strata with six random sites per stratum: North Fork (stratum 1), South Fork (stratum 2), Convergence Zone (stratum 3), Middle Estuary (stratum 4) and Lower Estuary (stratum 5). In 2001, sediment samples were analysed for contaminants, toxicity and benthic infauna at each of the 30 sites except for toxicity testing which was done at fifteen of the thirty sites (3 sites per stratum). These toxicity tests included amphipod survival assay (*Ampelisca abdita*), the HRGS P450 test and the sea urchin (*Arbacia punctulata*) survival test (Figure 2-1; Table 2-1). The amphipod assay was repeated in 2003 with samples collected from the same 15 sites using two species *Ampelisca abdita* and *Eohaustorius estuaries* (Figure 2-1; Table 2-1). Sediment chemistry, benthic characterization (macroinfauna and abiotic factors) and toxicity assays make up the three components of the sediment quality triad.

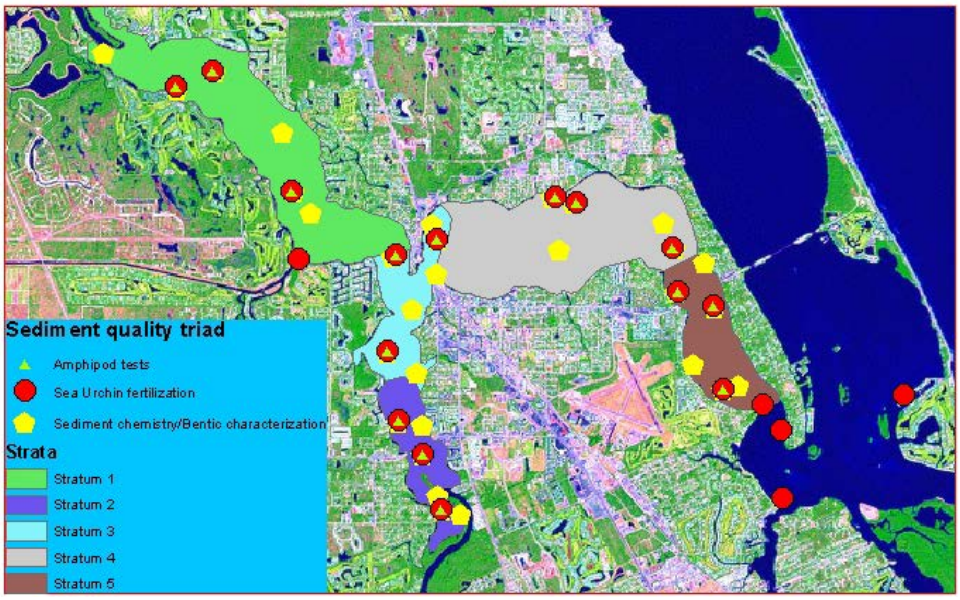

Figure 2-1. St. Lucie Estuary sediment quality triad sampling sites.

Table 2-1. Sediment quality triad site locations in the St. Lucie Estuary, FL.

Stratum	Random Sites	Assays	Latitude	Longitude	Location
1	1		27° 14' 26.8"	-80° 19' 12.0"	North Fork
1	2	Amp/SU/RGS	27° 14' 15.0"	-80° 17' 56.3"	North Fork
1	3	Amp/SU/RGS	27° 14' 4.6"	-80° 18' 21.9"	North Fork
1	4		27° 13' 38.4"	-80° 17' 10.2"	North Fork
1	5	Amp/SU/RGS	27° 13' 2.2"	-80° 17' 02.1"	North Fork
1	6		27° 12' 48.8"	-80° 16' 51.0"	North Fork
2	7	Amp/SU/RGS	27° 10' 42.0"	-80° 15' 52.2"	South Fork
2	8		27° 10' 39.8"	-80° 15' 36.5"	South Fork
2	9	Amp/SU/RGS	27° 10' 21.4"	-80° 15' 35.8"	South Fork
2	10		27° 09' 56.6"	-80° 15' 25.9"	South Fork
2	11		27° 09' 45.6"	-80° 15' 10.2"	South Fork
2	12	Amp/SU/RGS	27° 09' 48.2"	-80° 15' 23.1"	South Fork
3	13		27° 12' 41.7"	-80° 15' 28.3"	Convergence Zone
3	14	Amp/SU/RGS	27° 12' 32.0"	-80° 15' 24.5"	Convergence Zone
3	15	Amp/SU/RGS	27° 12' 21.9"	-80° 15' 55.3"	Convergence Zone
3	16		27° 11' 50.4"	-80° 15' 42.0"	Convergence Zone
3	17	Amp/SU/RGS	27° 11' 23.9"	-80° 15' 59.6"	Convergence Zone
3	18		27° 11' 11.9"	-80° 15' 40.1"	Convergence Zone
4	19	Amp/SU/RGS	27° 12' 57.7"	-80° 14' 06.5"	Middle Estuary
4	20		27° 12' 41.5"	-80° 12' 50.9"	Middle Estuary
4	21	Amp/SU/RGS	27° 12' 54.1"	-80° 13' 52.1"	Middle Estuary
4	22	Amp/SU/RGS	27° 12' 26.7"	-80° 12' 43.5"	Middle Estuary
4	23		27° 12' 25.9"	-80° 14' 02.3"	Middle Estuary
4	24		27° 12' 11.4"	-80° 15' 26.0"	Middle Estuary
5	25		27° 12' 17.2"	-80° 12' 23.5"	Lower Estuary
5	26	Amp/SU/RGS	27° 11' 59.6"	-80° 12' 42.6"	Lower Estuary
5	27	Amp/SU/RGS	27° 11' 49.6"	-80° 12' 16.70"	Lower Estuary
5	28		27° 11' 15.3"	-80° 12' 31.2"	Lower Estuary
5	29	Amp/SU/RGS	27° 11' 0.4"	-80° 12' 11.5"	Lower Estuary
5	30		27° 11' 2.4"	-80° 12' 00.4"	Lower Estuary

2.2 Sediment sampling

2.2.1 Chemistry and toxicity samples

Sediment samples were collected at 30 St. Lucie Estuary sites using a stainless steel 0.04-m^2 Young-modified van Veen grab sampler deployed with a davit and winch. Only the upper 2 - 3 cm of sediment from the sediment water interface was collected for chemical analyses and toxicity bioassays. Sediments were removed from the sampler with a new, acetone rinsed plastic scoop and transferred to a lined, acetone rinsed HDPE plastic bucket liner supported by a HDPE plastic bucket. A new scoop and bucket liner were used at each station. Sediments were homogenized using a Teflon® paddle prior to distribution into individual containers for grain size, carbon (TOC/ TIC/TC), contaminant chemistry and toxicity bioassays. The grain size and toxicity bioassay sediments were refrigerated (4^oC) and shipped to laboratories by overnight courier in cooler with blue ice. Contaminant chemistry and carbon samples were stored frozen and shipped by overnight courier in coolers with dry ice.

2.2.2 Benthic macroinfauna samples

A Young-modified Van Veen grab (area = 0.04 m^2) was used to collect bottom samples at each of the thirty stations during May, 2001. Three replicate samples were collected at 15 of the 30 sites and single bottom water samples were collected at the remaining 15 sites. Macroinfaunal samples were sieved through a 0.5–mm mesh screen, carefully transferred to HDPE containers and preserved with 10% formalin in the field. At the end of the 7-day field sampling effort the macroinfaunal samples were shipped by overnight courier to the Barry A Vittor & Associates, Inc. laboratory in Mobile, Alabama. In 2003, six sites were resampled in the North and South Forks and triplicate analyses were performed.

2.3 Sediment grain size analyses

Grain size parameters that were computed included percent gravel, sand, and silt /clay at half-phi intervals using the hydrometer technique for fractions smaller than 44 mm and nested sieves for larger particle fractions. Total organic carbon (TOC) content was measured as ash-free dry weight expressed as a percentage.

2.4 Sediment contaminant chemistry analyses

The sample processing protocol is described in detail in Lauenstein and Cantillo (1993, 1998) and is available from NOAA's National Status and Trends web site (http://NSandT.noaa.gov) or the National Environmental Methods Index (http://www.nemi.gov).

2.5 Sediment toxicity assays

2.5.1 Amphipod survival assay

The amphipod survival assay was done in 2001 with *Ampelisca abdita.* Due to high mortality in control samples, the amphipod assay was repeated in 2003 with two amphipod species (*Ampelisca abdita* and *Eohaustorius estuaries*) after collecting sediments from the same 15 sites. The original sediment bioassays were conducted simultaneously on material from the St. Lucie Estuary sediments and on control sediments collected from the Pettaquamscutt Cove, Narrow River, RI. Sediments were tested following procedures outlined in ASTM Method E 1367-92 (ASTM 1993), using *Ampelisca abdita* as a test species. Test organisms for the original (2001) bioassay were obtained from Eastern Aquatic Biosupply, Middleton, RI. For all retest, amphipods were obtained from Brezina and Associates, Dillon Beach, CA.

Measurements of environmental test conditions in the overlying waters (temperature, salinity, pH, dissolved oxygen and total ammonia) were made in two replicate test containers per sample on Day 2 and Day 8 of the test. Daily counts were made of emerged and dead animals on the sediment surface. At the end of the of the ten day exposure, the contents of each jar were poured through a 0.5 mm sieve and the number of surviving amphipods was counted twice.

For statistical comparison and assessment of samples versus control survival, data were analyzed by TOXSTAT® 3.5 (West, Inc., Cheyenne, WV). For normally-distributed data sets with homoscedastic variances, Dunnetts's test was used. For non-normally distributed data sets, or data sets with heteroscedastic variances, Steel's Test was the proposed alternative.

The amphipod bioassay was repeated by Northwest Aquatic Sciences (NAS), Newport Oregon with two species *Ampelisca abdita* and *Eohaustorius estuarius* with sediments collected in 2003 from the original 15 sites. In addition, a fine-grained reference sediment was tested with the *Eohaustorius estuarius* bioassay to estimate the contribution of potential test sediment grain size.

Eohaustorius estuarius control sediment was collected from the amphipod collection site in Yaquima Bay, Oregon. During a previous study, NAS obtained grain size analysis of 1.0 mm-sieved sediment from this site:

Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Fines (%)
0.0	100	0.0	0.0	0.0

Ampelisca abdita control sediment was collected from the *Ampelisca* collection site in San Francisco Bay, California. NAS obtained grain size data of 0.5 mm-sieved sediment from this site from the Marine Pollution Studies laboratory in Monterey, California:

Gravel (%)	Sand (%)	Silt (%)	Clay (%)	Fines (%)
0.0	4.5	36.8	58.8	95.5

The *Ampelisca abdita* control sediment was also used as the fine-grained reference sediment in the *Eohaustorius estuarius* tests.

Within each test, the percent amphipod survival at test termination was determined from the final observations according to the formula:

Percent Survival = 100 x (surviving amphipods/initial amphipods)

In each test sediment percent survival was compared against that in the control sediment, and in the *Eohaustorius estuarius* test also against that in the reference sediment. The software used for within-test statistical comparisons was BioStat (Beta v.4.1 (Excel)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Following determination of normality and homogeneity of variances, a one tailed Student T-test, Approximate T-test, 1-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance.

Following completion and analysis of all tests, between-species comparisons for each test sediment was made. Shapiro-Wilk's Test was used to test for the normality, and an F-test for equality of variances. Then significance between species responses was determined using a 2-tailed homoscedastic or heteroscedastic t-test at the 0.05 level of significance.

Dissolved organic carbon (DOC) was measured in all pore water samples collected. Samples were run in one batch with associated quality control standards. All analyses were run in triplicate with 1 ml auto sampled volumes. Blank values were acceptable and measured 0.18 mg/L. Percent recovery from laboratory controls run in conjunction with the batch was accepted at 99.56%. DOC values reflect the concentrations in the pore water before salinity adjustment at the time of extraction and prior to freezing. Preliminary data indicated that DOC concentration in freshly collected samples may range from 1 to 20% higher than samples that have been frozen. DOC concentration ranged from 4.67 to 84.43 mg/L.

2.5.2 Sea urchin fertilization

Sediment samples were collected and shipped as previously described to the U.S. Geological Survey (USGS) Marine Ecotoxicology Research Station (MERS) in Corpus Christi, Texas where the tests were performed. Sediment pore water was extracted with a

pneumatic apparatus similar to the one used in previous studies (Carr and Chapman, 1992; 1995; Carr et al., 1996a; 1996b; NBS, 1993; 1994; 1995a; 199USGS, 1997a; 1997b; 1998; 1999a; 1999b; 2000a; 2000b; 2000c). The extracted pore water samples were stored frozen. Prior to testing the samples were thawed and water quality parameters were measured and adjusted, if necessary. A dilution series (100, 50 and 25%) test design was used to determine the toxicity of sediment pore water samples. Additional subsamples of pore water were analyzed for dissolved organic carbon (DOC).

Surficial sediment samples were collected as previously described in section 2.2.1. Samples were placed in precleaned one gallon high density polyethylene containers (HDPE), chilled on water ice, and shipped by overnight courier in insulated coolers with blue ice to USGS MERS in Corpus Christi, Texas. Pore water samples were extracted within 5 days of the time of field collection of sediment.

Pore water was extracted from the sediment using a pressurized pneumatic extraction device. This extractor is made of polyvinyl chloride (PVC) and uses a 5 μm polyester filter. Sediment samples were held refrigerated (4^0C) until pore water was extracted. Pore water samples were extracted within 24 hours from the time of receipt. After extraction, the pore water was centrifuged in polycarbonate bottles at 1200 x g for 20 min to remove suspended particulate material; the supernatant was collected and frozen (-20^{o}C).

Two days before conducting a toxicity test, pore water samples were moved from the freezer to a refrigerator at 4^{o}C. One day prior to testing, samples were thawed in a tepid water bath then maintained at 20 ± 2^{o}C. Sample salinity was measured and adjusted to 30 ± 1%, if necessary; using purified deionized water or concentrated brine. Other water quality measurements (dissolve oxygen, pH, sulfide as S^{-2}), and total ammonia (TAN) were measured with Orion® meters and their respective probes. Unionized ammonium concentrations (UAN) were calculated for each sample using the respective salinity, temperature, pH, and TAN values. Samples with less than 80% DO saturation were gently aerated by stirring the sample on a magnetic stir plate. Following water quality measurements and adjustments, the samples were stored overnight at 4^0C returned to 20 ± 1^{o}C before the start of the toxicity tests.

Toxicity of sediment pore water was determined using the fertilization test with the sea urchin *Arbacia punctulata* following procedures outlined in SOP F10.9 (http://NSandT.noaa.gov). The sea urchins used in this study were obtained from Gulf Specimen Company, Inc. (Panacea, Florida). Each of the 21 pore water samples (water quality adjusted) was tested at three treatment levels including: 100, 50, and 25% pore water with 5 replicates per treatment. The 50% and 25% pore water treatment were prepared with 0.45 μm filtered sea water. A reference pore water sample collected from Redfish Bay, Texas, handled identically to the test samples, was included with each toxicity test as a negative control. This site is far removed from any known sources of contamination and has been used previously as a reference site (Carr and Chapman, 1992; Carr, 1993; NBS, 1993; 1994; 1995a; 1995b; USGS, 1997a; 1997b; 1998; 1999a; 1999b; 2000a; 2000b; 2000c). In addition, dilution blanks of filtered seawater and reconstituted brine (brine with purified deionized water) were also included. A dilution series test with sodium dodecyl sulfate (SDS) was included as a positive control.

Statistical comparison among treatments were made using GLM and Dunnett's one – tailed t-test (which controls the experimentwise error rate) on the arcsine square root transformed data with the aid of SAS (SAS, 1989). A further transformation was necessary in analyzing the data at the 25% pore water level to correct response scaling problems. The transformation used in this instance was the arcsine square root taken to the 1.5 power. The trimmed Spearman-Kaber method (Hamilton et al., 1977) with Abbott's correction (Morgan, 1992) was used to calculate EC50 (50% pore water level) values for dilution series test when possible. Prior to statistical analysis, the transformed data were screened for outliers (SAS, 1992). Outliers were detected by comparing the studentized residuals to a critical value from a t-distribution chosen using a Bonferroni-type adjustment. The adjustment is based on the number of observations, n, so that the overall probability of a type I error is at most 5%. The critical value, *cv*, is given by the following equation: $cv = t\ (df_{Error}, .05/2 \times n)$). After omitting outliers but prior to further analysis, the transformed data were tested for normality and for homogeneity of the variance using SAS/LAB® Software (SAS, 1992).

A second criterion was also used to compare test means to reference means. Detectable significance criteria (DCD) were developed to determine the 95% confidence value based on power analysis of all similar tests performed by the laboratory (Carr and Biedenbach, 1999). This value is the percent minimum significant difference from the reference that is necessary to accurately detect a difference from the reference. The DSC value for the sea urchin fertilization assay at $\alpha = 0.05$ are 15.5% and at $\alpha = 0.01$, 19%.

Dissolved organic carbon (DOC) was measured in the pore water samples using an OI Analytical Model 1010 Wet Oxidation Total Organic Carbon Analyzer following the model 1010 operators' manual (OI Analytical, 1998). Samples of freshly extracted pore water (20 ml approx.) were immediately filtered through a 0.45 µm Whatman nylon syringe filter and preserved with approximately 0.5 ml of phosphoric acid. Samples were stored refrigerated before analysis. Samples were analyzed in the TOC mode with 400 µl of acid (5% phosphoric acid) and 4000 µl of oxidant (200 g/L sodium persulfate). Total inorganic carbon (TIC) react and detect times were 2:00 (min:sec) and 2:00 (min:sec), respectively and total organic carbon (TOC) react and detect times were 8:30 (min:sec) and 2:00 (min:sec), respectively. At least one blank and laboratory control was run for each batch of 10 to 15 samples. Samples were reanalyzed if the percent recovery of the laboratory control failed to meet the 90-110% level or if concentrations were found to be in excess of the highest concentration used to calculate the calibration curve (50mg/L).

2.5.3 Human Receptor Gene System (HRGS) P450 bioassay on sediment extracts

Sediment samples from the 15 toxicity characterization sites, in addition to 6 additional samples, from other areas were extracted in the Jacksonville, Florida laboratory of Columbia Analytical Services using EPA Method 3540.

The P450 Reporter Gene System (HRGS) methodology has been described in detail elsewhere (APHA 1998, ASTM, 1999, EPA 1999). Briefly, approximately 20 g (wet weight) of sediment from each station was extracted with dichloromethane (DCM) to remove and concentrate organic contaminants. A small sub-sample of sediment was used

to determine the percent solids. Nineteen grams wet weight was exchanged into a 2:1:1 solvent mixture of DMSO: toluene: isopropyl alcohol for a final volume of 1 mL, and sent to the Columbia Analytical Services laboratory in Vista, CA for testing by EPA Method 4425.

Ten μL of sample extracts were applied to two replicate exposure wells and incubated for 16 hours. Cells were then washed, lysed, and the solution centrifuged. Fifty μL of the supernatant was then applied to a 96-well plate, followed by 100 μL of a cofactor solution, and then 100 μL of the enzyme substrate luciferin. Luminescence was then measured as relative light units (RLU) using a ML 2250 Luminometer. A solvent blank and reference inducers (2,3,7,8-TCDD and B[a]P) were used with each sample test run.

Equivalency Calculations

Benzo[a]pyrene Equivalents (B[a]PEq) were calculated for all sample 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 (μg/g) =

((fold induction / 60) * (volume factor/ dry weight))* (dilution factor)

Fold induction is calculated as mean relative light units (RLU) produced by the sample divided by mean RLU produced by the solvent blank. The factor of 60 represents the approximate fold induction produced by 1.0 μg of B[a]P/mL. The volume factor (100) represents the total extract volume (1 mL) divided by the volume of extract applied to the cells (10 μL). Dividing by the dry weight of each sample, calculated using percent solids of the 19 g samples, yields B[a]PEq in μg/g dry weight. If a dilution is used, the B[a]PEq value is multiplied by the dilution factor.

A standard curve for a dioxin/furan mixture has 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} (ng/g) =

((fold induction) * (volume factor/ 1000 * dry weight))* (dilution factor)

The extracts were lighter in color than most estuary studies tested thus far. The lighter color could be the results of larger particle size, lower organic matter, or lower concentrations of PAHs. Based on experience, it was determined that 10 μL, instead of the 2 μL used previously, would be applied from each extract to the test system. Test samples that produced a coefficient of variation greater than 20% were re-tested, along with SRM samples that required dilution.

2.6 Macroinfaunal sample analysis

In the laboratory of Barry A. Vittor & Associates, Inc., benthic samples were inventoried, rinsed gently 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 other St. Lucie samples.

Several numerical indices were chosen for analysis and interpretation of the macroinfaunal data. Infaunal abundance is reported as the total number of individuals per station and the total number of individuals per square meter (= density). Taxa richness is reported as the mean number of taxa represented in a given site location. Taxa diversity, which is often related to the ecological stability and environmental "quality" of the benthos, was estimated by the Shannon-Weaver Index (Pielou, 1966), according to the following formula:

$$H' = \sum_{i=1}^{S} p_i (\ln p_i)$$

where, S = the number of taxa in the sample, i = the i'th taxa in the sample, and p_i = the number of individuals of the i'th taxa divided by the total number ofindividuals in the sample.

Taxa diversity can be calculated using ln or $\log_2$ and both methods are common in the scientific literature. The taxa diversity calculated in this report using ln, can be converted to $\log_2$ diversity by multiplying the ln taxa diversity by 1.4427. 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 the equitability in the fauna to the taxa diversity for a given area, Pielou's Index J' (Pielou, 1966) was calculated as J' = H'/lnS, 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.

3.0 Results and Discussion

3.1 Sediment Chemistry

3.1.1 Metals

Sediment samples from thirty stratified random sites in the SLE were analyzed for nine metals (Table 3-1). All of the metals (arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver and zinc) have established threshold effects level (TEL) and probable effects level (PEL) sediment quality guidelines (SQG). Overall, thirty six percent of all metals with established sediment quality guidelines exceeded the TEL and 1% exceeded the PEL Sediment quality guideline exceedence was highly variable (10% arsenic, 0% cadmium, 67% chromium, 63% copper, 7% lead, 16% mercury, 73% nickel, 3 % silver, 23% Zinc) and the PEL (3% copper, 7% mercury) were exceeded at a small percentage of sites. The majority of sediments had metal concentrations below the TEL sediment quality guidelines (Table 3-1).

Table 3-1. Graphic depiction of TEL and PEL sediment quality guidelines and NS&T Mussel Watch Project sediment concentration quartiles for thirty stratified random sediment sites in the St. Lucie Estuary. Concentrations greater than the TEL and less than the PEL (T). Concentrations greater than the PEL (P). NS&T quartiles are represented by ∀ Quartile 1 ∀ Quartile 2 ∀ Quartile 3 ∀ Quartile 4.

Stratum	NS & T site	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Silver	Zinc
1	1									
1	2	T		T	T	T		T		T
1	3			T	P		P	T		T
1	4			T	T		T	T		T
1	5			T	T	T	T	T	T	T
1	6			T	T			T		T
2	7			T	T			T		
2	8									
2	9									
2	10				T					
2	11			T	T			T		T
2	12			T	T			T		T
3	13			T	T			T		
3	14				T			T		
3	15			T	T			T		
3	16			T	T			T		
3	17			T	T		T	T		
3	18			T	T			T		
4	19	T		T	T		P	T		
4	20			T				T		
4	21			T	T			T		
4	22							T		
4	23			T	T			T		
4	24									
5	25							T		
5	26	T		T	T			T		
5	27									
5	28									
5	29			T				T		
5	30			T						

Stratified Random Sampling

Strata comparisons using the rank sums Wilcoxon/Kruskal-Wallis tests found significant differences between stratum for aluminum, cadmium, copper, iron, lead, silver, tin and zinc (Figure 3-2; Table 3-3). The concentrations for copper in stratum 1 were the highest in the estuary. Stratum 1 is in the North Fork where most of the agriculture in the form of citrus groves is located. Copper is used as a fungicide in citrus groves and is one of the

main sources of copper to the North Fork. Overall, the highest metal concentrations were found in stratum 1, the North Fork (Table 3-2; Figure 3-1)

Table 3-2. Results for Sums Wilcoxon/Kruskal-Wallis test. Asterisks (*) denote where a significant difference was found. Mean concentrations are presented for each stratum in µg/g dry wt. Strata with dissimilar letter notations were found to be significantly different by the Tukey pairwise comparison statistical test.

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Metals	ChiSquare	Prob	Stratum 1	Stratum 2	Stratum 3	Stratum 4	Stratum 5
Arsenic	6.71	0.15	A	A	A	A	A
Cadmium	9.16	0.06	A	A	A	A	A
Chromium	8.33	0.08	A	A	A	A	A
Copper	12.12	0.02*	A	AB	AB	B	B
Mercury	5.00	0.29	A	A	A	A	A
Nickel	3.30	0.51	A	A	A	A	A
Lead	11.22	0.02*	A	AB	AB	AB	B
Silver	11.94	0.01*	A	AB	AB	AB	B
Zinc	10.16	0.04*	A	AB	AB	AB	B

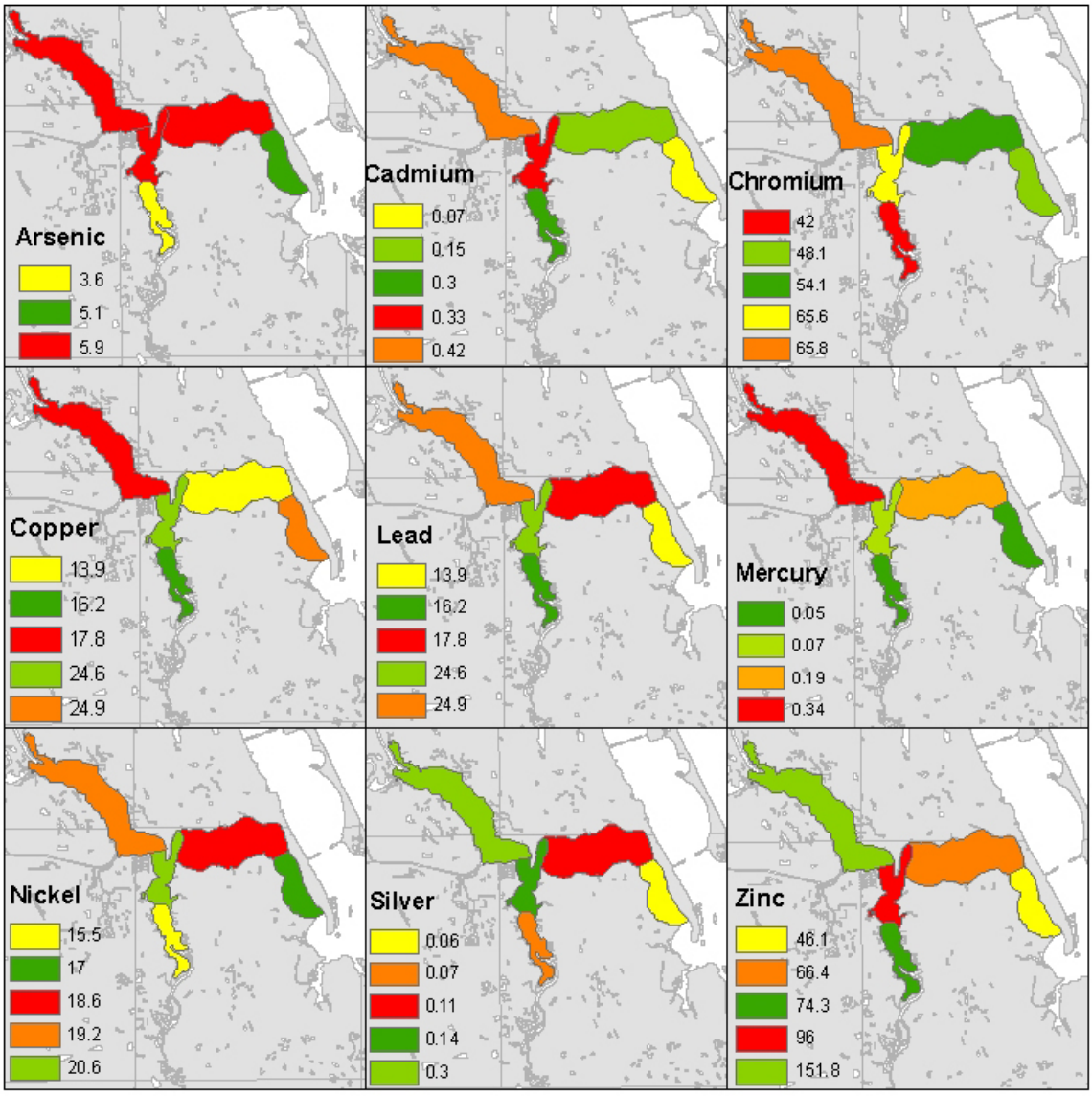
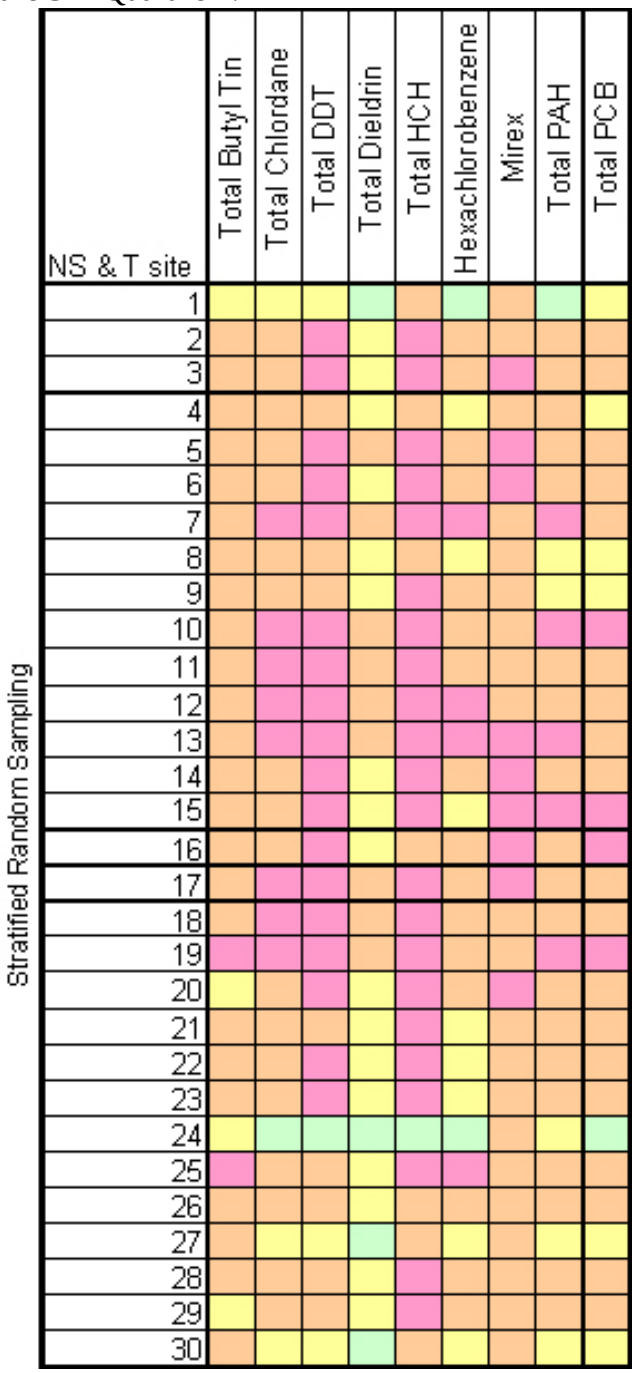


Figure 3-1. Mean metal concentrations (µg/g dry wt) by strata. for stratified random sites in the St. Lucie Estuary. The North Fork and convergence zone consistently have the highest metal concentrations.

3.1.2 Organics

Total organic measurements were compared to NS&T Mussel Watch percentiles. When contaminants in St. Lucie sediments were compared to NS&T-MW sediments (1996 & 1997), 27% exceeded 75th percentile and 79% exceeded the NS&T-MW 50th percentile (Table 3-3).Relative to National concentrations levels, St. Lucie sediments exhibited moderate to high levels of contamination (Table 3-3).

Table 3-3. Graphic depiction of NS&T Mussel Watch Project sediment concentration quartiles for thirty stratified random sediment sites in the St. Lucie Estuary. Concentrations greater than the PEL (P). NS&T quartiles are represented by ∀ Quartile 1 ∀ Quartile 2 ∀ Quartile 3 ∀ Quartile 4.



The spatial distribution of butyl tins, dieldrins and HCHs exhibited no significant difference between strata. Stratum 3 was significantly higher or amongst the highest of all contaminants while strata five was significantly lower or amongst the lowest of all the strata. (Table 3-4; Figure 3-2). For contaminants with significant differences found between strata, the convergence zone (stratum 3) had the highest mean concentrations.

Table 3-4. Results for Sums Wilcoxon/Kruskal-Wallis test. Asterisks (*) denote metals where a significant difference was found. Means are presented for each stratum in μg/g dry wt. Stratum with dissimilar letter notations were found to be significantly different by the Tukey pairwise comparison statistical test.

Total Organic Contaminants	ChiSquare	Prob	Stratum 1	Stratum 2	Stratum 3	Stratum 4	Stratum 5
Total Butyl Tins	2.38	0.67	A	A	A	A	A
Total Chlordanes	10.35	0.03*	AB	AB	A	AB	B
Total DDTs	12.8	0.01*	AB	AB	A	AB	B
Total Dieldrins	7.96	0.93	A	A	A	A	A
Total HCHs	6.06	0.19	A	A	A	A	A
Hexachlorobenzene	5.7	0.22	A	A	A	A	A
Mirex	13.56	0.01*	AB	ABC	A	BC	C
Total PAH	11.14	0.02*	B	AB	A	AB	B
Total PCB	9.89	0.04*	B	AB	A	AB	AB

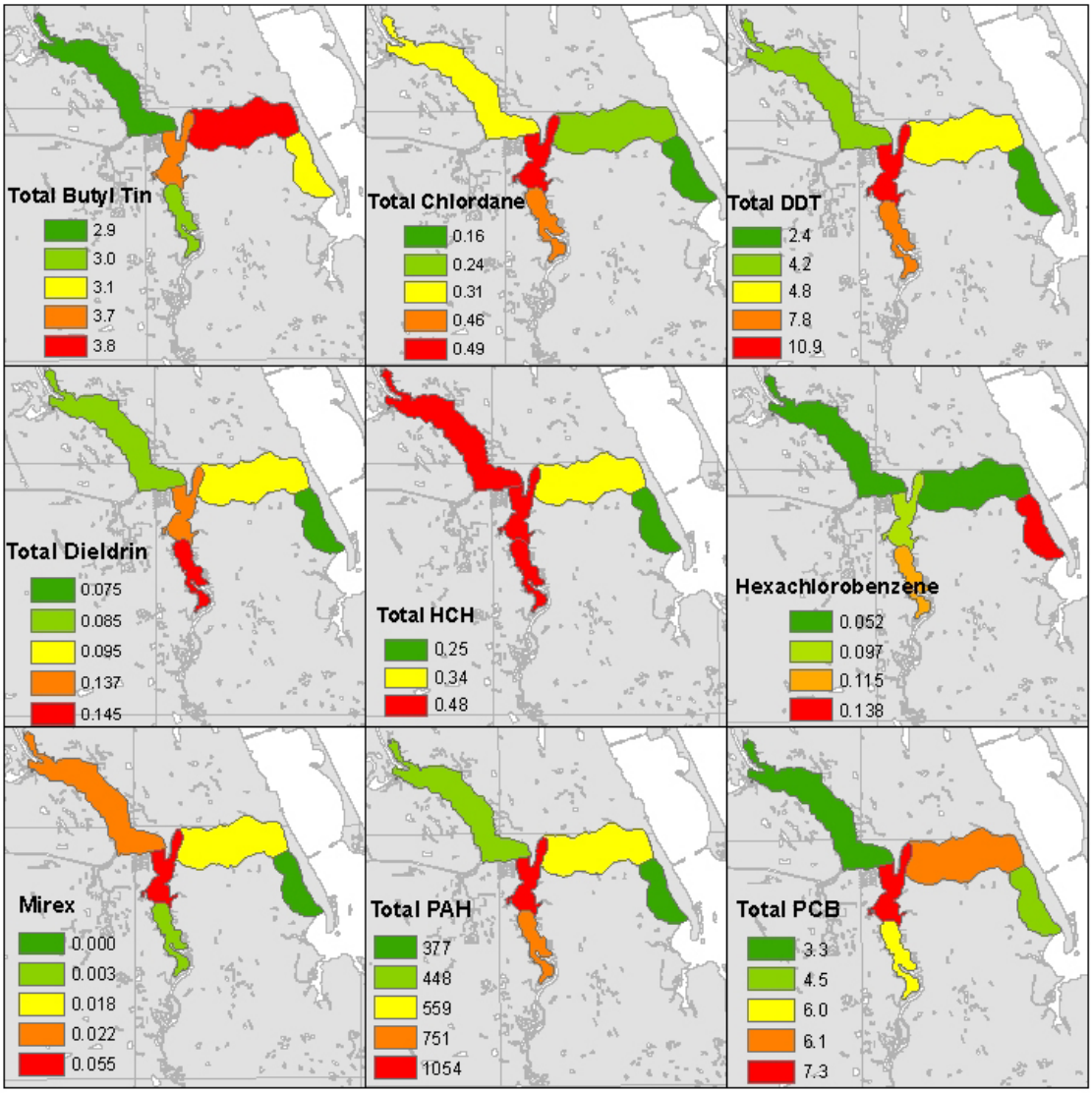


Figure 3-2. Mean concentrations (ng/g dry wt andng Sn/g dry wt. for total butyl tin) by chemical class and strata for stratified random sampling sites in the St. Lucie Estuary.

3.2 Bioassays

3.2.1 Amphipod survival

Amphipod tests were performed on a subset (15) of the thirty stratified random sites as part of the 2001 sediment quality triad assessment. The amphipod assay, performed in 2001 with *Ampelisca abdita*, resulted in no significant difference between control site sediment and SLE sediment due to low survival in controls. The 15 sites tested in 2001 were resampled in 2003 and tested using both Ampelisca *abdita* and *Eohaustorius estuarius*. Significant mortality relative to control sediments was found at three sites from the *Ampelisca abdita* tests and seven sites from the *Eohaustorius estuarius* tests. Only two sites exhibited significant mortality in both *Ampelisca abdita* and *Eohaustorius estuarius* (Figure 3-3).

The test negative control acceptance criteria for survival were met in all tests (≥ 90 % mean survival, not < 80 % survival in any one replicate). All reference toxicant tests were within the laboratory control chart limits. Mean *Eohaustorius* survival in the fine reference sediment (*Ampelisca* control sediment) was 80.0% and 86.0% in the first and second test, respectively. These values were significantly different from mean *Eohaustorius* control survival, but within the ≤ 20% absolute difference from the control recommended by some regulatory programs (USACE 2000).

F*or Eohaustorius estuarius*, seven test sediments (SLE5, SLE7, SLE9, SLE12, SLE15, SLE22, and SLE26) showed significantly lower survival than that in control sediment. However, only sediments SLE5 and SLE26 had survival lower than 90% (acceptable control survival), and these were 87% and 88% respectively. No test sediments had significantly lower survival than that in fine-grained reference sediment, suggesting the possibility of a grain-size component to the *Eohaustorius* response (Figure 3-3).

Only four test sediments showed significantly lower survival than in the control sediment in the *Ampelisca abdita* test (SLE3, SLE5, SLE14, and SLE22). Mean percent survival in these sediments ranged from 75% to 85%. One test sediment, SLE9, had mean percent survival of 80% but was not significantly less than the control survival. Replicate percent survival in sediment SLE9 was 100%, 95%, 10%, 100%, and 95%. At test termination, the replicate which had only 10% survival was carefully examined, and dark bright blue paint chips were discovered, along with an unusual odor. Sediments were thoroughly homogenized before adding them to the test beakers, but discrete particles may still partition into a particular replicate.

Four sediments showed significantly different percent survival in the *Eohaustorius vs.* the *Ampelisca* test: SLE3 (97% *Eohaustorius*, 85% Ampelisca), SLE14 (98% *Eohaustorius*, 75% *Ampelisca*), SLE21 (97% *Eohaustorius*, 89% *Ampelisca*), and SLE22 (90% *Eohaustorius*, 75% *Ampelisca*). Although these species have shown good agreement in sediment toxicity comparisons (Schlekat et al. 1995), differences may reflect species differences in response to particular toxicants in these sediments. For example, *E. estuarius* is known to be less sensitive to cadmium than other commonly-tested amphipod

species (e.g. DeWitt et al. 1989), but is more sensitive to tributyltin than *Rhepoxnius* (Meador et al 1993).

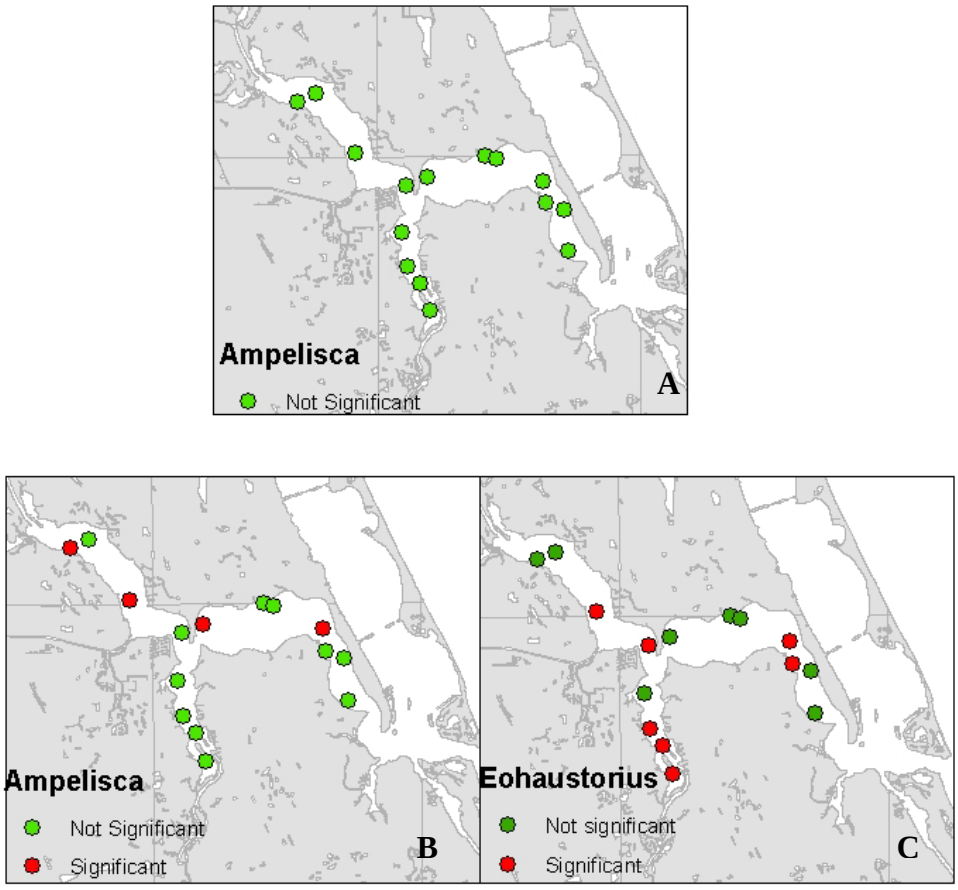


Figure 3-3. Results from 2001 (A) and 2003 (B & C) amphipod sediment toxicity tests depict significance relative to control sediment.

3.2.2 Sea urchin fertilization

Sea urchin fertilization tests were conducted on sediment collected from the same 15 sites used in for amphipod testing, and six additional sites associated with the fish tissue contaminant assessment. As with the *Eohaustorius* and *Ampelisca* tests, toxicity is primarily found in the North and South Forks (Figure 3-3 and 3-4). Twenty one sediment samples from the St. Lucie Estuary were extracted and tested for toxicity. Salinity of the pore water ranged from 22 to 38 ppt. Initial dissolved oxygen of all samples exceeded 80% saturation and did not require aeration prior to testing. Sulfide measurements were below detection limits (0.01 mg/L) in all but three samples and pH ranged from 7.73-8.00. Total ammonia measured ranged from <0.1 to 12.30 mg/L and the unionized fraction ranged from < 3.1 to 308.2 μg/L.

The sea urchin fertilization test of the 21 samples revealed 10 of the 21 samples (47.6%) were toxic in the undiluted (100%) pore water. Eight of 10 samples with 50% pore water and six of the 10 samples with 25% pore water were also found to be toxic. In four samples (SLE-15, SLE-22, SLE-27, and SLE-29) atypical dilution responses were observed. These samples became toxic as they were diluted. Dilution water blanks run with the test showed no evidence of toxicity from the dilution water itself and while there was a slight toxicity response in the reconstituted brine blank (probably due to a lack of trace elements), this would not explain the results observed as brine was not added to these samples (Figure 3-4). Further testing would be required to determine if pH shifts or other factors were the cause of the results. Toxic responses observed in diluted samples were not considered when undiluted sample was not toxic. The majority of toxic samples occurred in the North and South Forks of St. Lucie Estuary with fewer toxic responses observed proceeding towards Indian River Lagoon. However, the most toxic station (SLE 19) was found in the mid estuary region with an EC50 value <25%. None of the samples exceeded the NOEC for ammonia (400 µg/L) for the fertilization test indicating that other contaminants are likely responsible for the observed toxicity (Figure 3-4).

EC50 values could be calculated for six stations. Quality controls used in the test resulted in acceptable values. EC50 values for the SDS positive control was 5.51mg/L (95% confidence intervals 5.15-5.89) which falls within the control charts for the MERS laboratory.

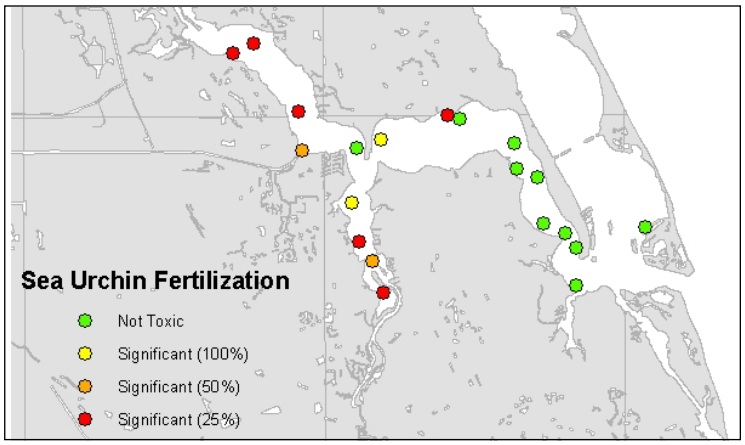


Figure 3-4. Sea urchin fertilization toxicity test.

3.2.3 HRGS P450 bioassay on sediment extracts

None of the samples produced responses of 60 µg/g B[a]PEq or greater (Figure 3-5), which has been associated with degraded benthic communities (Fairey et al. 1996). In addition, no samples were at the level of response (about 32 µg/g B[a]P Eq) where biological effects might be expected to occur (Anderson et al. 1999a). All but three samples were below 11 µg/g B[a]PEq, the level where impacts on the benthos would not be expected. It is interesting to note that all samples with B[a]PEq above11 µg/g were located in the lower portion of the South Fork of the estuary where high volume freshwater discharges from lake Okeechobee can deposit large quantities of sediment over a short time period.

The HRGS analysis is a test for specific organic contaminants including: PAHs and HRGS inducing PCBs. Only three sites, all in the South Fork, have B[a]p eqv > 11.3, a level found to be toxic (Anderson and McCoy 2002). The South Fork PAH levels exceed the TEL and could be responsible for the toxicity seen in the HRGS 450 tests.

Since total PAH concentrations were low in most of the estuary low B[a]PEqs were expected. Also, no correlation was found by Spearmans Rho between PAH (total, LMW or HMW) and HRGS P450.

An overview of the distribution of HRGS responses, on a basis of µg/g of B[a]P equivalents is shown in Figure 3-5. The three samples with the highest B[a]PEqs were above 11 µg/g (benthic impacts not expected) and below 32 µg/g (benthic impacts might be expected).

Fifty parts per trillion (ppt) of TCDD produced fold induction values of 60.3 and 67.9 on the two test periods. Three-hundred ppb of B[a]P produced fold induction values of 10.5 and 12.2 on the two test periods. The solvent blanks produced B[a]P equivalent values of 2.0 and 0.5 µg/g B[a]PEq, approximately the level of the lowest sediment samples. The laboratory control samples (20g) were spiked with 2.5 ng TEQ of a dioxin/furan mixture, which should produce a calculated TEQ of 0.125 ng/g (ppb).

In comparison to several previous studies, the samples from St. Lucie Estuary contain much lower amounts of the types of chemicals that induce the CYP1A1 gene (PAHs, coplanar PCBs, dioxins, furans). The 7.0 µg B[a]PEq/g mean and 10.4 upper 99 % CI observed in this study are nearly as low as the South Florida Study conducted in 1999. It is certainly possible that chemicals other than those that attached to the Ah receptor are present in these samples, and are producing toxic effects on the biota.

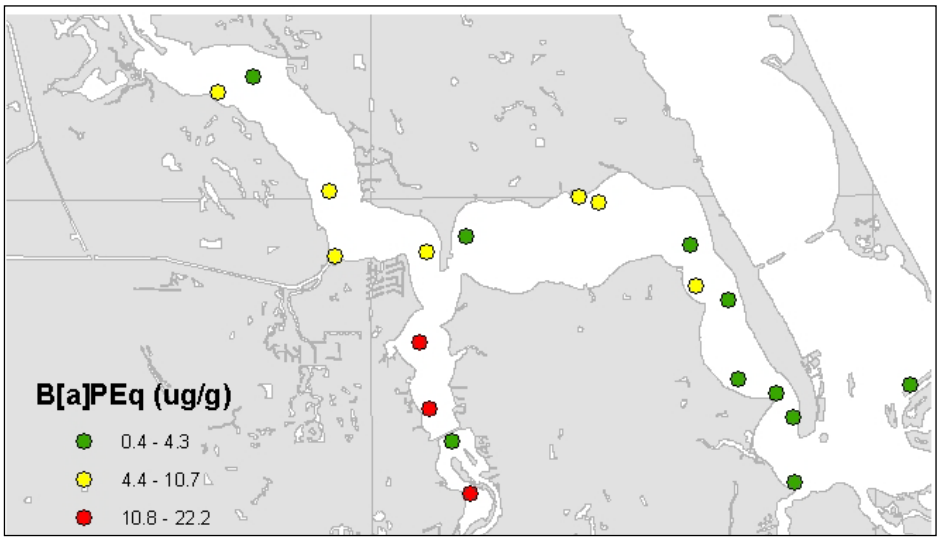


Figure 3-5. HRGS P450 reported in benzo[a]pyrene equivalent units. Toxicity was found in the South Fork and convergence zone where the highest PAH concentrations exist.

3.3 Benthic characterization

3.3.1 Habitat characterization

Sediment data for the 30 St. Lucie stations are given in Figure 3-6. Sediment composition varied considerably and ranged from 98% sand at Station 24 to 87% silt+ clay at Station 18 (Figure 3-6). Muck sediments defined by clay/silt > 60%, TOC > 6%, and water content > 75 % were only found in the North Fork, Convergence Zone and South Forks (Figure 3-6). Salinities varied from 17 ppt at Station 1 to 38.1 ppt at Station 28 (Figure 3-7).

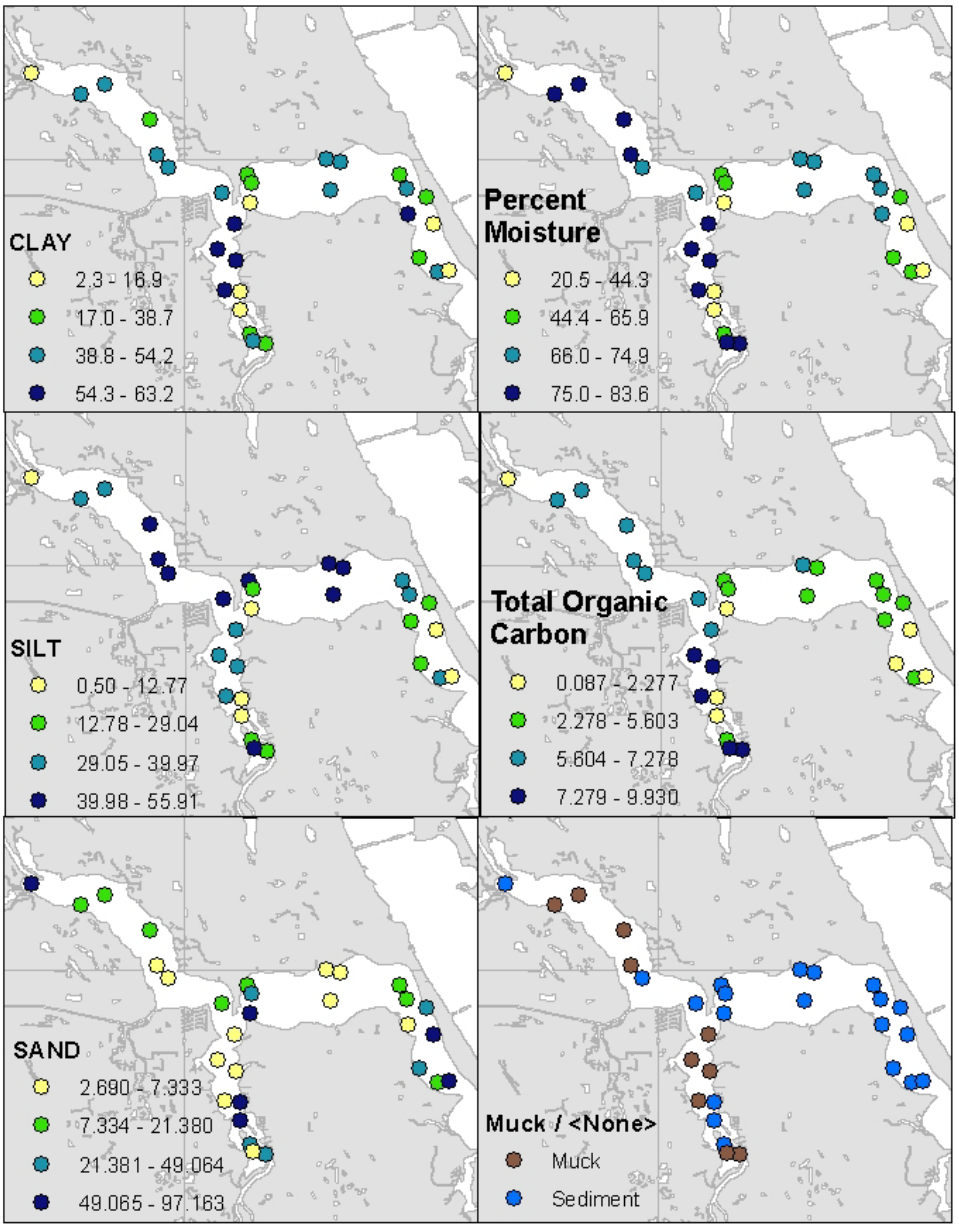


Figure 3-6. Sediment grain size characterization for thirty stratified random sites.

3.3.2 Benthic community characterization

A total of 7429 organisms, representing 148 taxa, were identified from the 30 St. Lucie sites. Polychaetes were the most numerous organisms present representing 48.9% of the total assemblage, followed in abundance by bivalves (27.2%) and malacostracans (17.8%). Polychaetes represented 41.9% of the total number of taxa followed by malacostracans (25.0%) and bivalves (17.6%). The number of taxa per station ranged from 3 to 54. The number of organisms per station ranged from 4 to 1224.

The dominant taxa collected from were the polychaete, *Sternaspis scutata*, the bivalve, *Mulinia lateralis*, the malacostracans, *Ampelisca abdita*, and the polychaete, *Mediomastus* (LPIL) representing 23.4%, 21.7%, 13.7%, and 10.8% % of the total individuals collected. The polychaete, *Glycinde solitaria* was the most widely distributed taxon being found at 83% of the stations.

Taxa richness varied and ranged from 2.0 (± 1.0) to 53. Taxa richness was positively correlated with the percent gravel + sand in the sediments (Spearman's Rho = 0.748, P > Rho = < 0.0001) and inversely correlated with the percent silt + clay (Spearman's Rho = -0.746, P > Rho = <0.0001) and salinity (Spearman's Rho = -0.382, P > Rho = 0.0449). Station mean densities exhibited considerable variation ranging from 66.7 organisms/m^2 (± 52.0) to 12275 organisms/m^2 (Figure 3-8). Densities were also positively correlated with the percent gravel + sand in the sediments (Spearman's Rho = 0.466, P > Rho = 0.0095) and inversely correlated with the percent silt + clay (Spearman's Rho = -0.462, P > Rho = 0.0102) and salinity (Spearman's Rho = -0.380, P > Rho = 0.0458).

Taxa diversity (H') ranged from 0.64 to 2.98 (Figure 3-8). Taxa evenness (J') ranged from 0.33 to 1.00.

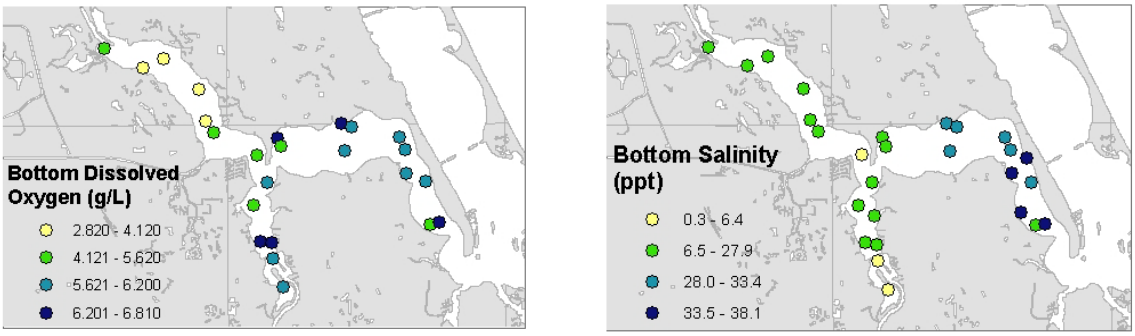


Figure 3-7. Bottom water dissolved oxygen and salinity concentrations.

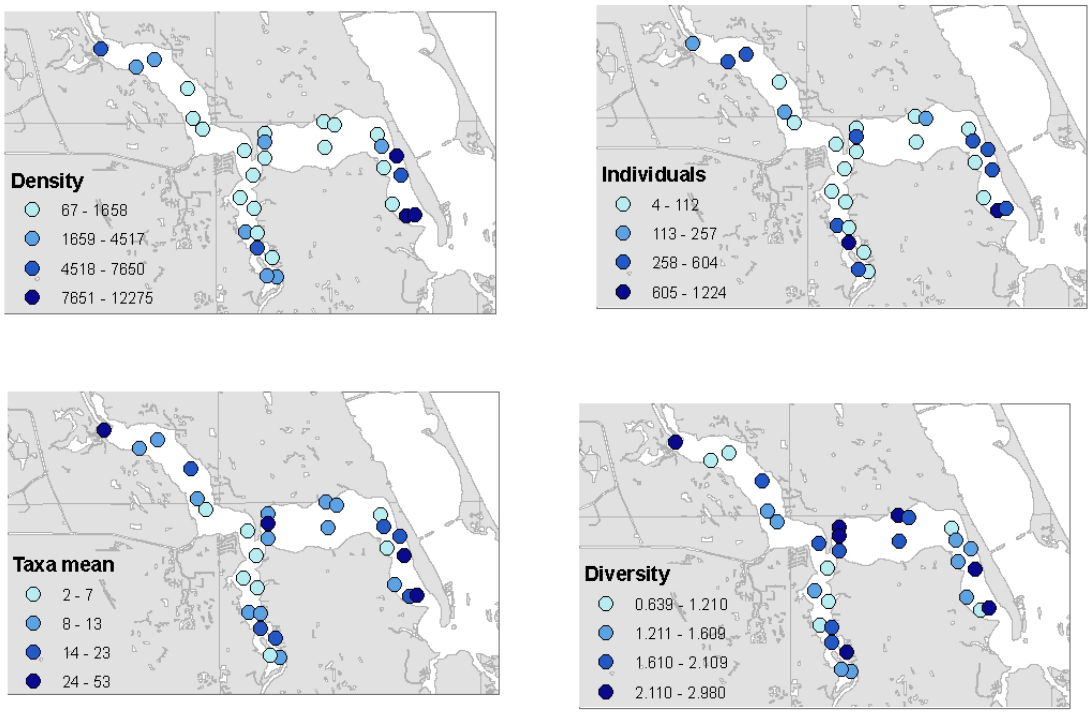


Figure 3-8. Benthic macroinfauna characterization.

4.0 Conclusions

This study has characterized the St. Lucie Estuary in terms of toxic contamination and environmental toxicity using the sediment quality triad approach that has previously been used in assessing the condition of several estuaries and coastal bays (Long, et al., 1996; Long, 2000). The primary study has been supplemented with laboratory-based experiments to elucidate cause-effect relationship between contaminant levels and their adverse biological effects, using spiked-sediment assays (Fulton …) and a suite of biomarkers (Ringwood, et al., 2006). Based on the study results, it can be surmised that sediment contamination in the estuary is low to moderate. Unlike other toxic chemicals, copper levels in the sediment were generally high. Sediment toxicity data, based on samples collected in 2001 and 2003, showed a varied response depending on the test species and toxicological end points. However, in nearly all cases there was greater incidence of toxicity in samples from the North Fork and South Fork and much less at the mouth of the estuary and at sites in the Indian River Lagoon and Jupiter Inlet.

The study results point to copper as a contaminant of concern. The observed levels of copper in surficial sediments of the estuary often exceeded threshold values of copper-related effects, i.e., the Effects Range Low, 34 mg/kg (Long, et al., 1995) and Threshold Effect Level, 19 mg /kg (MacDonald, et al, 1996) and approached sediment quality criteria adopted in a number of North American and European countries, i.e., ca. 100

mg/kg. The copper concentration of 100 mg/kg in sediment equates to a 0.52 probability of causing adverse biological effects according to a logistics regression model (Field, et al., 2002). It should be noted that the logistics regression model was based on data on amphipod toxicity and, therefore, does not account for toxicity to more sensitive species or life stages.

The most likely means of copper input to the estuary is canals and tributaries that drain the coastal watershed with leaching of copper from antifouling paint on boat hulls probably also contributing a significant amount. In the citrus orchards and row crops within the estuary's watershed, copper based fungicides are used extensively. In addition to the publicly-owned treatment plans (POTWs), other land-based sources of copper may include algaecides (used in swimming pools and ponds), roof runoff, vehicle brake pads and soil erosion.

It is also evident that copper entering the estuary is biologically available and readily accumulates in the tissues. The observed levels of copper in the oyster deployed in different parts of the estuary for about four months accumulated from 373 to 505 ppm (dry weight) of copper, exceeding the 50^{th} percentile (150 ppm) and 85^{th} percentile (360 ppm) of NOAA's nationwide data for oysters (Mussel Watch data). Potentially adverse, sub-lethal biological effects of such high copper concentration on oysters have been documented, including increased cellular damage in deployed oysters (Ringwood, et al., 1998; 2006).

Since the majority of data under this study are based on static observations and laboratory tests, they are insufficient in detail for describing the dynamic behavior of copper in the estuary. To address this limitation, a hydrodynamic model has been formulated to examine the transport and fate of copper in St. Lucie Estuary and south Indian River Lagoon (Applied Environmental Engineering, 2006). This mode is built upon the Environmental Fluid Dynamics Computer (EFDC) code that has previously been used to simulate effects of high fresh water inflow events in the northern portion of the Indian River Lagoon. As it is further refined and uncertainty in the model results can be properly attributed, it will be transferred for use by natural resource management agencies in Florida to gain a better understanding of the ecological processes and water quality issues in this highly altered and complex estuarine system.

Despite substantive scientific evidence that points to copper as a contaminant of concern in the St. Lucie Estuary, it is debatable whether the observed levels of copper in the estuary are adversely affecting the flow of products (such as fish) or services (such as recreation) of the estuary, based on established criteria or guidelines. With the limited scope of the present study, it is uncertain whether the copper levels may imply signals of change in relationships among ecological attributes that would affect future delivery of products and services, such as those resulting from human population growth, extreme natural events, and global climate change.

A logical next step in the study of the St. Lucie Estuary is to synthesize information from the suite of studies that have been funded by SLRIT, preferably using the "integrated

assessment" (IA) protocols that focus on management needs of scientific data and provide a means of data integration and determining priority of research needs. Accordingly, the IA consists of the following four elements:

a) Characterization of the status and trends of ecosystem conditions and stressors;
b) Description of the ecological and economic consequences of change for each of the selected stressors;
c) Prediction of outcomes of management options for the most important stressors; and
d) Offering guidance for implementing a range of potential action.

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