FLORIDA FISH & WILDLIFE CONSERVATION COMMISSION
MEMORANDUM

REPORT SUBMISSION MEMORANDUM
FOR ALL REPORTS OTHER THAN DEPARTMENT OF INTERIOR

DATE: July 15, 2004

TO: Linda Torres, FWC/FMRI Grants Office, Administrative Svc, J2N

THRU: Jennifer Wheaton, Administrator, FWC/FMRI/EAR

FROM: Dr. Karen A. Steidinger, Principal Investigator, FWC/FMRI

SUBJECT: FINAL Report for Grant # **F2203/F0007**
Reporting Period: 10/01/1999 to 02/28/2004 Due 07/31/2004
Funding Agency Identifier: NA97OA0361
Project Title: Intensive monitoring for *Pfiesteria* and *Pfiesteria*-like organisms in the St. Johns River

Please submit 1 original and 2 copies of this report to:

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FWC: FMRI File Code: FO F2203/F0007-99-04-F

TITLE: Intensive monitoring for *Pfiesteria* and *Pfiesteria*-like organisms in the St. Johns River

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ORGANIZATION: Florida Fish & Wildlife Conservation Commission, Fish & Wildlife Research Institute
GRANT NUMBER: NA97OA0361
DATE: July 15, 2004

ABSTRACT
The goal of this research was to determine if there are relationships between water quality/sediment variables and the occurrence and abundance of *Pfiesteria* and *Pfiesteia* –like species (PLS) and fish disease events in the Lower St. Johns River (LSJR) basin, Florida. Probes were developed to detect PLS in natural waters. MARVIN, a portable *in situ* monitoring platform, was implemented and became a major success of the program. No PLS blooms occurred during the study period, however, the PLS *Karlodinium micrum* was identified as an important dinoflagellate species in this system that may cause harmful algal blooms. Flagellates were numerically abundant in the natural phytoplankton assemblages of the SJR. A variety of these flagellates were found to be suitable prey for PLS in culture. Many water quality parameters were measured; including nutrients, microbial indicators and benthic organism diversity and sediment quality. All parameters measured indicated the SJR is an estuary impacted by anthropogenic activities, including urbanization and industrialization. Despite these impacts and the potential for HAB outbreaks - specifically PLS blooms - the cause of fish disease events in the SJR was determined to be fungal infection.

EXECUTIVE SUMMARY

Description: Sudden death fish kills and seasonal occurrences of fish with lesions and ulcers in shallow, poorly-flushed estuarine areas are recognized events in coastal waters from northeast Florida to Mexico. These events have also occurred along the eastern seaboard up to New York. Such resource impacts were blamed for many years on oxygen depletion and possibly eutrophication in the shallow areas. North Carolina State University scientists discovered that a small, heterotrophic dinoflagellate called *Pfiesteria piscicida* Steidinger et Burkholder was associated with fish kills and implicated in causing fish skin lesions. This species and other related *Pfiesteria*-like species (PLS) have recently caused concern in the mid- and south Atlantic states because of their association with natural resource impacts, potential public health risks, and subsequent economic losses. The goal of this research was to determine if there are relationships between water quality/sediment variables and the occurrence and abundance of *Pfiesteria* and PLS and fish disease events in the Lower St. Johns River (LSJR) basin.

Objectives The specific objectives of this project were as follows: 1.) To evaluate the historical data within the LSJR and its drainage basin to determine status and trends of environmental variables, including pollutants, over time/space and correlate with distribution patterns of identified PLS or PLS events. 2.) To conduct laboratory studies to determine the significance of different flagellate prey, including cryptophytes, in the growth of *Pfiesteria* and PLS organisms and conduct field studies to monitor flagellate composition and abundance. 3.) To establish water quality parameters in relation to water-inflows, point/non-point source nutrient inputs, and phytoplankton distribution throughout the LSJR estuary. 4.) To implement a portable, *in-situ* instrument platform (A.K.A. MARVIN) to autonomously monitor water quality variables. 5.) To determine if there was any correlation between the schooling of planktivorous fish and blooms of *Pfiesteria* and PLS. 6.) To determine if there was a potential correlation between the distribution of fish lesions described as ulcerative mycosis (UM) with PLS distribution. 7.) To produce nucleic acid probes for the PLS organisms in the SJR and compare the results to other PLS organisms in Florida, North Carolina and Maryland.

Summary of Findings The oligohaline sites (Doctor's Lake, Cedar/Ortega River) in the upper reaches were more similar (in terms of chemical/physical parameters) than the four mesohaline (Clapboard Creek, Mill Cove, Dames Point, Tallyrand, Trout River) sites in the middle and lower reaches. Chlorophyll *a* concentration within sub-surface and bottom waters were equivalent ($p>0.05$), indicating a well mixed column. Phytoplankton assemblages were comprised mostly of diatoms, chlorophytes, cryptophytes, and cyanobacteria. Phytoplankton populations within the

lower SJRB were highly dynamic. A pronounced seasonal variation in phytoplankton abundance (as total chlorophyll *a*) occurred, with indications that conditions for phytoplankton accumulation were most favorable during summer and within the upper reaches of the estuary.

Results to date suggest that the PLS, including *K. micrum*, were more frequent in bottom samples than surface samples. Probe results were checked against count and incubation results - in some cases at least two agreed, in others they did not. We recommend that all methods be standardized and compared between laboratories in order to determine the most accurate method and protocol for identifying the presence of specific PLS. The most frequently occurring and most abundant *Pfiesteria*-like cells are *K. micrum* and the cryptoperidiniopsoids, of which there are several species.

Flagellates (potential PLS prey) were numerically significant in phytoplankton composition, if not biomass. Abundance tended to be greater in the more brackish stations. Some of these species were effective food for PLS during the feeding experiments, including: *Isochyrysis, Rhodomonas, Tetraselmis, Plagioselmis* and *Pyramimonas*. Growth rate among the PLS predators fed flagellate prey was highest in *K. micrum*, followed by *P. shumwayae* and *P. piscicida*.

Using diel physical/chemical data derived from MARVIN (Merhab Autonomous Research Vessel for IN-situ sampling), deployed within the Trout River tributary of the SJR, from May 2001 to May 2003 indicated that production and respiration sources and potential within this segment of the SJRS were extremely variable and resulted in highly-dynamic trophic conditions (as derived from NEM values). During 2001, 2002, and 2003, the Trout River tributary was phototrophic only ca. 52, 41, and 24% of the days during which diel oxygen flux were assessed.

Chemical contamination of sediment at levels likely of having adverse effects on benthic fauna was observed at four stations near the center of Jacksonville (Cedar/Ortega, Tallyrand, Trout River, and Dames Point). Concentrations of man-made pesticides or other chemical substances typically associated with human activities (e.g., PCBs) were detectable at all stations, though not always present at concentrations likely of causing significant bioeffects. Variable and often high levels of porewater un-ionized ammonia and hydrogen sulfide were detected at the MERHAB Florida sampling stations and indicate the potential for problems associated with eutrophication. Doctors Lake, Cedar/Ortega, and Tallyrand were stations that exhibited the highest levels of porewater un-ionized ammonia and hydrogen sulfide. Five sites (Doctors Lake, Cedar/Ortega, Tallyrand, Trout River, and Dames Point) are dominated by species indicative of polluted environments. One additional site (Mill Cove) shows degraded condition based on values of several benthic community measures.

Fecal coliforms were detected at all sites throughout the study, increasing in abundance with proximity to urban areas. Enterococci were never detected at the Clapboard Creek site, but when detected they also increased in abundance with proximity to urban areas. *Aeromonas* sp. were detected at all sites throughout the study, with elevated abundances near the confluence of the Cedar and Ortega Rivers. Coliphage and *Vibrio* sp. were detected at all sites except for Doctors Inlet due to the low salinity. Source tracking for the fecal coliforms indicated that all seven sites showed a significant percentage isolates of human origin, probably as a result of septic systems.

During the course of the study in the LSJR, no fish kills or lesion events were ever attributed to blooms of *P. piscicida* or *P. shumwayae*. In fact, no blooms of *P. piscicida* or *P. shumwayae* were documented. Of those fish that were processed for stomach content incubations, none were positive for PLS. Neither were any ulcerative lesions detected in these fish samples. During the course of this project, and in parallel with other federal grant-supported projects, the cause of the ulcerative mycosis lesions in mullet and menhaden and other estuarine fish in the LSJR and other coastal areas of Florida was verified. The same fungus (*Aphanomyces invadans)* causing UM in menhaden along the eastern seaboard was confirmed in the field, from experimental isolations and exposures confirming Koch's postulates, and by molecular characterization. Data management activities focused on development of the framework for capturing and disseminating continuous monitoring data from MARVIN at the Trout River station. These data were transferred via the GOES satellite system every three hours and retrieved by the Coastal Ecology Program (http://www.chbr.noaa.gov/cep/default.aspx) of the CCEHBR in Charleston, using an automated process (the MERHAB Data Management Suite written in Perl and hosted on a Linux server). The near real-time data on a suite of chemical, physical and biological variables were processed and stored in a database for subsequent use by research partners and all other interested users. AMERHAB Florida website (http://www.merhabfl.org) has been developed to facilitate the dissemination of this information to as wide a user audience as possible.

PLS probes were developed by sequencing the ITS region. Working with this region provided important taxon information at the same time it allowed identification of unique primer binding sites for species-specific PCR assay

development. This taxonomic information may prove useful, particularly in uncharacterized dinoflagellate groups. ITS sequence data will also likely prove useful for developing species-specific PCR assays in other algal groups.

PURPOSE

A. Detailed description of any problems or impediments of research project that were addressed: No problems or impediments were encountered during the project.

B. The specific objectives of this project were as follows: 1.) To evaluate the historical data within the LSJR and its drainage basin to determine status and trends of environmental variables, including pollutants, over time/space and correlate with distribution patterns of identified PLS or PLS events. 2.) To conduct laboratory studies to determine the significance of different flagellate prey, including cryptophytes, in the growth of *Pfiesteria* and PLS organisms and conduct field studies to monitor flagellate composition and abundance. 3.) To establish water quality parameters in relation to water-inflows, point/non-point source nutrient inputs, and phytoplankton distribution throughout the LSJR estuary. 4.) To implement a portable, *in-situ* instrument platform (A.K.A. MARVIN) to autonomously monitor water quality variables. 5.) To determine if there was any correlation between the schooling of planktivorous fish and blooms of *Pfiesteria* and PLS. 6.) To determine if there was a potential correlation between the distribution of fish lesions described as ulcerative mycosis (UM) with PLS distribution. 7.) To produce nucleic acid probes for the PLS organisms in the SJR and compare the results to other PLS organisms in Florida, North Carolina and Maryland.

APPROACH

A Detailed description of the work that was performed **Study Site:** The St. Johns River and estuary, a 300 mile-long system located in northeastern Florida, USA where phytoplankton blooms have been associated with localized fish kills (from hypoxia/ anoxia), loss of submerged vegetation (from reduced water clarity), wildlife mortalities and human health issues.

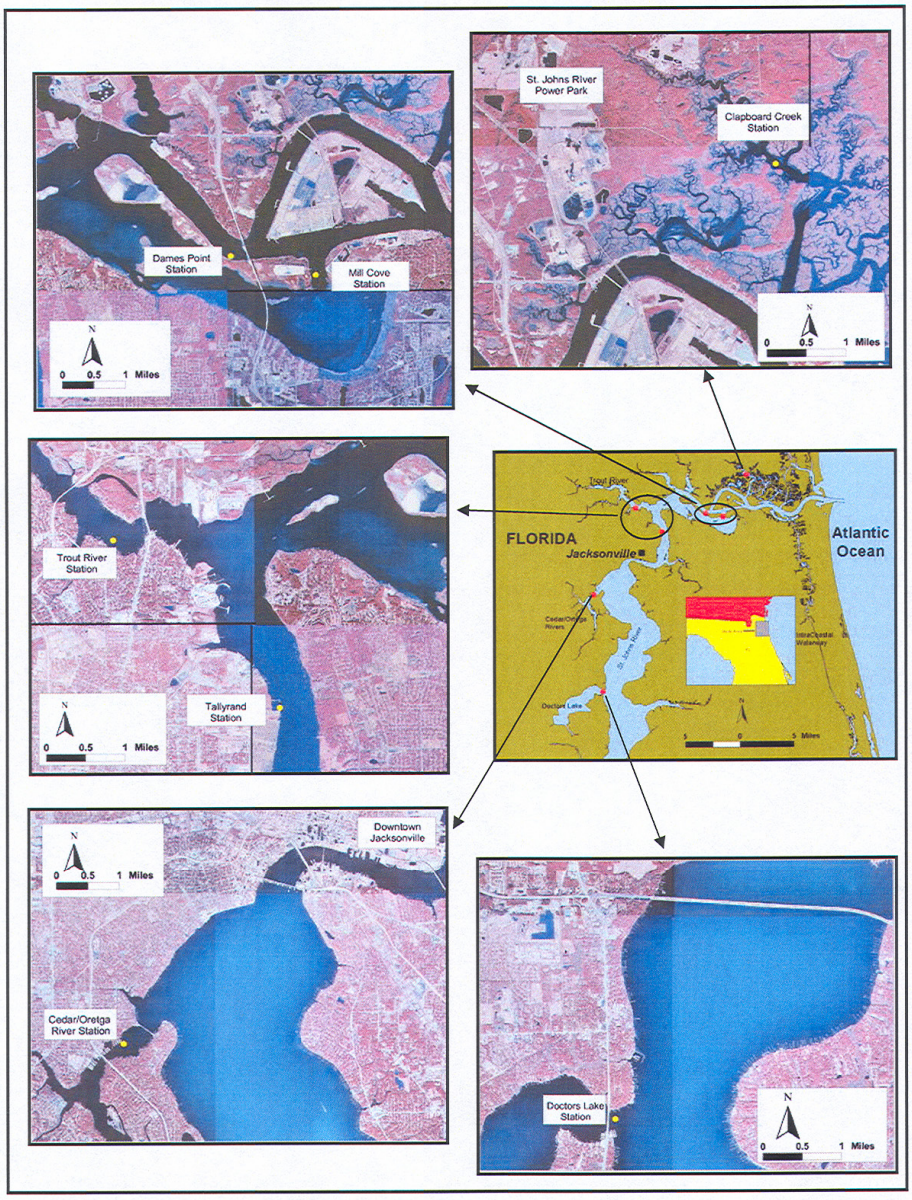

Figure 1. Station locations for MERHAB Florida monitoring in the lower St. Johns River, Florida.

Water quality: From July 2000 to July 2002, monitoring of water-quality and phytoplankton was conducted on a seasonal (Spring-Summer-Fall) basis. Water-quality and phytoplankton were characterized within sub-surface and bottom waters at seven sites (Clapboard Creek, Mill Cove Entrance, Dames Point, Trout River, Tallyrand, Cedar/Ortega River, Doctors' Inlet) situated along an oligo- to mesohaline gradient throughout the lower basin. Continuous (time-series) monitoring of a suite of physical, chemical, and biological variables at multiple water depths from an instrumented platform (MARVIN) also was conducted at one of the fixed stations. The instrumented platform was connected to a HDR GOES (High Data Rate Geostationary Operational Environmental Satellites) satellite transmitter that provides real-time, remotely accessed data. NOAA's National Ocean Service/National Centers for Coastal Ocean Science/Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina was responsible for maintaining these data and developing an associated web site. Physical (temperature, salinity, pH, turbidity) and chemical (dissolved oxygen, inorganic and organic nitrogen, inorganic phosphate concentrations) parameters were measured using water quality multiprobes (YSI and

HydroLab) and standard methods (Parsons et al. 1984). Historical data from this system, analyzed by various state and local agencies, were compiled into a CD-ROM database.

Microbial indicators: Fecal coliform, enterococci, *Clostridium perfringens, Aeromonas* sp., coliphage and *Vibrio* sp. levels were measured from 1-L samples taken at each of the 7 fixed stations seasonally. 1, 5, 10, 25, 50, or 100 ml samples were filtered through 0.45µm membrane filters. Fecal coliform samples were incubated on m-FC agar at 44.5°C for 24 hours and counted. *C. perfringens* were incubated on m-CP media, anaerobically for 24 h at 45°C, exposed to ammonium hydroxide vapors for 30s and counted. Enterococci were incubated on m-EI agar for 24 h at 41°C and counted. *Aeromonas* sp. were grown on ADA for 24 h at 37°C and counted. Ten percent of these samples were then tested for a positive oxidase reaction. *Vibrio* sp. were incubated on TCBS agar for 24 h at 37°C. Coliphage assays were conducted with 2 ml of sample and 0.5 ml of *Escherischia coli* 15597 added to 3 ml TSA, incubated for 24 h and counted. If coliphage was not detected in the standard coliphage assay, the F specific coliphage assay was conducted in 900 ml of sample and 100ml of bacteriophage host and incubated for 48 h. One milliliter of this solution was then added to an agar plate, incubated for 12 h and presence/absence of coliphage was noted.

Phytoplankton: Phytoplankton biomass (as chlorophyll *a*) was determined using chemotaxonomic photopigments derived using high-performance liquid chromatography (HPLC). Absolute and relative phylogenetic group contributions to Chl *a* were derived from chemotaxonomic photopigments using CHEMTAX software. Pigments used for delineating groups included: fucoxanthin (diatoms), peridinin (dinoflagellates), alloxanthin and b,e-carotene (cryptophytes), neoxanthin, violaxanthin, lutein, chl *b* (chlorophytes), zeaxanthin (cyanobacteria), and chl *a* (all algae). Surface and bottom water samples were also collected, preserved with unacidified Lugol's, archived in brown plastic bottles, and refrigerated at 5° C. Seventy-six of those samples at the core seven stations were analyzed for dinoflagellate species composition. Separate analyses were done on the same sample for diatoms and cyanobacteria using different methods. In trials, 25 ml of surface water was the minimum sample volume required to detect PLS at 160X magnification. Bottom water samples were limited to 15 ml because of detritus and other particles. Samples were analyzed on a Zeiss Axiovert 100 inverted microscope with a universal mount. Counts were conducted at two levels of magnification, 160X and 640X. Smaller dinoflagellates such as the PLS, *Prorocentrum minimum*, and *Heterocapsa rotundatum* were counted at 640X, as were the cryptomonads. Forty fields of view at that magnification were quantified for final area and volume measurements to derive per milliliter counts. The whole chamber bottom was counted at 160X for the larger dinoflagellates such as *Takayama sanguinea (=Gymnodinium splendens, G. sanguineum)* and *Ceratium hircus*. Sediment incubations for PLS consisted of 5ml of sediment and 90 ml filtered seawater adjusted for salinity in a 250ml Erlenmeyer flask and monitored three times per week for excystment of PLS. Organisms found above background levels in incubations were analyzed with scanning electron microscopy in an attempt to identify them as cryptoperidiniopsoids, *P. piscicida, P. shumwayae*, "Lucy", *K. micrum,* or other small heterotrophic dinoflagellates. SEM was also used for differentiating PLS if they were detectable in live water samples.

Flagellate prey bioassays: Water samples for bioassay dilution cultures were collected at each fixed station during seasonal field sampling. Four dilutions of each sample were incubated in quadruplicate in Erdschreibers medium. Bioassays were incubated for 5 to 14 days and observed daily for growth measurements and to determine the natural flagellate assemblage at each station. Subsamples were removed from the bioassays for cultures and some were preserved for subsequent microscopy observations. Twenty-three flagellate culture clones were fed to dinoflagellate predators (*P. piscicida* CCMP Clone 1830, *P shumwayae* clone 2089, and *K. micrum*). Predator growth rate and prey clearance rates were monitored to determine prey suitability. In addition, sediments from North Carolina estuaries known for *Pfiesteria* blooms were incubated for a month with suitable flagellate prey species (23°C, continuous light) and monitored every other day for the emergence of PLS.

Benthic community and sediment quality: Samples were collected seasonally for the analysis of benthic community structure and composition (macro-infauna > 0.5 mm), porewater un-ionized ammonia and sulfide, and basic habitat parameters (depth, sediment grain size and TOC, dissolved oxygen, salinity, temperature, and pH). Sediment samples for macro-infaunal analysis were collected at each station in triplicate using a 0.04 m^2 Young grab sampler. Each replicate was sieved in the field through a 0.5-mm mesh screen and preserved in 10% buffered formalin with rose Bengal. All infaunal samples were transferred to 70% ethanol once in the laboratory. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest practical taxonomic level (usually to species).

The upper 2 – 3 centimeters of sediment from additional multiple grabs were taken at each station, combined into a single station composite, and then subsampled for analysis of metals, organic contaminants (PCBs, pesticides, PAHs), total organic carbon (TOC), and grain size. TOC and grain size were analyzed using protocols modified

from Plumb (1981). TOC content of sediment was measured on a CHN elemental analyzer (at 950˚ C combustion temperature). Methods for analysis of chemical contaminants followed those of Sanders (1995), Fortner et al. (1996), Kucklick et al. (1997), and Clum et al. (2002). Metal analyses were performed using inductively coupled plasma mass spectrometry (ICP/MS) for the following suite of metals: Al, Cr, Cu, Fe, Mn, Ni, Sn, As, Cd, Pb and Zn. Ag and Se were analyzed using graphite furnace atomic absorption (GFAA). Cold vapor atomic absorption (CVAA) was used for analysis of Hg. The organic PCBs and pesticides were analyzed by dual-column gas chromatography with electron capture detection (GC-ECD). An ion-trap mass spectrometer equipped with a gas chromatograph (GC/MS-IT) was used for analysis of PAHs.

Sediment quality guidelines (SQG) for each corresponding chemical were used (where available) to help in interpreting the biological significance of the observed contaminant levels (Appendix B). Two types of SQGs were used: (1) Effects Range-Low (ERL) and Effects Range-Median (ERM) values of Long et al. (1995, updated from Long and Morgan 1990); and (2) Threshold Effects Level (TEL) and Probable Effects Level (PEL) values of MacDonald et al. (1996). ERL and TEL values are both lower-threshold bioeffect limits, below which adverse effects of the contaminants on sediment-dwelling organisms are not expected to occur. In contrast, ERM and PEL values both represent mid-range concentrations of chemicals above which adverse effects are more likely to occur. Concentration-to-SQG comparisons were based on the ERL and ERM values for most chemicals; in some cases, however (e.g., where updated ERL and ERM values were not available), the alternative TEL and PEL values were used.

Fish behavior: In March, July, and November of 2000 mullet and Atlantic menhaden (*Brevoortia tyrannus*) were sampled with a trammel net. In addition to morphometrics, abundance, fish health status, and photographic documentation, fish were collected for stomach content analysis. Fish were evaluated for lesion characterization, pathology, and microbiological work-up under the ongoing FMRI fish health project supported by USFWS. Individuals of mullet and menhaden were examined in parallel with fish health evaluations for the potential presence of PLS in stomach contents. Approximately 20 fish from two schools of fish were examined for stomach contents. Stomach contents were removed, measured volumetrically, and approximately 6 ml added to 90ml artificial seawater, run in duplicate, and incubated with microalgal prey for emergence of PLS, as described for water and sediment samples elsewhere.

PLS probe development: Sediment samples for genetic characterization of and development of molecular-based probes for heterotrophic dinoflagellates were collected and shipped to Dr. Patricia Tester (NOAA-NOS). An approximately 3600 bp section of the ribosomal region, including the ITS regions, was sequenced from multiple clones of *P. piscicida*, *P. shumwayae* Glasgow et Burkholder, Florida "Lucy" species, two cryptoperidiniopsoid spp., "PLO21" and "H/V14," and *K. micrum* (Leadbeater et Dodge) J. Larsen comb. nov. (formerly *Gyrodinium/Gymnodinium galatheanum*). *P. piscicida*, *P. shumwayae*, Florida "Lucy" species, and cryptoperidiniopsoid species "H/V14" and "PLO21" are heterotrophs and were cultured in sterile Gulf Stream water diluted to 15 psu, 23+/-1 ^{o}C, L/D 14:10, ~50 µE $m^{-2}s^{-1}$. *Karlodinium micrum* is a mixotrophic species and was cultured in F/40 at 15-20 psu, 23+/-1 ^{o}C, L/D 14:10, ~50 µE $m^{-2}s^{-1}$. All species were fed a small amount of the prey species *Rhodomonas* sp. (CCMP767) every 2-3 days. Cultures were transferred to new flasks with fresh media every 8-12 days. The autotrophic species, *Karenia brevis* Hansen and Moestrup comb. nov (=*Gymnodinium breve*), was used during the PCR assay development as a negative control. This species was cultured in F/2 (Guillard and Ryther 1962) or K media (Keller et al.1987) at 23+/-1^{o}C, L/D 14:10, ~100 µE $m^{-2}s^{-1}$, 30 psu. The prey species *Rhodomonas* CCMP767 was grown in F/2-Si, 1 L glass or polycarbonate bottles, 30 psu, 25+/-0.2^{o}C, L/D 14:10, ~80 µE $m^{-2}s^{-1}$.

DNA extraction, PCR amplification and DNA sequencing procedures. Approximately 50 mL of each heterotrophic culture (~1 X 10^{3} cell·mL^{-1}) were concentrated by filtration onto a 47 mm, 3 µm pore size Nucleopore™ polycarbonate filter (Whatman, Clifton, New Jersey). These heterotrophs were allowed to graze down the *Rhodomonas* prior to harvesting. As controls, 50 mL of log phase *K. brevis* and the *Rhodomonas* strain CCMP767, were similarly filtered onto a 3 µm Nucleopore filter. DNAs were extracted from the filters using a DNeasy Tissue Kit following the manufacturer's protocol (Qiagen™, Valencia, California). Filters that were not immediately processed were stored at –80^{o}C. The SSU, ITS1, 5.8S, ITS2, and first 66 bp of the LSU were amplified using the Dino5'UF and ITSR2 primers (Table 1). The amplification reaction mixtures contained 20 mM Tris-HCl, pH 8.4, 3 mM $MgCl_2$, 50 mM KCl, 25 pmoles of each primer, 200 µM of dNTPs, 0.5 units Platinum® *Taq* DNA polymerase (Invitrogen™ Life Technologies, Rockville, Maryland), and 20 ng of genomic DNA in a total volume of 50 µL. The DNA was amplified in a Robocycler® (Stratagene®, LaJolla, California) using the following cycling conditions: 2 min. at 95^{o} C followed by 35X (30 sec. at 95^{o} C, 45 sec. at 60^{o} C, 2.5 min. at 72^{o} C) with a final extension of 7 min. at 72^{o} C. The primers 5.8SF and LSU-B were used to amplify the region containing the last ~100 bp of the 5.8S gene through the first ~900 bp of the LSU. The amplification mixture was a 50 µL volume identical to that described above. These reactions were amplified using an initial

denaturation of 2 min. at 95° C followed by 30X (30 sec. at 95° C, 40 sec. at 63° C, 1.25 min. at 72° C) with a final extension of 5 min. at 72° C. The Dino5'UF and LSU-B primers were capable of amplifying the entire SSU-5'LSU region. However, this primer pair also amplified the *Rhodomonas* CCMP767 DNA equally well. This made it impossible to obtain a single PCR product spanning the entire SSU-LSU gene regions that was not contaminated with *Rhodomonas* DNA.

A 5 µL aliquot of each PCR reaction was checked for the presence of a specific amplification product by agarose gel electrophoresis (1% TAE, 50 V) and ethidium bromide staining. PCR reactions containing specific products were cleaned using the QIAquick™ PCR purification kit (Qiagen), quantified spectrophotometrically, and sequenced on an ABI377 DNA sequencer using the Deoxy™ Terminator Cycle sequencing kit (Applied Biosystems – ABI™, Foster City, California). Sequencing was performed following the manufacturer's instructions and using approximately 10 ng of DNA for each 100 bp of template DNA and 25 pm of primer. DNA templates were sequenced completely in both directions using the primers listed in Table 1. The resulting SSU to 5' LSU sequence for each species was assembled using the Vector NTI® program (Informax Inc., Bethesda, Maryland).

Species-specific PCR assay development. The SSU – 5' LSU sequences obtained in this study and other related dinoflagellate sequences available from GenBank were aligned using the CLUSTAL-W algorithm (Thompson et al. 1994) included in the MacVector 7.0™ software package (Oxford Molecular Ltd., Oxford, U.K.). These alignments were used to identify unique ITS sequences and to develop species-specific PCR assays.

Each of the PCR assays used the conserved forward primer, DinoDUF1, located ~150 bp from the 3' end of the SSU (Table 2). Species-specific reverse primer sites in the ITS regions were selected for evaluation (Innis et al. 1999, McPherson et al. 2000). All primer pairs were designed to anneal at 56° C (Table 2). Each PCR reaction contained 25 pmoles of each primer, 200 µM of dNTPs, 0.5 units Platinum™ *Taq* DNA polymerase (Invitrogen™) and ~20 ng of genomic DNA in a total volume of 50 µL. The pH, Tris-HCl, $MgCl_2$ and KCl concentrations were optimized with the 12 buffer optimization kit (Opti-Prime™, Stratagene) (Table 3). Amplifications were carried out in a Robocycler® with the following profile: 2 min. at 95° C followed by 35X (20 sec. at 95° C, 30 sec. at 56° C, 45 sec. at 72° C) with a final extension of 5 min. at 72° C. Aliquots (5 µL) from each amplification were separated on 3% 3:1 Nusieve (BioWhittaker™, Walkersville, Maryland) TAE agarose gels run at 50V. The size of the PCR products was verified using either a 100 or 123 bp molecular weight ladder (Promega™, Madison, Wisconsin and Roche™, Basel, Switzerland, respectively). Following optimization, the primer pairs were tested for possible cross-reactivity with a panel of DNAs, including the other species for which assays were being developed, *K. brevis*, and the *Rhodomonas* sp. used as food for the heterotrophic dinoflagellates. The *Rhodomonas* controls were particularly important because residual DNA from these food species contaminated each of the heterotrophic DNA preparations.

Determining minimal limits of detection for each assay. Water samples were collected from the Neuse River estuary, North Carolina, USA. These samples came from a region of the estuary where the salinity was ~15 psu, the same salinity as used to culture each dinoflagellate species. Cells from each of the six PLO species were added to individual Neuse River water samples such that a series of cell concentrations ranging from 1 cell·mL^{-1} to 50 cells·mL^{-1} in a final volume of 100 mL were generated. These spiked samples were gently filtered (<10 cm Hg) though a 47 mm, 3 µm Nucleopore polycarbonate filter and extracted using the Mo Bio UltraClean™ soil DNA extraction kit (Mo Bio Laboratories, Inc., Solana Beach, California). Blank cell addition treatments were included to identify contaminating target cells already present in the Neuse River water. Precise *Pfiesteria* or PLO DNA concentrations could not be measured spectrophotometrically due to the presence of other contaminating DNAs from micro-organisms in the Neuse River water. We therefore used a 2 µL volume of extracted DNA per PCR reaction. DNAs from these samples were amplified using the species-specific primers and the reaction conditions described above. The limits of detection were determined using agarose gel electrophoresis and ethidium bromide staining.

B. Project Management: List individuals and/or organizations actually performing the work. Florida Fish and Wildlife Conservation Commission, Florida Marine Research Institute: Brian Bendis, Ryan Pigg, Karen Steidinger, Jan Landsberg, Paul Carlson, Behzad Mahmoudi, Jay Abbott. Florida Institute of Oceanography/FWRI: Jennifer Wolny, David Millie. University of North Carolina, Wilmington, Carmelo Tomas, Tatum Neeley, Amanda Bridgers, Ellen McConnell. AMJ Equipment, Inc. of Lakeland, Florida. Oscar Schofield, Rutgers University. NOAA, Cynthia Cooksey, Wayne Litaker, Patricia Tester. University of South Florida, College of Marine Science: Joan Rose, Stephanie Shehane. St. Johns River Water Management District: John Burns, BCI Engineers and Scientists, Lakeland, FL.

FINDINGS

A. Actual Accomplishments and Findings **Water Quality**: The oligohaline sites (Doctor's Lake, Cedar/Ortega River) in the upper reaches were more similar (in terms of chemical/physical parameters) than the four mesohaline (Clapboard Creek, Mill Cove, Dames Point, Tallyrand, Trout River) sites in the middle and lower reaches. Chlorophyll *a* concentration within sub-surface and bottom waters were equivalent ($p>0.05$), indicating a well mixed column.

Phytoplankton populations within the lower SJRB were highly dynamic. A pronounced seasonal variation in phytoplankton abundance (as total chlorophyll *a*) occurred, with indications that conditions for phytoplankton accumulation were most favorable during summer and within the upper reaches of the estuary. Phytoplankton assemblages were comprised mostly of diatoms, chlorophytes, cryptophytes, and cyanobacteria. Phytoplankton composition was most diverse during Spring and Summer, with a dominance shift from diatoms to cyanobacteria during Summer at the oligohaline sites. The greatest relative abundances of cyanobacteria occurred in July at the most oligohaline sites (Cedar/Ortega Rivers; ca. 45% and Doctor's Lake; ca. 65%)- as expected, cyanobacterial was inversely associated with salinity, particularly in March/April and July, but had no consistent association with temperature.

Modeling Phytoplankton and Trophic Status: Assessing the status or health of coastal systems is key for the accurate characterization of chemical/biological responses resulting from natural- and/or human-derived disturbance. The parameter, Net Ecosystem Metabolism (NEM), represents the balance between production and respiration; as such, values can be used as a proxy for trophic condition. Positive NEM values indicate internal production of organic matter dominates the system (phototrophism) whereas negative values signify that external sources of organic matter dominate system (heterotrophism).

Table 1. Trophic conditions for the Trout River tributary from May, 2001 to May 2003, as indicated from Net Ecosystem Metabolism values derived from monitoring of diel oxygen flux. Data represent the annual number of days. Numbers in parentheses indicate annual percentages (of total days assessed) for each trophic condition.

Year/Trophic Condition	Heterotrophic	Phototrophic	Total Days Monitored
2001	62 (48.4)	66 (51.6)	128
2002	174 (58.8)	41.2 (122)	296
2003	108 (76.1)	23.9 (34)	142

Using diel physical/chemical data derived from MARVIN (Merhab Autonomous Research Vessel for IN-situ sampling), deployed within the Trout River tributary of the SJR, from May 2001 to May 2003 indicated that production and respiration sources and potential within this segment of the SJRS were extremely variable and resulted in highly-dynamic trophic conditions (as derived from NEM values). During 2001, 2002, and 2003, the Trout River tributary was phototrophic only ca. 52, 41, and 24% of the days during which diel oxygen flux were assessed (Table 1). Examination of NEM dynamics over short time intervals (hours to days) indicated that both gross production and net oxygen flux (synonymous with net production) were highly variable. Overall, respiration typically exceeded gross production and as a result, both autochthonous and allochthonous sources of organic matter appeared to dominate for short-time intervals.

Microbial indicators: Fecal coliforms were detected at all sites throughout the study, increasing in abundance with proximity to urban areas. Enterococci were never detected at the Clapboard Creek site, but when detected they also increased in abundance with proximity to urban areas. *Aeromonas* sp. were detected at all sites throughout the study, with elevated abundances near the confluence of the Cedar and Ortega Rivers. Coliphage and *Vibrio* sp. were detected at all sites except for Doctors Inlet due to the low salinity. Source tracking for the fecal coliforms indicated that all seven sites showed a significant percentage isolates of human origin, probably as a result of septic systems.
Phytoplankton: Results to date suggest that the PLS, including *K. micrum*, were more frequent in bottom samples than surface samples. Probe results were checked against count and incubation results - in some cases at least two agreed, in others they did not. We recommend that all methods be standardized and compared between laboratories in order to determine the most accurate method and protocol for identifying the presence of specific PLS. At the light microscopy level of resolution, *Karlodinium* and cryptoperidiniopsoids can be differentiated, but *P. piscicida, P. shumwayae* and "Lucy" are difficult. The most frequently occurring and most abundant *Pfiesteria*-like cells are *K.*

micrum and the cryptoperidiniopsoids, of which there are several species. None of the methods to date determine toxicity or potential toxicity of the PLS.

Dinoflagellates in the samples so far total > 50 recognizable taxa: *K. micrum*, PLS, cryptoperidiniopsoids, *Heterocapsa rotundatum, H.* cf. *niei*, *H. triquetra*, *Prorocentrum minimum, P. scutellum, P. compressum, P. micans, P. gracile, P. cf. lima, Kryptoperidinium foliaceum, Oxyphysis oxytoxoides, Protoperidinium conicum, P. cf. leonis, P. quinquecorne, P. divergens, P. pellucidum, P.cf. pallidum, P. pentagonum, P. cf. abei, P.* spp., *Bysmatrum* sp., *Dinophysis caudata, Ceratium hircus, C. fusus, C. kofoidii, C. tripos, Podolampas palmipes, scrippsielloids, Scrippsiella* cf. *trochoidea, Oxytoxum* cf. sp., *Corythodinium tesselatum, Gonyaulax polygramma, G. digitale, G. spinifera, G. verior, Polykrikos kofoidii, Amphidinium* sp., *A. carterae, Oxyhris marina*, *Gymnodinium* spp., *Gyrodinium* spp., *Gyrodinium spirale, G.* cf. *pinque, Gyrodinium instriatum, Takayama sanguinea*, *Katodinium glaucum, Oblea*-like sp (diplopsalid), *Diplopsalis* cf. *lenticula*, *Lingulodinium polyedrum*, peridinioids that possibly could include *Woloszynskia*, spiny and smooth dinocysts , and unidentified dinoflagellates. The list will undoubtedly increase with analysis of summer samples.

The smaller size classes dominated and, particularly with cryptomonads, *Heterocapsa* can codominate. Cryptomonads, a recognized food source for PLS in the field as well as in culture, had peaks of abundance. These peaks will be analyzed in the future in relation to the frequency and abundance of PLS depth, season and hydrography time series. There is no apparent within sample count correlations, but the processed sample size is too low. Future analyses will be performed using the entire dataset. In addition, cell counts will be compared to results of coinvestigator Tomas' dilution method for incubating flagellates and determining most probable number (MPN). Whether there is a correlation or not is ecologically significant since a hypothesis is that PLS, (heterotrophs) feed on chlorophyll-bearing phytoplankton like cryptomonads. Future analyses to determine the forcing functions for community structure and species associations are planned.

Flagellate prey for PLS results: Diatoms were common in the field samples, dominating the phytoplankton biomass, and sometimes dominated the dilution bioassays. Flagellates were numerically significant in phytoplankton composition, if not biomass. While most flagellates from the field samples were unidentifiable and did not preserve well, some species were common and able to be identified either to the species or genus or level. *Karlodinium micrum* was the only commonly found dinoflagellate and thus was used as an additional predator in the feeding experiments. Fouty-five species of flagellates from 8 families were found during the study. Abundance tended to be greater in the more brackish stations. *Apedinella spinifera, Plagioselmis prolonga, Pryamimonas amylifera, Tetraselmis striata* and *Teleaulax acuta* were the most common species at most stations throughout the study. In some instances, the dilution assays produced populations of flagellates not present in the discrete fixed samples. Some of these species were effective food for PLS during the feeding experiments, including: *Isochyrysis, Rhodomonas, Tetraselmis, Plagioselmis* and *Pyramimonas*. Growth rate among the predators was highest in *K. micrum*, followed by *P. shumwayae* and *P. piscicida*. A number of flagellate prey sustained PLS populations and lead to varying growth rates among predators. The flagellate prey cultures isolated from the SJR basin were effective in stimulating PLS growth from North Carolina estuary sediments.

Benthic organisms and sediment quality: Chemical contamination of sediment at levels likely of having adverse effects on benthic fauna was observed at four stations near the center of Jacksonville (Cedar/Ortega, Tallyrand, Trout River, and Dames Point). Concentrations of man-made pesticides or other chemical substances typically associated with human activities (e.g., PCBs) were detectable at all stations, though not always present at concentrations likely of causing significant bioeffects. The widespread distribution of these contaminants is most likely attributable to the proximity to a major metropolitan area with potential pollutant inputs from shipping, marinas, commercial shipbuilding and repair, military bases, pulp and paper manufacturing, petroleum storage facilities, power generation, fishing, urban and high-density residential development, and recreation. Other sources include a wide variety of agricultural activities throughout the LSJR watershed and several Superfund sites. Variable and often high levels of porewater un-ionized ammonia and hydrogen sulfide were detected at the MERHAB Florida sampling stations and indicate the potential for problems associated with eutrophication. Doctors Lake, Cedar/Ortega, and Tallyrand were stations that exhibited the highest levels of porewater un-ionized ammonia and hydrogen sulfide. Benthic macroinfaunal communities at five of the seven sites sampled in the LSJR show indication of stress that could be associated with anthropogenic activities. Five sites (Doctors Lake, Cedar/Ortega, Tallyrand, Trout River, and Dames Point) are dominated by species indicative of polluted environments. One additional site (Mill Cove) shows degraded condition based on values of several benthic community measures. No catastrophic HAB events occurred in the LSJR during the course of this study so it was not possible to evaluate the impacts of HABs on benthic communities. However, in a heavily developed system such as the St. Johns River any evaluation of the impacts of Harmful Algal Blooms on benthic communities would have to take into account the relative contributions of impacts resulting from multiple stressors.

Fish behavior: In the late 1990s (at the time of proposal submission) investigations of the role of PLS, specifically *Pfiesteria piscicida*, in the initiation of fish lesions relied on field-based observations of lesioned fish and/or fish kill events in relation to the abundance of PLS, and preliminary exposure studies demonstrating that lesions could be initiated by fish exposure to external "*Pfiesteria* toxin". Laboratory-based exposures using *P. piscicida* demonstrated that tilapia exposed to tank water containing *P. piscicida* developed external lesions involving several opportunistic species of bacteria and fungi (Noga et al. 1996). This scenario was not quite the same as that being observed in the field, where typically a dominant fungal pathogen, *Aphanomyces invadans* was primarily detected in fish lesions and was subsequently confirmed to be the primary etiological agent of ulcerative mycosis in menhaden (Blazer et al., 1999, 2002; Kiryu et al., 2002, 2003).

We investigated the statewide distribution of fish lesions attributed to UM in a number of estuarine fish species and conducted a number of field and laboratory based exposure studies to verify the cause of UM in the SJR and elsewhere. UM in estuarine fish has been known in Florida since at least the late 1970s. Unlike the rest of the eastern seaboard in the United States, where menhaden are primarily affected, multiple estuarine and freshwater fish species with UM have been reported in Florida (Hargis 1985; Grier and Quintero 1987; Te Strake and Lim 1987; McGarey et al. 1990, 1991; Florida Fish and Wildlife Conservation Commission, unpublished data); predominantly in striped mullet, *Mugil cephalus*, silver mullet, *Mugil curema*, bluegill, *Lepomis macrochirus*, and American shad, *Alosa sapidissima*. UM is characterized by deep, penetrating ulcers, chronic inflammation, and the presence of a fungus, usually *Aphanomyces* spp.

Pfiesteria piscicida has been implicated in UM. Fish experimentally exposed to *P. piscicida* showed hemorrhaging and epithelial sloughing which can further result in deep ulcerations that become secondarily invaded by opportunistic bacteria and fungi. In active kills in North Carolina and Maryland in 1997, 30-95% of the fish exhibited deep, chronic ulcers. As the pathology of these mature lesions indicated that they are at least a week old, it suggested that *P. piscicida* could not be involved in lesion initiation at the time of the fish kill.

In certain geographical locations, *P. piscicida* has been associated with active fish kills that are coincident with fish exhibiting lesions. This has led to the inference that *P. piscicida* is also responsible for the lesions. The spatial and temporal distribution of *P. piscicida* and *P. shumwayae* in Florida did not support this conclusion. In some situations *P. piscicida* may initiate lesions, but this may not always be the case. In Florida and South Carolina, for example, fish with lesions can occur in the absence of active fish kills or *P. piscicida*.

During the course of the study in the LSJR, no fish kills or lesion events were ever attributed to blooms of *P. piscicida* or *P. shumwayae*. In fact, no blooms of *P. piscicida* or *P. shumwayae* were documented. Our initial efforts to sample mullet and menhaden in the Mill Cove area in the first year were not successful and only minimal numbers of fish were located. Additionally of those fish that were processed for stomach content incubations, none were positive for PLS. Neither were any ulcerative lesions detected in these fish samples. Mill Cove was therefore dropped as a parallel study site and efforts were focused towards determining if fish with lesions were present in the Trout River – the location sited for MARVIN. Again, efforts to correlate fish lesions with increased PLS in the water column were not successful and sampling efforts were discontinued after one year of monitoring. During the course of this project, and in parallel with other federal grant-supported projects, the cause of the ulcerative mycosis lesions in mullet and menhaden and other estuarine fish in the LSJR and other coastal areas of Florida was verified. The same fungus, *Aphanomyces invadans*, causing UM in menhaden *Brevoortia tyrannus*, along the eastern seaboard, was confirmed in the field, from experimental isolations and exposures confirming Koch's postulates, and by molecular characterization

PLS Probe Development: Sequencing of the ITS regions revealed that there were only minor polymorphisms within individual genomes or between individuals of the same species. By contrast, the between species DNA sequence variation was large. This made it possible to identify and test a number of unique oligonucleotide primer binding sites. The PCR assays which were developed had a minimum sensitivity of 100 cells in a 100 mL sample (1 cell mL^{-1}) and were successfully used to detect PLS from the SJR. This study showed that species-specific PCR assays based on unique ITS sequences could be developed for dinoflagellate species. Targeting the ITS was advantageous because, though relatively short and economical to sequence, this region contained a significant number of species-specific primer binding sites. ITS sequence data reduced the time and money needed to develop new species-specific PCR assays relative to sequencing SSU or LSU regions. As has been reported for other dinoflagellate species (Adachi et al. 1994, 1995, Hudson and Adlard 1996, Adachi et al. 1997, Baillie et al. 2000), the ITS region of PLS dinoflagellates enabled us to distinguish closely related species. Sequencing the ITS region therefore provided important taxon information at the same time it allowed identification of unique primer binding sites for species-specific PCR assay development. This taxonomic information may prove useful, particularly in uncharacterized dinoflagellate groups. ITS sequence data will also likely prove useful for developing species-

specific PCR assays in other algal groups.

MARVIN Data Management Data management activities focused on development of the framework for capturing and disseminating continuous monitoring data from MARVIN at the Trout River station. These data were transferred via the GOES satellite system every three hours and retrieved by the Coastal Ecology Program (http://www.chbr.noaa.gov/cep/default.aspx) of the CCEHBR in Charleston, using an automated process (the MERHAB Data Management Suite written in Perl and hosted on a Linux server). The near real-time data on a suite of chemical, physical and biological variables were processed and stored in a database for subsequent use by research partners and all other interested users.

A MERHAB Florida website (http://www.merhabfl.org) has been developed to facilitate the dissemination of this information to as wide a user audience as possible. The website provides direct access to various types of information including the near-real time data on water-quality variables measured by the platform (raw data are available for download as well as graphs for display); data archives associated with the additional seasonal intensive monitoring at the various fixed stations; and general information on scientific approaches, significant developments and products. In addition, this website incorporated features to be fully W3C standard and Section 508 compliant.
B. Description of need, if any, for additional work. Phytoplankton identification of samples will continue beyond the project period. Statistical analysis of the historical data along with data collected during this study will be ongoing.
EVALUATION
A. Describe the extent to which the project goals and objectives were attained. All of the project objectives were attained. The volume of data collected during this project necessitates ongoing analysis efforts beyond the project period. In addition, the development and deployment of MARVIN (in-situ autonomous sampling platform delivering near-real time data) was so successful that it will continue to be utilized in data collection efforts in Florida. The advancements made during this research will be used to further PLS culture and molecular probe studies.
B. Dissemination of Project Results. FMRI participated in a demonstration project at the Kennedy Space Center with the Navy, NOAA, NASA and USF from August 16-23, 2003. A NASA newsletter with an article about the project was produced. P.I. David Millie was a participant at a workshop, *Harmful Algal Blooms Observation System (HABSOS)*, in Pensacola, FL (11/2000). Dr. Millie also presented oral and poster presentations summarizing data generated from MARVIN at annual meetings of the Phycological Society of America (8/2002 and 7/2003), the 10th International Conference on Harmful Algae (10/2002), the annual Meeting of the Southeastern Phycological Colloquy (10/2003) and the American Society of Limnology and Oceanography (6/2004) . An oral paper presentation is also planned for annual meeting of the Phycological Society of America (8/2004).

Bendis, B.J., Steidinger, K.A., Millie, D. MARVIN:MERHAB Autonomous Research Vessel for In-Situ sampling. AASLO 2002, Victoria, BC Canada, Oral.
Bendis, B.J., Steidinger, K.A., Pigg, R.J., Millie, D. An Autonomous instrumentation platform in the St. Johns River, FL, ERF 2001, St. Petersburg, FL, poster.
Pigg, R.J., Steidinger, K.J., Millie, D.F., & Bendis, B.J. Seasonal impact on phytoplankton growth along the lower St. Johns River Basin, ERF 2001, St. Petersburg, FL, poster.
Pigg, R.J., Millie, D.F., Bendis, B.J. Autonomous instrumentation in conjuntion with adaptive sampling routines to characterize the lower St. Johns Basin, USA, ASLO Savannah 2004, Oral.
Pigg, R.J., Millie, D.F., Steidinger, K.A., Bendis, B.J. Relating phytoplankton composition to environmental forcing in the lower St. Johns River estuary. ASLO 2003, Salt Lake City, oral.
Trader, T.H., Bendis, B.J., Steidinger, K.A. Real-time remote water quality monitoring in the St. Johns River, FL, ASLO 2003 Salt Lake City, Oral.

P.I. Jan Landsberg presented the following oral and poster presentations:

Landsberg J.H., Steidinger, K.A., Cook, S., Singh, E.S., Sosa, E.R., Forstchen, A.B., Wood, R.L., Rublee, P.A., Scott, P.S., Wolny, J.L., Winner, B.L., Whittington, J.A. and Perry, N. E. 2000. *Pfiesteria*, *Pfiesteria*-like species, and fish health in Florida.Poster presentation at the CDC National Conference on *Pfiesteria*: from Biology to Public Health. 18-20 October 2000, Stone Mountain, Georgia. Abstr. p. 78
Landsberg, J. H. 2000. The effect of harmful algal blooms on the health of Florida's fish Presented at the Florida Chapter of the American Fisheries Society, 20th Annual Meeting, Brooksville, Florida, March 28-30, 2000.

Landsberg, J.H., E. Sosa, S. Cook, A. Forstchen, N. Perry, B. Winner, J. Whittington, K. A. Steidinger, and J. W. Bernstein. Fish lesions and *Pfiesteria* in Florida Oral presentation at the American Fisheries Society Fish Health Section meeting, Pensacola, Florida, 6-8 September 2000.

Landsberg, J. H., Steidinger, K.A., Cook, S., Singh, E., Sosa, E., Forstchen, A., Wood, R., Rublee, P., Scott, P., Wolny, J., and Bendis, B. 2000. *Pfiesteria*, *Pfiesteria*-like species and fish health in Florida: an update.Presentation at the Symposium on Harmful Marine Algae in the U.S. December 4-9, 2000, Marine Biological Laboratory, Woods Hole, Massachusetts. Abstr. p. 45.

Landsberg, J.H., Steidinger, K.A., and Cook, S. 2000. *Pfiesteria* and *Pfiesteria*-like species in Florida.. Poster presentation at the Harmful Algal Blooms Ninth International Conference, Hobart, Tasmania, 6 – 11 February 2000

Sosa, E. R., Landsberg, J.H., Forstchen, A., Perry, N. Whittington, J., Winner, B. and Bernstein, J. 2000. Occurrence of fish lesions in Florida.Poster presentation at the American Fisheries Society Fish Health Section meeting, Pensacola, Florida, September 6-8, 2000.

Sosa, E. R., Landsberg, J. H., Litaker, R. W., and Forstchen, A. B. 2002. Lesions of estuarine fish in Florida: are they caused by the same pathogen? Poster presentation at the Fourth International Symposium on Aquatic Animal Health, September 1-5, 2002, New Orleans, Louisiana.

Stephenson, C. and Landsberg, J. H. 2002. Ulcerative mycosis: salinity, and granuloma formation in Florida fish. Poster presentation at the Fourth International Symposium on Aquatic Animal Health, September 1-5, 2002, New Orleans, Louisiana.

P.I. Joan Rose and her students made the following oral and poster presentations:

Shehane, Stephaney D., Angela Gennaccaro, John Whitlock, Valerie J. Harwood, and Joan B. Rose. The Influence of Climate on the Incidence of Microbial Fecal Indicators and the Source of Fecal Contamination with the Lower St. Johns River Basin, Florida. Poster Presentation at the 103rd General meeting of the American Society for Microbiology, Washington, D.C. May 18-22, 2003.

Shehane, Stephaney D., Angela Gennaccaro, John Whitlock, Valerie J. Harwood, and Joan B. Rose. The Occurrence of Microbial Fecal Indicators and the Determination of the Source of Fecal Contamination in the St. Johns River, Florida. Poster Presentation at the 102nd General meeting of the American Society for Microbiology, Salt Lake City, Utah. May 19-23, 2002.

Shehane, Stephaney D., Angela Gennaccaro, Molly R. McLaughlin, and Joan B. Rose. Incidence of Indicator Species and Virulence Genes of *Aeromonas hydrophila* from the St. Johns River Basin, Florida. Poster Presentation at the International Water Association World Water Congress, Melbourne, Australia. April 7-11, 2002.

Shehane, Stephaney D., Angela Gennaccaro, Molly R. McLaughlin, and Joan B. Rose. Incidence of Indicator Species and Virulence Genes of *Aeromonas hydrophila* from the St. Johns River Basin, Florida. Oral Presentation at the Annual Meeting of the Florida Branch of the American Society for Microbiology, Cocoa Beach, Florida. February 15-16-2002.

Shehane, Stephaney D., Molly R. McLaughlin, Jennifer Jarrell and Joan B. Rose. Incidence and Distribution of Indicator SPecies and Virulence Genes Isolated from the St. Johns River, Florida. Oral Presentation at the 101st General Meeting of the American Society for Microbiology,Orlando, Florida. May 20-24, 2001.

Shehane, Stephaney D., Molly R. McLaughlin, Jennifer Jarrell and Joan B. Rose. Incidence and Distribution of Indicator SPecies and Virulence Genes Isolated from the St. Johns River, Florida. Poster Presentation at the 101st General Meeting of the American Society for Microbiology,Orlando, Florida. May 20-24, 2001.

To date, manuscripts summarizing data acquired from this program include:

Kane, A.S., Dykstra, M.J., Noga, E.J., Reimschuessel, Baya, A., Driscoll, C., Paerl, H.W. and Landsberg, J.H. 2000. Etiologies, observations and reporting of estuarine finfish lesions. *Mar. Environ. Res*., 50:473-477.

Litaker, R. W., M. W. Vandersea, S. R. Kibler, K. S. Reece, N. A. Stokes, K. Steidinger, D. F. Millie, B. J. Bendis, R. M. Pigg, and P. A. Tester. 2003. Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using ITS-specific PCR assays. Journal of Phycology 39: 754-761

Litaker, R. W., Vandersea, M. W., Kibler, S. R., Reece, K. S., Stokes, N. A., Steidinger, K. A., Millie, D. F., Bendis, B., Pigg, R., & Tester, P. A. 2003. Variability among dinoflagellate ITS region sequences: Identification of *Pfiesteria piscicida* (Dinophyceae) and *Pfiesteria*-like organisms using ITS-specific PCR assays. *Journal of Phycology*. 39: 754-761.

M. W. Vandersea, R. W. Litaker, B. Yonnish, E. Sosa, J. Landsberg, C. Pullinger, P. Moon-Butzin, J. Morris, H. Kator, E. J. Noga, P. A. Tester, Detection of *Aphanomyces invadans* and *Aphanomyces* sp. 84-1240 using its-specific PCR assays and fluorescent peptide nucleic acid *in situ* hybridization probes in environmental samples, Submitted to Applied Environmental Microbiology

Millie, D., Weckman, G., Paerl, H., Pinckney, J., Bendis, B., & Pigg, R. Statistical-based characterization of estuarine indicators: predicting phytoplankton biomass and net ecosystem production using linear models and neural networks. In preparation for *Journal of Phycology* (to be submitted 8/04).

Paerl, H. W., Dyble, J., Pinckney, J. L., Valdes, L. M., Millie, D. F., Moisander, P. H., Morris, J. T., Bendis, B., and Piehler, M. F. 2003 (Submitted). Using microalgal indicators to assess human and climactically-induced ecological change in estuaries. *Proceedings of the Estuarine Indicators Workshop*; CRC Press.

Pigg, R. J., Millie, D. F., Bendis, B. J., and Steidinger, K. A. 2004 (In Press). Relating cyanobacterial abundance to environmental parameters in the lower St. Johns River Estuary. *Proceeding of the 10th International Conference on Harmful Algae*. Steidinger, K. A., Landsberg, J. H., Tomas, C. R., and Vargo, G. A. (Eds). Florida Fish and Wildlife Commission and Intergovernmental Oceanographic Commission of UNESCO.

Shehane, Stephaney D., and Joan B. Rose. (in preparation) Occurrence of Indicator Species and Virulence Genes of *Aeromonas hydrophila* within the St. Johns River.

Shehane, Stephaney D., Angela Gennaccaro, John Whitlock, Valerie J. Harwood, and Joan B. Rose. (in preparation) The Influence of Climate on the Incidence of Microbial Fecal Indicators and the Source of Fecal Contamination with the Lower St. Johns River Basin, Florida.

PRINCIPLE INVESTIGATOR SIGNATURE

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Jan H. Landsberg, Ph.D. Date

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Appendix D

Final Report
Cynthia Cooksey
NOAA
Benthic community and sediment quality

Benthic Infauna Monitoring and Autonomous Platform Data Management

Introduction

NOAA's National Ocean Service/National Centers for Coastal Ocean Science/Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) in Charleston, South Carolina has been actively involved in this project since its beginning, with key responsibilities in data management and implementation of a benthic monitoring component. The soft-bottom benthos is a key component of coastal ecosystems, playing vital roles in detrital decomposition, nutrient cycling, and energy flow to higher trophic levels. Moreover, because of their relatively stationary existence within the sediments, benthic infauna can serve as reliable indicators of potential environmental disturbances. The benthic monitoring component also includes the analysis of chemical contaminants and other stressors (e.g., ammonia, sulfide) in sediments. These analyses are important in providing the program with a basis for interpreting potential biological impacts in the Lower St. Johns River (LSJR) in relation to multiple stressor inputs.

A primary objective of the Monitoring and Event Response for Harmful Algal Blooms (MERHAB) Florida Monitoring Program was to evaluate potential relationships between water- and sediment-quality variables, occurrences of harmful algal blooms, and the incidence of fish diseases and other biological impacts in the LSJR estuary. The benthic component of this program was designed to address the following additional supporting objectives: (1) to evaluate potential effects of HABs and associated environmental changes (e.g., nutrient enrichment, organic loading, oxygen depletion) on the composition and structure of benthic assemblages; (2) to provide information on effects of catastrophic HAB events on economically valuable species of benthic molluscs and crustaceans; (3) to provide an opportunity for monitoring the incidence of HAB cysts in sediments; (4) to provide a basis for examining potential relationships between HAB events and processes occurring in bottom substrates; and (5) to provide a basis for comparing the relative contributions of HABs and other symptoms of eutrophication versus chemical contamination of sediments as potential sources of impacts in the benthos. The latter objective is especially important to address given the proximity to a major metropolitan area with potential pollutant inputs from a variety of sources including shipping, marinas, commercial shipbuilding and repair, military bases, pulp and paper manufacturing, petroleum storage facilities, power generation, commercial and recreational fishing, urban and high-density residential development, and water-based recreation. The river also supports a wide variety of agricultural activities throughout its watershed and includes several Superfund sites.

This report provides a brief summary of activities and accomplishments of the CCEHBR team's component of the MERHAB Florida Monitoring Program.

Methods

Field sampling was conducted over a two-year period (July 2000 to July 2002) and included: (1) seasonal sampling (July, November/December, March) of biotic and environmental variables in both sediments and the water column at seven fixed stations (Fig. 1), and (2) continuous (time-series) monitoring of a suite of physical, chemical, and biological variables at multiple water depths from an instrumented platform at one of the fixed stations. The instrumented platform

was connected to a HDR GOES (High Data Rate Geostationary Operational Environmental Satellites) satellite transmitter that provides real-time, remotely accessed data. The CCEHBR team is responsible for maintaining these data as part of their data management role and developing an associated web site.

As mentioned above, this project also contained a benthic monitoring component. Benthic samples were collected three times a year over a two-year period at each of the seven fixed monitoring stations. Sampling occasions were as follows: July 2000, December 2000, March 2001, July 2001, November 2001, March 2002 and July 2002. Samples were collected for the analysis of benthic community structure and composition (macroinfauna > 0.5 mm), porewater un-ionized ammonia and sulfide, and basic habitat parameters (depth, sediment grain size and TOC, dissolved oxygen, salinity, temperature, and pH). Sediment samples earmarked for contaminant analysis (metals, pesticides, PCBs, and PAHs) were collected at each station and sampling occasion; however, only two sample collections have been processed, July 2000 and July 2002. The remaining samples will be archived for possible subsequent analysis pending results of other monitoring activities.

Sediment samples for macroinfaunal analysis were collected at each station in triplicate using a 0.04 m^2 Young grab sampler. Each replicate was sieved in the field through a 0.5-mm mesh screen and preserved in 10% buffered formalin with rose bengal. All infaunal samples were transferred to 70% ethanol once in the laboratory. Animals were sorted from sample debris under a dissecting microscope and identified to the lowest practicaltaxonomic level (usually to species).

The upper 2 – 3 centimeters of sediment from additional multiple grabs were taken at each station, combined into a single station composite, and then subsampled for analysis of metals, organic contaminants (PCBs, pesticides, PAHs), total organic carbon (TOC), and grain size. TOC and grain size were analyzed using protocols modified from Plumb (1981). TOC content of sediment was measured on a CHN elemental analyzer (at 950˚ C combustion temperature). Methods for analysis of chemical contaminants followed those of Sanders (1995), Fortner et al. (1996), Kucklick et al. (1997), and Clum et al. (2002). Metal analyses were performed using inductively coupled plasma mass spectrometry (ICP/MS) for the following suite of metals: Al, Cr, Cu, Fe, Mn, Ni, Sn, As, Cd, Pb and Zn. Ag and Se were analyzed using graphite furnace atomic absorption (GFAA). Cold vapor atomic absorption (CVAA) was used for analysis of Hg. The organic PCBs and pesticides were analyzed by dual-column gas chromatography with electron capture detection (GC-ECD). An ion-trap mass spectrometer equipped with a gas chromatograph (GC/MS-IT) was used for analysis of PAHs.

Sediment quality guidelines (SQG) for each corresponding chemical were used (where available) to help in interpreting the biological significance of the observed contaminant levels (Appendix B). Two types of SQGs were used: (1) Effects Range-Low (ERL) and Effects Range-Median (ERM) values of Long et al. (1995, updated from Long and Morgan 1990); and (2) Threshold Effects Level (TEL) and Probable Effects Level (PEL) values of MacDonald et al. (1996). ERL and TEL values are both lower-threshold bioeffect limits, below which adverse effects of the contaminants on sediment-dwelling organisms are not expected to occur. In contrast, ERM and PEL values both represent mid-range concentrations of chemicals above which adverse effects

are more likely to occur. Concentration-to-SQG comparisons were based on the ERL and ERM values for most chemicals; in some cases, however (e.g., where updated ERL and ERM values were not available), the alternative TEL and PEL values were used.
The total amount of sediment contamination within a sample is expressed as the mean of the ratios of individual contaminant concentrations to their corresponding ERM values. Therefore, of all the chemical contaminants that were measured, only those for which SQGs have been developed were included in this computation (Table 1). Concentrations of chemical contaminants below analytical detection limits were treated here as zeros and included in the computations. Methods used to compute mean ERM quotients are the same as those used by Long et al. (1998; 2000) and Hyland et al. (2003). Mean ERM quotients > 0.058 have been associated with a high incidence of stress in benthic communities in southeastern estuaries (Hyland et al., 1999).

Spatial patterns in the distribution of benthic infauna among the seven locations were examined using normal (Q mode) cluster analysis (Boesch 1977). Group-average sorting (= unweighted pair-group method; Sneath and Sokal 1973) was used as the clustering method and Bray-Curtis similarity (Bray and Curtis 1957) was used as the resemblance measure. The analysis was run on log(x+1) transformed abundances using the PRIMER software package (Clarke and Gorley 2001). Results were expressed as a dendrogram in which samples were ordered into groups of increasingly greater similarity based on resemblances of component-species abundances.

Canonical discriminant analysis was used to determine whether the separation of the cluster groups could be explained by other measured abiotic environmental factors (*sensu* Green and Vascotto 1978, Hyland et al. 1991). Only abiotic variables that displayed significant mean differences across the cluster groups (at $p < 0.05$) were included in the canonical discriminant analysis. The analysis sought to derive a reduced set of discriminant (canonical) functions that best described the separation of the pre-declared station groups based on data represented by the different abiotic environmental variables. Total structure coefficients (TSC), which are the correlations between the original variables and the discriminant scores on each function, provided a measure of the relative contribution of each variable to group separation.

Results and Discussion

Chemical Contamination and Benthic Infauna Communities

Key habitat characteristics of the seven sampling sites (Figs. 2 and 3; Appendix A) can be summarized as follows: (1) relatively shallow water depths of 1.4 m to 9.2 m; (2) a wide range in salinity (oligohaline to euhaline salinities); (3) dissolved oxygen levels averaging 6.7 mg/L, which is above a reported benthic hypoxic effect threshold of 1.4 mg/L (Diaz and Rosenberg 1995); (4) total organic carbon in sediments ranging from low (Mill Cove – 0.1 mg/g) to very high (Cedar/Ortega River – 91.0 mg/g); and (5) sediment texture ranging from fine (Cedar/Ortega River – 81.1% Silt-Clay) to coarse (Mill Cove – 0.0% Silt-Clay). Variation is evident in the measured water quality parameters including temperature, salinity, pH, and dissolved oxygen over the course of the study (Fig. 2). Porewater hydrogen sulfide and un-ionized ammonia were measured at each station and showed a high degree of variation both by station and sampling cruise (Fig. 4). Cedar/Ortega had the highest measured value for hydrogen

sulfide during this study at 1.8 mg/L while Tallyrand had the highest measured value for un-ionized ammonia at 0.33 mg/L.

Appendix B lists means and ranges in concentrations of various chemical contaminants measured in this study (i.e., pesticides, PAHs, PCBs, and metals) and, where available, corresponding sediment quality guidelines (SQG) for interpreting the biological significance of the observed contaminant levels. Sediments exhibited varying degrees of chemical contamination. Only three of the seven sites, including the reference station Clapboard Creek, had sediments with all measured contaminants below corresponding, lower-threshold ERL/TEL guidelines. Four stations, Cedar/Ortega, Tallyrand, Trout River and Dames Point, exhibited "high" levels of contamination — defined here as one or more contaminants present at concentrations above upper-threshold ERM/PEL guideline values, or multiple (three or more) contaminants present at moderate concentrations between these lower and upper bioeffect thresholds (Table 2). Of particular note are the levels of sediment contamination at the Cedar/Ortega River and Tallyrand stations with 8 and 13 lower-threshold ERL/TEL guideline exceedences, respectively in 2000, and 6 and 13 lower-threshold ERL/TEL guideline exceedences, respectively in 2002. This amount of contamination is exceptionally high in comparison to conditions throughout southeastern estuaries. Only 1.2% of stations sampled randomly in 1994 and 1995 as part of the Environmental Monitoring and Assessment Program (EMAP) had 13 or more ERL/TEL exceedences and only 4.8% of EMAP stations had 8 or more ERL/TEL exceedences (Hyland et al 1996, Hyland et al. 1998). At the Tallyrand station, two of the 13 chemicals in excess of ERL/TEL guidelines, Acenaphthene and Phenanthrene, also exceeded the higher ERM/PEL guidelines in 2000. At levels above ERM/PEL values adverse bioeffects on sediment-dwelling organisms are likely to occur. No ERM/PEL values were exceeded in July 2002.

Three stations in July 2000 (Cedar/Ortega, Tallyrand, and Trout River) and two stations in July 2002 (Cedar/Ortega and Tallyrand) had mean ERM quotients > 0.058 indicating the potential for stress to the benthic communities from chemical contamination (Table 3). Using mean ERM quotients, Wilcoxon's signed rank test for paired observations was used to evaluate any overall change in levels of contamination between July 2000 and July 2002 for the seven stations sampled in this study. There was no significant difference in mean ERM quotients between the two sampling periods ($S = 7$, $df = 6$, $p = 0.2969$).

Appendix C provides a list of the benthic infaunal species collected throughout the course of this study at the MERHAB Florida sampling stations. Polychaete worms and molluscs dominated these benthic fauna. The polychaete, *Streblospio benedicti*, the most abundant species collected in all samples combined, is a species indicative of polluted environments (Pearson and Rosenberg 1978, Hyland et al. 1985). Dominant species, however, varied among sampling stations. Doctors Lake, Cedar/Ortega, Tallyrand, Trout River and Dames Point were dominated by species indicative of polluted environments (*Streblospio benedicti*, *Macoma* spp., and *Neanthes succinea*) while the remaining two stations, inclusive of the reference station Clapboard Creek, were not (Table 4). Recall that four of these stations that were dominated by pollution indicators (Cedar/Ortega, Tallyrand, Trout River, and Dames Point) were also highly contaminated by chemicals (Table 2).

The macroinfaunal assemblages in the St. Johns River displayed varying measures of species richness, diversity and density both between stations and over the course of this study within a station (Fig. 5). Dames Point had the highest species richness and diversity while Cedar/Ortega and Mill Cove consistently had the lowest values for richness, diversity and density. Mill Cove also was the only station where no biota were collected on at least one sampling occasion, November 2001. The low values of the various benthic community measures suggest that Mill Cove (in addition to Doctors Lake, Cedar/Ortega, Tallyrand, and Trout River) also exhibits evidence of impaired benthic condition. The degraded benthos at Mill Cove may be explained by its close proximity to dredge spoil sites (Fig. 6) as chemical contaminants at Mill Cove were at relatively low levels below ERL/TEL SQGs (Table 2; Appendix B).

Using a Bray-Curtis similarity value of 0.11 as a separation rule yielded three major site groups, denoted as A, B and C (Fig. 7). Group A is comprised of Doctors Lake and Cedar/Ortega stations. Groups B is comprised of Tallyrand, Trout River, Dames Point and Clapboard Creek stations. Group C is the smallest cluster group and only consists of Mill Cove. All sampling events for a station are contained within that station's cluster group. Results of the canonical discriminant analysis showed that the first canonical function was significant (CAN 1: $p < 0.0001$, $df = 12$) and accounted for 95% of the among-group variation in abiotic variables. The second canonical function was significant at an alpha level of 0.1 (CAN 2: $p = 0.0604$, $df = 5$) and accounted for 5% of the among-group variation in abiotic variables. A plot of the discriminant scores on each of these two functions showed a clear separation of site groups (Fig. 8). TSCs (Table 5) reveal that the first canonical function (CAN 1) is most highly correlated with latitude, thus explaining the separation of the two stations furthest upriver, Doctors Lake and Cedar/Ortega from the remainder of stations. Group A also had the lowest average salinities, but salinity was highly variable over the course of the study which may explain why it was not more highly correlated. The canonical plot further reveals that the second canonical function explains most of the variation between Group B vs. Group C. TSCs for CAN 2 (Table 5) confirm that the strongest correlation on this function is with percent silt/clay. Group C, Mill Cove, was closely associated with two dredge spoil islands and consisted of very coarse sediments.

Data Management

Data management activities have focused on development of the framework for capturing and disseminating continuous monitoring data from the instrumented platform located at the Trout River station. These data are being transferred via the GOES satellite system every three hours and are currently being retrieved by the Coastal Ecology Program (http://www.chbr.noaa.gov/cep/default.aspx) of the CCEHBR in Charleston, using an automated process (the MERHAB Data Management Suite written in Perl and hosted on a Linux server). The near real-time data on a suite of chemical, physical and biological variables are then being processed and stored in a database for subsequent use by research partners and all other interested users.

A MERHAB Florida website (http://www.merhabfl.org) also has been developed to facilitate the dissemination of this information to as wide a user audience as possible. The website provides direct access to various types of information including the near-real time data on water-quality

variables measured by the platform (raw data are available for download as well as graphs for display); data archives associated with the additional seasonal intensive monitoring at the various fixed stations; and general information on scientific approaches, significant developments and products. In addition, this website incorporate features to make it fully W3C standard compliant and Section 508 compliant.

Conclusions

- Chemical contamination of sediment at levels likely of having adverse effects on benthic fauna was observed at four stations near the center of Jacksonville (Cedar/Ortega, Tallyrand, Trout River, and Dames Point). Concentrations of man-made pesticides or other chemical substances typically associated with human activities (e.g., PCBs) were detectable at all stations, though not always present at concentrations likely of causing significant bioeffects. The widespread distribution of these contaminants is most likely attributable to the proximity to a major metropolitan area with potential pollutant inputs from shipping, marinas, commercial shipbuilding and repair, military bases, pulp and paper manufacturing, petroleum storage facilities, power generation, fishing, urban and high-density residential development, and recreation. Other sources include a wide variety of agricultural activities throughout the LSJR watershed and several Superfund sites.
- Variable and often high levels of porewater un-ionized ammonia and hydrogen sulfide were detected at the MERHAB Florida sampling stations and indicate the potential for problems associated with eutrophication. Doctors Lake, Cedar/Ortega, and Tallyrand were stations that exhibited the highest levels of porewater un-ionized ammonia and hydrogen sulfide.
- Benthic macroinfaunal communities at five of the seven sites sampled in the LSJR show indication of stress that could be associated with anthropogenic activities. Five sites (Doctors Lake, Cedar/Ortega, Tallyrand, Trout River, and Dames Point) are dominated by species indicative of polluted environments. One additional site (Mill Cove) shows degraded condition based on values of several benthic community measures.
- No catastrophic HAB events occurred in the LSJR during the course of this study so it was not possible to evaluate the impacts of HABs on benthic communities. However, in a heavily developed system such as the St. Johns River any evaluation of the impacts of Harmful Algal Blooms on benthic communities would have to take into account the relative contributions of impacts resulting from multiple stressors.
- Data were remotely retrieved from the autonomous instrumented platform via the GOES satellite system over the course of this study. A MERHAB Florida website for disseminating these and other related monitoring data was developed and can be found at http://www.merhabfl.org. Additionally, all benthic infauna data are available via the National Benthic Inventory (http://www.nbi.noaa.gov).

Acknowledgements

This work is being sponsored by the NOAA Center for Sponsored Coastal Ocean Research/ Costal Ocean Program. Special recognition is extended to the MERHAB Florida Coordinating P.I., Dr. Karen Steidinger and Co-P.I. Mr. Brain Bendis (Florida Marine Research Institute) for program coordination and sampling support; to Barry Vittor & Associates (Mobile, AL) for analysis of macroinfaunal samples, TOC, and particle-size; to Allan Clum, Aaron Dias, Scott

Sivertsen, and Brian Shaddrix (NOAA/NOS/CCEHBR) for analysis of contaminants in sediments; and to A.K. Leight (NOAA/NOS/CCEHBR) for boat operation and sample collection support.

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Table 1. Chemicals and corresponding sediment quality guideline (SQG) values used in the calculation of mean ERM quotients.

Chemical	ERM
Metals (*ug/g dry wt.*)	
Arsenic	70
Cadmium	9.6
Chromium	370
Copper	270
Lead	218
Mercury	0.71
Silver	3.7
Zinc	410
Pesticides *(ng/g dry wt.)*	
4,4'-DDE	27
Total DDT[f]	46.1
PAHs *(ng/g dry wt.)*	
Acenaphthene	500
Acenaphthylene	640
Anthracene	1100
Benzo(a)anthracene	1600
Benzo(a)pyrene	1600
Dibenz(a,h+a,c)anthracene	260
Fluoranthene	5100
Fluorene	540
2-Methylnaphthalene	670
Naphthalene	2100
Phenanthrene	1500
Pyrene	2600
Total PAHs[b]	44792
PCBs *(ng/g dry wt.)*	
Total PCBs	180

Table 2. St. Johns River stations with sediment contaminant concentrations exceeding ERL/TEL or ERM/PEL sediment quality guidelines in July 2000 or July 2002. -- = did not exceed ERL/TEL for that year.

Station	Analyte	Concentration 2000	Concentration 2002	ERL/TEL[a]	ERM/PEL[b]
Cedar/ Ortega	Acenaphthene (ng/g)	26.7	--	16.00	500.00
	Copper (μg/g)	109.0	45.8	34.00	270.00
	4′,4'-DDE (ng/g)	3.5	--	2.20	27.00
	Fluorene (ng/g)	39.0	22.7	19.00	540.00
	Mercury (μg/g)	0.50	0.58	0.15	0.71
	Lead (μg/g)	99.2	67.4	46.70	218.00
	Zinc (μg/g)	--	168		
	Total DDTs[c] (ng/g)	5.0	--	1.58	46.10
	Total PCBs (ng/g)	125.4	49.9	22.70	180.00
Tallyrand	Acenaphthene (ng/g)	503	194	16.00	500.00
	Acenaphthylene (ng/g)	46.6	45.9	44.00	640.00
	Anthracene (ng/g)	542	304	85.30	1100.00
	Benzo(a)anthracene (ng/g)	723	807	261.00	1600.00
	Benzo(a)pyrene (ng/g)	435	517	430.00	1600.00
	Fluoranthene (ng/g)	2740	2430	600.00	5100.00
	Fluorene (ng/g)	444	228	19.00	540.00
	2-Methylnaphthalene (ng/g)	281	390	70.00	670.00
	Naphthalene (ng/g)	676	824	160.00	2100.00
	Phenanthrene (ng/g)	1920	945	240.00	1500.00
	Pyrene (ng/g)	1550	1950	665.00	2600.00
	Total PAHs[d] (ng/g)	12791.0	11431.8	4022.00	44792.00
	Total PCBs (ng/g)	37.8	26.8	22.70	180.00
Trout River	Mercury (μg/g)	0.3	--	0.15	0.71
	Total DDTs[c] (ng/g)	1.8	--	1.58	46.10
	Total PCBs (ng/g)	66.8	22.8	22.70	180.00
Dames Point	Acenaphthene (ng/g)	--	26.2	16.00	500.00
	Fluorene (ng/g)	--	62.1	19.00	540.00
	2-Methylnaphthalene (ng/g)	--	186	70.00	670.00
	Naphthalene (ng/g)	--	316	160.00	2100.00

[a] SQG value is the ERL value from Long et al. (1995), unless noted otherwise; [b] SQG value is the ERM value from Long et al. (1995), unless noted otherwise; [c]Total DDTs = 2′4′-DDD + 4′4′-DDD + 2′4′-DDE + 4′4′-DDE + 2′4′-DDT + 4′4′-DDT; [d]Without Perylene.

Table 3. Mean ERM quotients for the St. Johns River stations, July 2000 and July 2002. * = mean ERM quotient > 0.058.

Station	Mean ERM Quotient	
	2000	2002
Doctors Lake	0.0089	0.0095
Cedar/Ortega	0.1807*	0.1319*
Tallyrand	0.3206*	0.2453*
Trout River	0.0981*	0.0504
Dames Point	0.0065	0.0486
Mill Cove	0.0034	0.0012
Clapboard Creek	0.0014	0.0024

Table 4. Top five numerically dominant taxa collected at each station, July 2000 - July 2002. A=Anthozoa, B=Bivalve, G=Gastropod, I=Insecta, M=Malacostraca, O=Oligocheate, P=Polychaete, Ph= Phoronida, and T=Tunicate.

Station	Taxa Name	Abundance (number/grab)	Cumulative % Abundance
Doctors Lake	*Mytilopsis leucophaeata* (B)	566	30.1
	Streblospio benedicti (P)	403	51.5
	Coelotanypus sp. (I)	80	55.7
	Littoridinops sp. (G)	75	59.7
	Ischadium recurvum (B)	70	63.4
Cedar/Ortega	*Macoma mitchelli* (B)	113	38.7
	Streblospio benedicti (P)	50	55.8
	Rangia cuneata (B)	22	63.4
	Chironomus sp. (I)	18	69.5
	Tellinidae (B)	17	75.3
Tallyrand	*Mediomastus* sp. (P)	565	26.4
	Streblospio benedicti (P)	499	49.8
	Mulinia lateralis (B)	445	70.6
	Tubificoides heterochaetus (O)	128	76.6
	Tubificidae (O)	61	79.4
Trout River	*Mediomastus* sp. (P)	118	14.8
	Neanthes succinea (P)	114	29.0
	Nereis sp. (P)	100	41.5
	Boonea impressa (G)	89	52.6
	Streblospio benedicti (P)	55	59.5
Dames Point	*Streblospio benedicti* (P)	382	17.2
	Sabellaria vulgaris (P)	283	29.9
	Paracaprella pusilla (M)	144	36.4
	Actiniaria (A)	130	42.2
	Apocorophium lacustre (M)	100	46.7
Mill Cove	*Paraonis fulgens* (P)	50	28.2
	Tellinidae (B)	16	37.3
	Bivalvia (B)	8	41.8
	Nephtys picta (P)	8	46.3
	Protohaustorius wigleyi (M)	8	50.8
Clapboard Creek	*Mediomastus* sp. (P)	366	16.1
	Gemma gemma (B)	295	29.1
	Phoronis sp. (Ph)	238	39.6
	Nassarius obsoletus (G)	131	45.4
	Ascidiacea (T)	102	49.9

Table 5. Total structure coefficients (TSC) of abiotic environmental variables on the first two canonical functions associated with variations among site groups. Coefficients considered important in each function are underlined.

Abiotic Variable	TSC	
	Can1	Can2
Salinity	0.72	-0.27
Percent Silt/Clay	-0.18	<u>0.90</u>
Percent TOC	-0.35	0.41
Un-ionized Ammonia	-0.39	0.23
Latitude	<u>0.97</u>	0.13
Longitude	-0.86	0.44

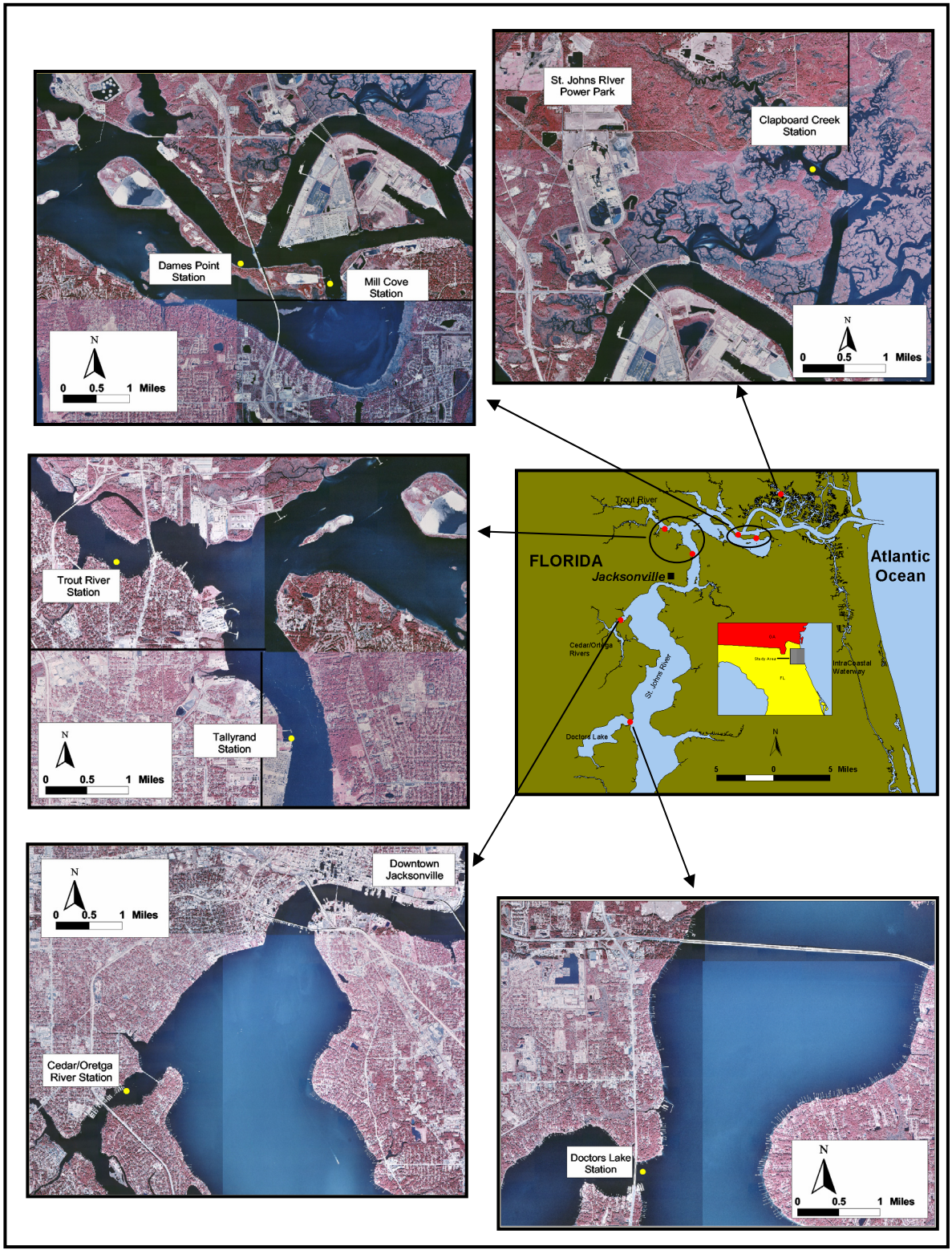

Figure 1. Station locations for MERHAB Florida monitoring in the lower St. Johns River, Florida.

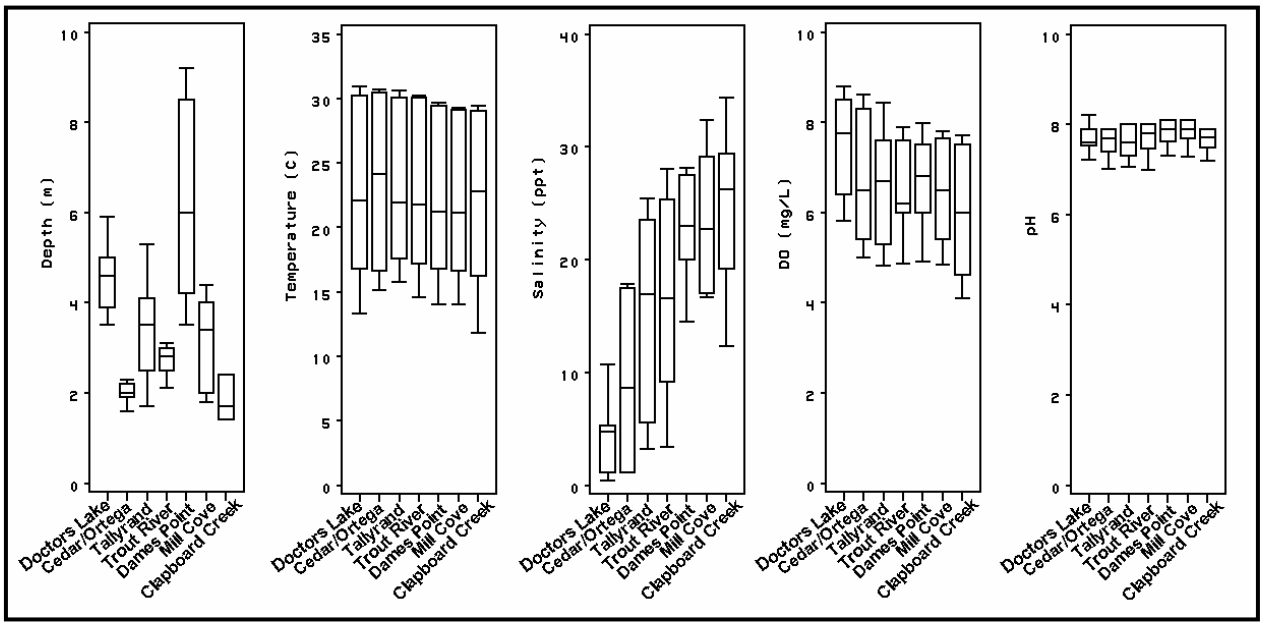

Figure 2. Key habitat characteristics for the seven LSJR stations based on bottom water measurements, July 2000 - July 2002 (n = 7 sampling events). Boxes are interquartile ranges, horizontal lines within boxes are medians and wisker endpoints are high/low extremes.

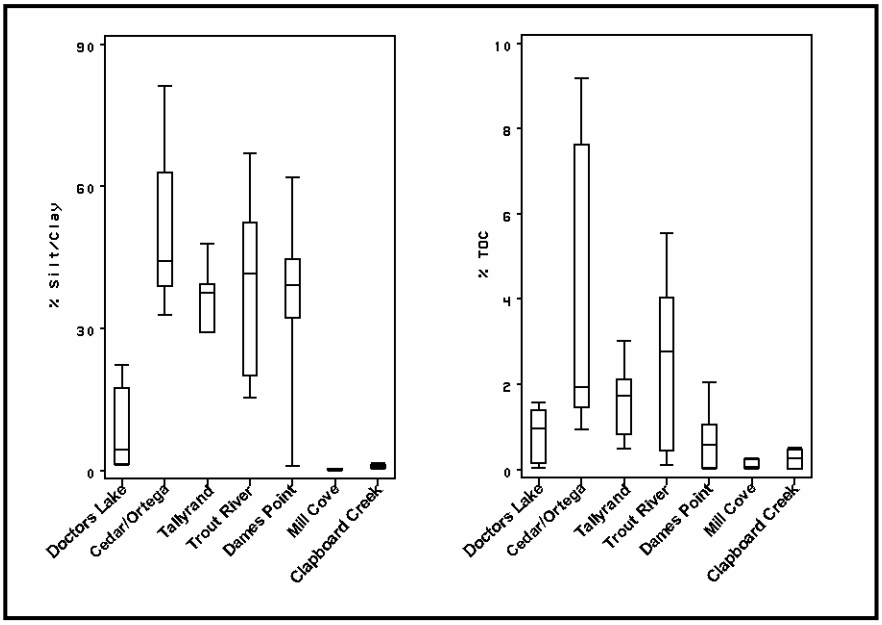

Figure 3. Sediment characteristics for the seven LSJR stations, July 2000 - July 2002 (n = 7 sampling events). Boxes are interquartile ranges, horizontal lines within boxes are medians and wisker endpoints are high/low extremes.

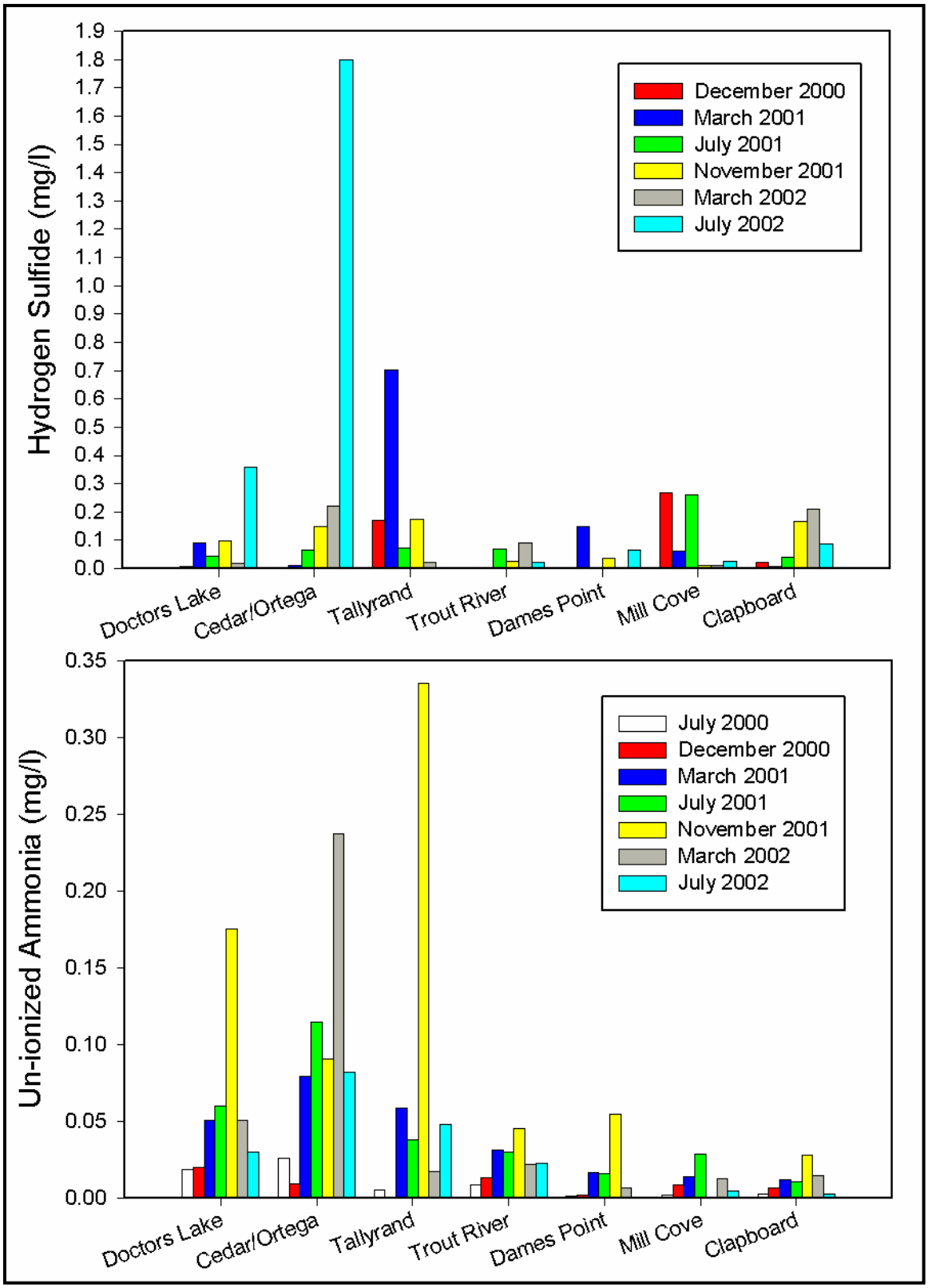

Figure 4. Porewater un-ionized ammonia and sulfide concentrations for the seven LSJR stations, July 2000 - July 2002. No sulfide measurements are reported for July 2000 because readings were not corrected for turbidity at that time.

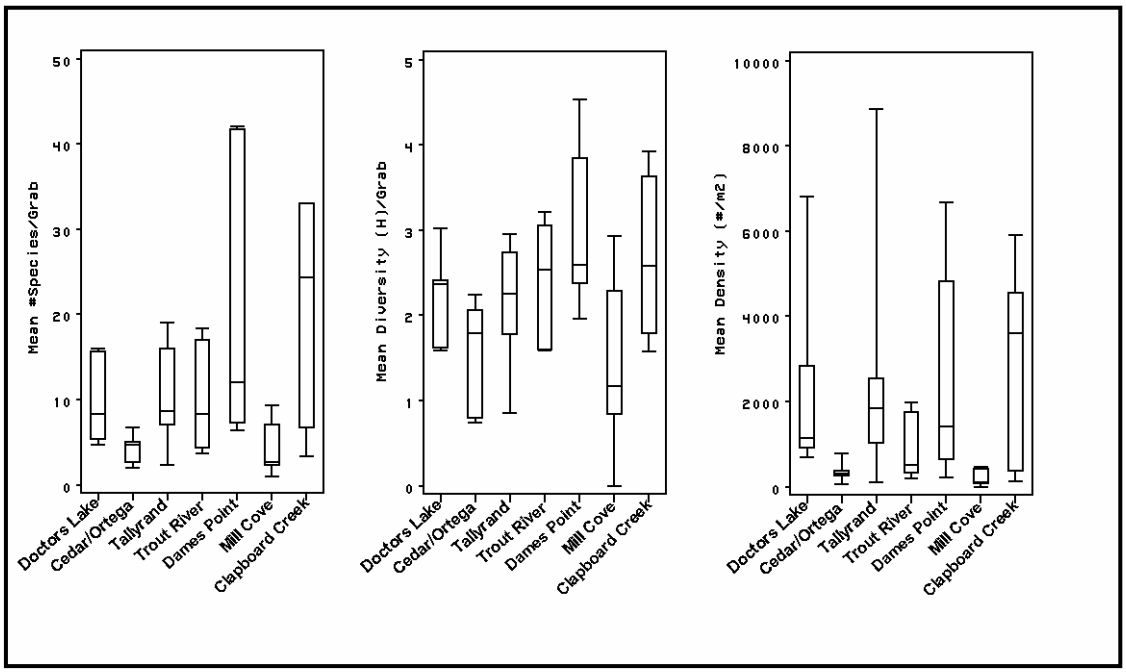

Figure 5. Benthic species richness, diversity and density for the seven LSJR stations, July 2000 - July 2002 (n = 7 sampling events). Boxes are interquartile ranges, horizontal lines within boxes are medians and wisker endpoints are high/low extremes. Base 2 logarithms were used to calculate H´.

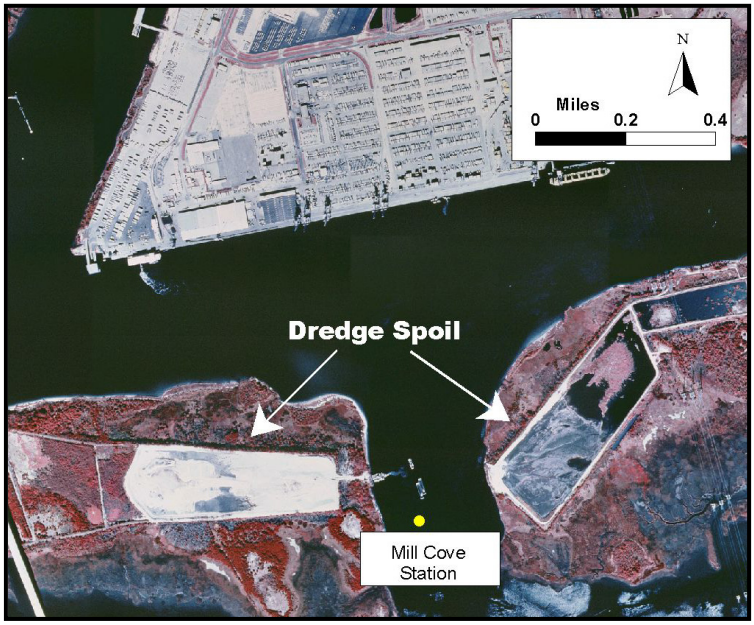

Figure 6. Mill Cove Station, Lower St. Johns River, Florida.

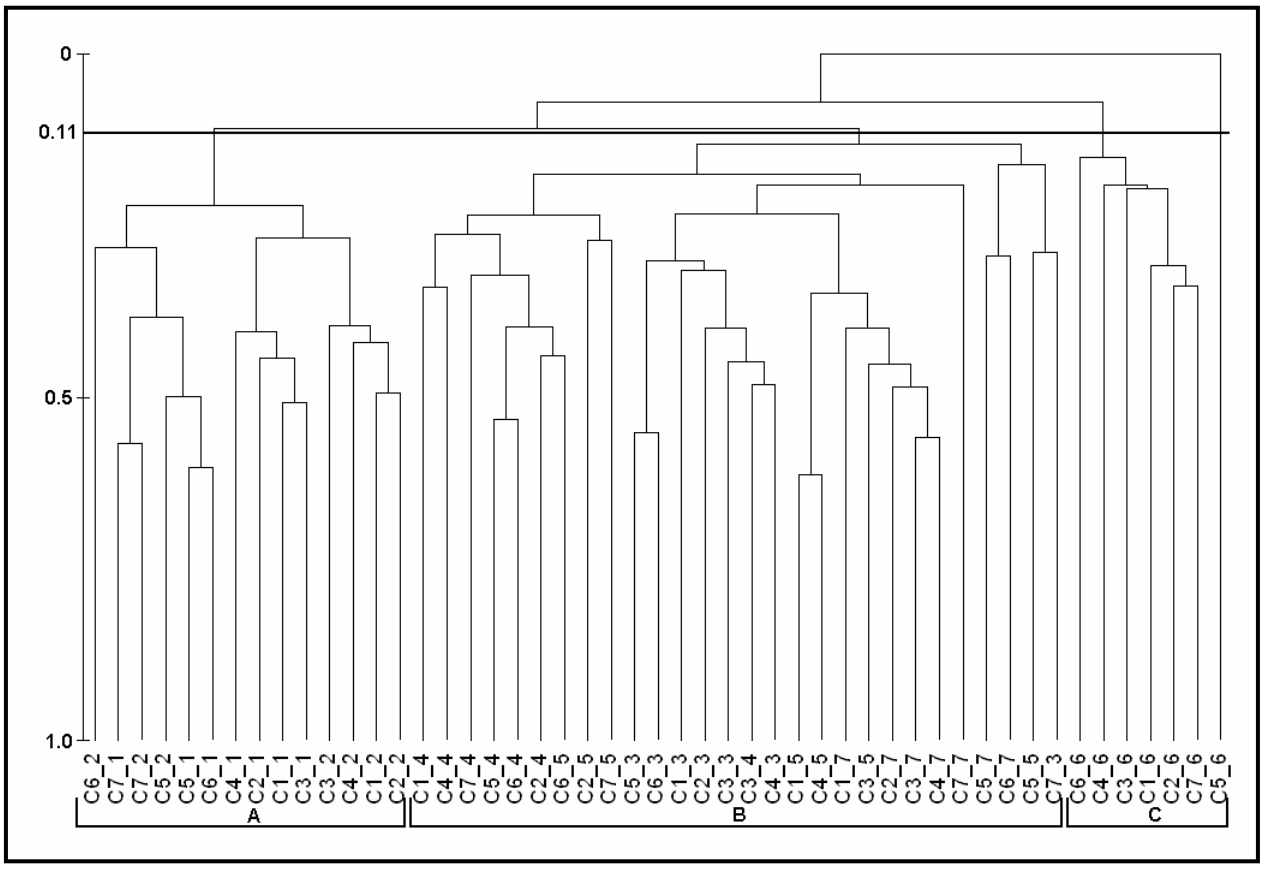

Figure 7. Dendrogram resulting from clustering of benthic samples using group-average sorting and Bray-Curtis similarity. A similarity level of 0.11 was used to define the three major site groups, A, B and C. C1=July 2000, C2=December 2000, C3=March 2001, C4=July 2001, C5=November 2001, C6=March 2002, C7=July 2002. 1=Doctors Lake, 2=Cedar/Ortega, 3=Tallyrand, 4=Trout River, 5=Dames Point, 6=Mill Cove, 7=Clapboard Creek.

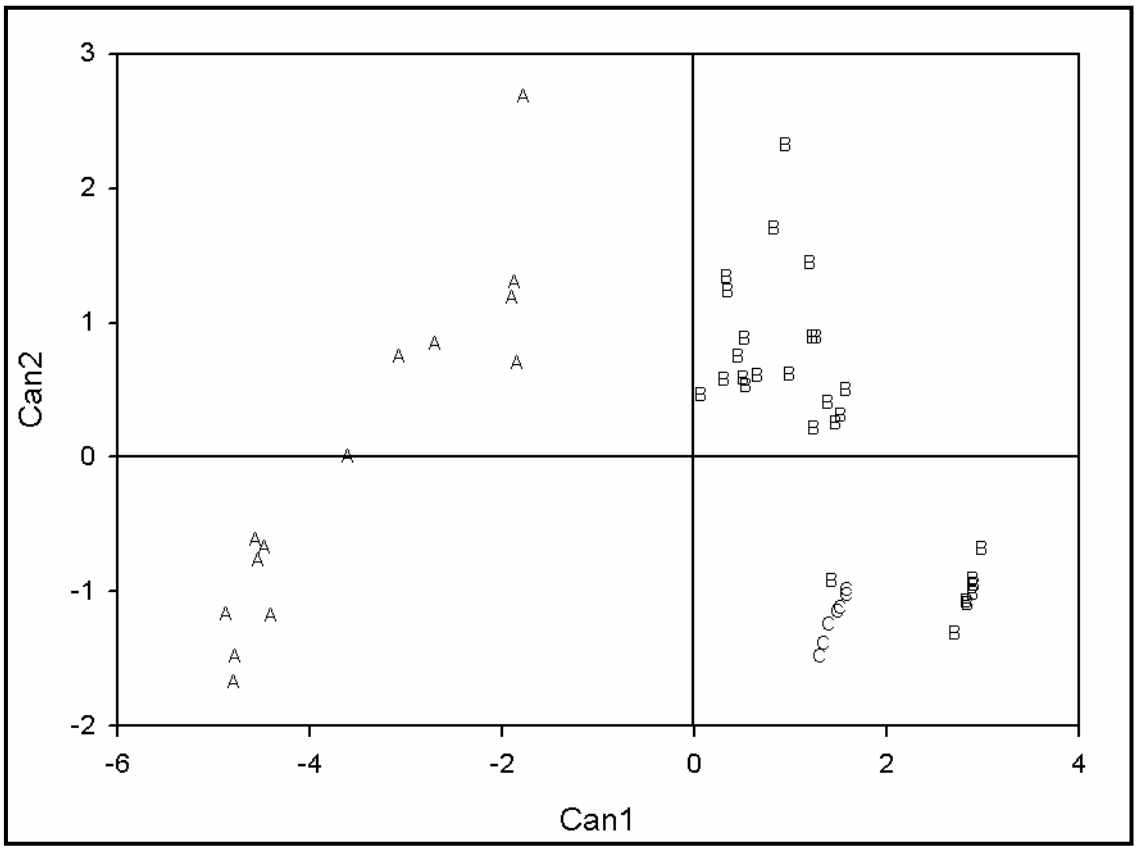

Figure 8. Separation of site groups on the first and second canonical function derived from canonical discriminant analysis performed on abiotic environmental variables. Can 1 = first canonical function (95% of variability). Can 2 = second canonical function (5% of variability).

Appendix A. Summary of station location and water quality data for stations sampled in the St. Johns River in July 2000 - July 2002.

Station	Latitude	Long.	Bottom Water				
			Depth (m)	Temperature (°C)	Salinity (ppt)	D.O. (mg/L)	pH
			Average (min – max)	Average (min – max)	Average (min – max)	Average (min – max)	Average (min – max)
Doctors Lake	30.1495°	81.6992°	4.5 (3.5 – 5.9)	23.4 (13.3 – 30.9)	4.1 (0.4 – 10.7)	7.4 (5.8 – 8.8)	7.7 (7.2 – 8.2)
Cedar/ Ortega	30.2768°	81.7113°	2.0 (1.6 – 2.3)	24.0 (15.1 – 30.7)	8.5 (1.1 – 17.8)	6.8 (5.0 – 8.6)	7.6 (7.0 – 7.9)
Tallyrand	30.3598°	81.6195°	3.5 (1.7 – 5.3)	23.8 (15.8 – 30.6)	15.1 (3.2 – 25.4)	6.6 (4.8 – 8.4)	7.6 (7.1 – 8.0)
Trout River	30.3913°	81.6550°	2.7 (2.1 – 3.1)	23.6 (14.6 – 30.2)	16.2 (3.4 – 28.0)	6.6 (4.9 – 7.9)	7.7 (7.0 – 8.0)
Dames Point	30.3831°	81.5613°	6.4 (3.5 – 9.2)	22.9 (14.0 – 29.7)	22.7 (14.5 – 28.1)	6.7 (4.9 – 7.8)	7.8 (7.3 – 8.1)
Mill Cove	30.3792°	81.5388°	3.2 (1.8 – 4.4)	22.8 (14.0 – 29.3)	23.3 (16.6 – 32.4)	6.6 (4.8 – 7.8)	7.8 (7.3 – 8.1)
Clapboard Creek	30.4342°	81.5075°	1.8 (1.4 – 2.4)	22.7 (11.8 – 29.4)	24.2 (12.3 – 34.4)	6.0 (4.1 – 7.7)	7.6 (7.2 – 7.9)

Appendix B. Summary of contaminant concentrations at St. Johns River sites in July 2000 and July 2002. Concentrations of analytes below method detection limits are reported as < MDL.

Analyte	Doctors Lake		Cedar/Ortega		Tallyrand		Trout River		Dames Point		Mill Cove		Clapboard Cr.	
	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002
Metals (µg/g dry wt., unless otherwise indicated)														
Aluminum (%)	0.29	0.419	1.69	1.87	1.44	1.64	2.32	3.29	1.3	2.01	0.02	0.0872	0.11	0.258
Arsenic	0.35	0.661	5	6.08	4.99	3.2	6.96	5.26	1.44	6.6	<MDL	0.187	0.18	0.501
Cadmium	0.02	<MDL	0.74	0.759	0.18	0.0998	0.33	0.358	0.06	0.149	<MDL	<MDL	<MDL	<MDL
Chromium	<MDL	6.04	37.8	54.5	30.9	25.6	47.5	39.7	7.6	42.4	<MDL	1.83	<MDL	3.3
Copper	1.38	2.15	109	45.8	16.2	9.33	27.2	19.8	0.53	5.04	0.21	0.207	<MDL	0.642
Iron (%)	0.29	0.273	2.72	2.93	1.98	1.08	3.11	2.33	0.78	2.43	0.04	0.0426	0.12	0.118
Lead	5.25	6.53	99.2	67.4	44.5	25	43.6	29.3	6.61	12.1	0.54	0.737	0.88	1.98
Manganese	32	26.5	157	160	180	101	208	148	30.1	211	8.42	5.26	4.89	7.28
Mercury	0.02	0.0271	0.5	0.576	0.07	0.0552	0.29	0.0507	0.01	0.0169	<MDL	0.0013	<MDL	0.0026
Nickel	2.21	1.3	14	13.9	11.8	6.91	17.2	11.2	3.36	10.7	0.4	0.0691	0.74	0.672
Selenium	0.09	0.0521	0.65	0.412	0.32	0.149	0.07	0.123	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Silver	<MDL	0.0762	0.47	0.337	0.18	0.17	0.26	0.239	<MDL	0.273	<MDL	0.0424	<MDL	0.0341
Tin	<MDL	<MDL	4.93	7.04	1.88	0.738	4.35	3.3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Zinc	<MDL	10.7	124	168	42.6	53	126	77.8	<MDL	39.1	19.7	<MDL	<MDL	3.05
PAHs (ng/g dry wt.)														
Acenaphthene	<MDL	<MDL	26.7	<MDL	503	194	<MDL	<MDL	<MDL	26.2	<MDL	<MDL	<MDL	<MDL
Acenaphthylene	<MDL	<MDL	16.5	13	46.6	45.9	12.1	7.59	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Anthracene	<MDL	4.26	41.9	38	542	304	17.1	27.4	<MDL	15.7	<MDL	<MDL	<MDL	<MDL
Benzo(a)anthracene	<MDL	11.2	151	122	723	807	71.6	55.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Benzo(a)pyrene	<MDL	14.9	195	124	435	517	112	69.8	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Benzo(b)fluoranthene	11.8	19.5	255	176	495	624	123	95.4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Benzo(e)pyrene	9.9	14	167	117	296	336	103	66	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Benzo(g,h,i)perylene	11.7	15.4	147	102	201	210	95.1	58.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Benzo(j+k)fluoranthene	12.1	14	193	136	417	479	132	75	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Biphenyl	<MDL	<MDL	25.4	<MDL	95.7	135	<MDL	<MDL	10.6	55.8	<MDL	<MDL	<MDL	<MDL
Chrysene+Triphenylene	10.2	15.4	216	151	712	622	89.1	74.8	3.75	5.45	<MDL	2.35	<MDL	<MDL
Dibenz(a,h+a,c)anthracene	2.04	2.59	23.5	19.6	38.6	34.5	23	7.59	1.94	<MDL	<MDL	<MDL	<MDL	<MDL
Dibenzothiophene	<MDL	<MDL	15.2	7.23	181	64.5	<MDL	<MDL	<MDL	8.77	<MDL	<MDL	<MDL	<MDL
2,6 Dimethylnaphthalene	<MDL	<MDL	11.2	<MDL	102	45	<MDL	<MDL	<MDL	13.5	<MDL	<MDL	<MDL	<MDL
Fluoranthene	20.8	26.5	468	332	2740	2430	151	147	9.16	16.2	<MDL	<MDL	<MDL	<MDL
Fluorene	<MDL	<MDL	39	22.7	444	228	10.2	11.6	<MDL	62.1	<MDL	<MDL	<MDL	<MDL
Indeno(1,2,3-cd)pyrene	12.4	<MDL	162	80.7	246	187	91	51.5	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

Analyte	Doctors Lake		Cedar/Ortega		Tallyrand		Trout River		Dames Point		Mill Cove		Clapboard Cr.	
	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002	2000	2002
1-Methylnaphthalene	<MDL	<MDL	19.9	11.2	138	86.3	15.9	<MDL	<MDL	41.2	<MDL	<MDL	<MDL	<MDL
1-Methylphenanthrene	<MDL	<MDL	24.1	<MDL	136	26.1	11.9	10.3	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
2-Methylnaphthalene	<MDL	<MDL	33.7	21.1	281	390	19.9	12.1	<MDL	186	<MDL	<MDL	<MDL	<MDL
Naphthalene	<MDL	<MDL	131	66	676	824	60	27.3	<MDL	316	<MDL	<MDL	<MDL	<MDL
Perylene	28.2	26.6	173	167	141	149	55.2	66.7	5.21	71.4	<MDL	<MDL	<MDL	<MDL
Phenanthrene	6.76	6.71	157	71.3	1920	945	49.3	45.4	<MDL	82.5	<MDL	<MDL	<MDL	<MDL
Pyrene	20.7	28.9	454	284	1550	1950	187	145	8.73	12.4	<MDL	3.03	<MDL	3.69
1,6,7 Trimethylnaphthalene	<MDL	<MDL	16.2	<MDL	53.1	12	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Total PAHs[a]	118.4	173.36	2974.1	1887.6	12791	11431.8	1374.2	987.48	34.18	833.05	<MDL	5.38	<MDL	3.69
PCBs (ng/g dry wt.)														
Total PCBs	17.1477	4.6975	125.399	49.9232	37.8651	26.7881	66.7731	22.8505	7.2927	5.12898	4.9494	2.4265	4.6647	2.6499
Pesticides (ng/g dry wt.)														
Aldrin	<MDL	<MDL	<MDL	0.144	0.02	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Alpha-chlordane	<MDL	<MDL	0.25	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Chlorpyrifos	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	1.4	<MDL	0.21	<MDL	<MDL	<MDL	<MDL	<MDL
Dieldrin	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Endosulfan ether	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Endosulfan I	<MDL	<MDL	<MDL	<MDL	0.24	<MDL	0.21	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Endosulfan II	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Endosulfan lactone	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Endosulfan Sulfate	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Heptachlor	<MDL	<MDL	0.04	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Heptachlor epoxide	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Hexachlorobenzene	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Lindane[b]	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	0.08	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Mirex	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Trans-nonachlor	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4,4'-DDD	<MDL	<MDL	1.21	0.98	<MDL	0.48	0.43	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
4,4'-DDE	0.29	<MDL	3.5	<MDL	0.81	0.187	1.31	<MDL	0.05	<MDL	<MDL	<MDL	<MDL	<MDL
4,4'-DDT	0.16	<MDL	0.14	0.0313	0.1	<MDL	0.06	<MDL	0.04	<MDL	<MDL	<MDL	<MDL	<MDL
DDD[c]	<MDL	<MDL	1.33	0.98	0.17	0.48	0.43	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
DDE[c]	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
DDT[c]	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
Total DDTs[d]	0.45	<MDL	4.97	1.0113	1.08	0.667	1.8	<MDL	0.09	<MDL	<MDL	<MDL	<MDL	<MDL

[a]Without Perylene; [b]Gamma BHC; [c]DDD = 2´4´-DDD + 4´4´-DDD; DDE = 2´4´-DDE + 4´4´-DDE; DDT = 2´4´-DDT + 4´4´-DDT; [d]Total DDTs = 2´4´-DDD + 4´4´-DDD + 2´4´-DDE + 4´4´-DDE + 2´4´-DDT + 4´4´-DDT.

Appendix C. Summary of benthic infauna collected at all seven St. Johns River stations, July 2000 - July 2002.

Taxa Name	Abundance	% Abundance	% Cumulative Abundance
Streblospio benedicti	1492	15.25	15.25
Mediomastus sp.	1147	11.72	26.98
Mytilopsis leucophaeata	568	5.81	32.78
Mulinia lateralis	461	4.71	37.49
Gemma gemma	295	3.02	40.51
Sabellaria vulgaris	285	2.91	43.42
Phoronis sp.	243	2.48	45.91
Neanthes succinea	214	2.19	48.09
Actiniaria	202	2.06	50.16
Ascidiacea	188	1.92	52.08
Nereis sp.	183	1.87	53.95
Macoma mitchelli	156	1.59	55.55
Paracaprella pusilla	156	1.59	57.14
Tubulanus sp.	153	1.56	58.70
Tubificoides heterochaetus	143	1.46	60.17
Nassarius obsoletus	135	1.38	61.55
Rhynchocoela	127	1.30	62.84
Tubificidae	116	1.19	64.03
Ampelisca sp.	107	1.09	65.12
Apocorophium lacustre	103	1.05	66.18
Batea catharinensis	99	1.01	67.19
Dipolydora socialis	98	1.00	68.19
Tellinidae	95	0.97	69.16
Boonea impressa	94	0.96	70.12
Coelotanypus sp.	92	0.94	71.06
Bivalvia	83	0.85	71.91
Melitidae	76	0.78	72.69
Texadina sphinctostoma	76	0.78	73.46
Littoridinops sp.	75	0.77	74.23
Rangia cuneata	73	0.75	74.98
Ischadium recurvum	72	0.74	75.71
Mactridae	71	0.73	76.44
Hydrobiidae	69	0.71	77.14
Parvilucina multilineata	63	0.64	77.79
Sphenia antillensis	60	0.61	78.40
Porifera	56	0.57	78.97
Corophiidae	55	0.56	79.54
Capitella capitata	54	0.55	80.09
Scoloplos rubra	52	0.53	80.62
Paraonis fulgens	51	0.52	81.14
Anadara transversa	49	0.50	81.64
Marenzelleria viridis	49	0.50	82.14
Cirrophorus sp. C	47	0.48	82.62
Diopatra cuprea	47	0.48	83.10
Xanthidae	45	0.46	83.56
Ampelisca vadorum	43	0.44	84.00
Mediomastus ambiseta	40	0.41	84.41
Spionidae	40	0.41	84.82
Abra aequalis	35	0.36	85.18
Eusarsiella zostericola	35	0.36	85.54

Taxa Name	Abundance	% Abundance	% Cumulative Abundance
Polypedilum scalaenum group	35	0.36	85.89
Clinotanypus sp.	34	0.35	86.24
Hydroides dianthus	34	0.35	86.59
Grandidierella bonnieroides	33	0.34	86.93
Serpulidae	31	0.32	87.24
Melita sp.	29	0.30	87.54
Chironomus sp.	28	0.29	87.83
Cyathura polita	28	0.29	88.11
Acteocina canaliculata	27	0.28	88.39
Nereis micromma	27	0.28	88.66
Cyclaspis varians	25	0.26	88.92
Heteromastus filiformis	25	0.26	89.18
Sigambra tentaculata	25	0.26	89.43
Panopeus herbstii	24	0.25	89.68
Leptosynapta tenuis	23	0.24	89.91
Leitoscoloplos sp.	22	0.22	90.14
Melita longisetosa	22	0.22	90.36
Podarkeopsis levifuscina	21	0.21	90.58
Oxyurostylis smithi	20	0.20	90.78
Paraprionospio pinnata	19	0.19	90.97
Polydora cornuta	19	0.19	91.17
Spiophanes bombyx	19	0.19	91.36
Ampelisca cristata	18	0.18	91.55
Asabellides oculata	17	0.17	91.72
Cirratulidae	17	0.17	91.89
Mytilidae	17	0.17	92.07
Leucon americanus	16	0.16	92.23
Lucina sp.	15	0.15	92.38
Monoculodes sp. D	15	0.15	92.54
Pista quadrilobata	15	0.15	92.69
Amphipoda	14	0.14	92.83
Amygdalum papyrium	14	0.14	92.98
Magelona sp. H	14	0.14	93.12
Nassarius vibex	14	0.14	93.26
Nucula proxima	13	0.13	93.40
Eurypanopeus depressus	12	0.12	93.52
Hypereteone sp.	12	0.12	93.64
Phyllodocidae	12	0.12	93.76
Armandia maculata	11	0.11	93.88
Assiminea succinea	11	0.11	93.99
Eobrolgus spinosus	11	0.11	94.10
Aeginellidae	10	0.10	94.20
Glycinde solitaria	10	0.10	94.31
Mediomastus californiensis	10	0.10	94.41
Nereis lamellosa	10	0.10	94.51
Prionospio sp.	10	0.10	94.61
Americhelidium americanum	9	0.09	94.71
Edotia triloba	9	0.09	94.80
Ophiuroidea	9	0.09	94.89
Owenia fusiformis	9	0.09	94.98
Petricolidae	9	0.09	95.07
Spiochaetopterus oculatus	9	0.09	95.17
Chironomidae	8	0.08	95.25

Taxa Name	Abundance	% Abundance	% Cumulative Abundance
Corophium sp.	8	0.08	95.33
Demonax microphthalmus	8	0.08	95.41
Diplodonta semiaspera	8	0.08	95.49
Nephtys picta	8	0.08	95.57
Protohaustorius wigleyi	8	0.08	95.66
Tharyx acutus	8	0.08	95.74
Ampelisca abdita	7	0.07	95.81
Amphilochidae	7	0.07	95.88
Cirrophorus sp.	7	0.07	95.95
Exogone sp.	7	0.07	96.02
Leitoscoloplos robustus	7	0.07	96.10
Monocorophium acherusicum	7	0.07	96.17
Odostomia sp.	7	0.07	96.24
Ogyrides alphaerostris	7	0.07	96.31
Ostreidae	7	0.07	96.38
Pectinaria gouldii	7	0.07	96.45
Aglaophamus verrilli	6	0.06	96.51
Ampelisca sp. C	6	0.06	96.58
Ampharetidae	6	0.06	96.64
Capitella sp.	6	0.06	96.70
Cryptochironomus sp.	6	0.06	96.76
Epitonium multistriatum	6	0.06	96.82
Geukensia demissa	6	0.06	96.88
Mitrella lunata	6	0.06	96.94
Podarke obscura	6	0.06	97.01
Syllidae	6	0.06	97.07
Syllis beneliahui	6	0.06	97.13
Terebellidae	6	0.06	97.19
Turbonilla sp.	6	0.06	97.25
Aoridae	5	0.05	97.30
Cerapus benthophilus	5	0.05	97.35
Nephtys sp.	5	0.05	97.40
Oxyurostylis sp.	5	0.05	97.45
Spiophanes missionensis	5	0.05	97.51
Streptosyllis pettiboneae	5	0.05	97.56
Turbonilla interrupta	5	0.05	97.61
Vitrinella floridana	5	0.05	97.66
Amphicteis floridus	4	0.04	97.70
Demonax sp.	4	0.04	97.74
Gastropoda	4	0.04	97.78
Lepidonotus sublevis	4	0.04	97.82
Lineidae	4	0.04	97.86
Melinna maculata	4	0.04	97.90
Nereis falsa	4	0.04	97.95
Paraonis sp.	4	0.04	97.99
Sabaco americanus	4	0.04	98.03
Sabellidae	4	0.04	98.07
Tellina sp.	4	0.04	98.11
Automate sp.	3	0.03	98.14
Boonea seminuda	3	0.03	98.17
Calotrophon ostrearum	3	0.03	98.20
Capitellidae	3	0.03	98.23
Cerapus sp.	3	0.03	98.26

Taxa Name	Abundance	% Abundance	% Cumulative Abundance
Chione intapurpurea	3	0.03	98.29
Cirrophorus sp. A	3	0.03	98.32
Corbulidae	3	0.03	98.35
Corophium lacustre	3	0.03	98.38
Crassostrea virginica	3	0.03	98.42
Exogone rolani	3	0.03	98.45
Glycera americana	3	0.03	98.48
Gyptis pluriseta	3	0.03	98.51
Hemipholis elongata	3	0.03	98.54
Hesionidae	3	0.03	98.57
Hydrozoa	3	0.03	98.60
Latreutes parvulus	3	0.03	98.63
Macoma sp.	3	0.03	98.66
Marphysa sp.	3	0.03	98.69
Nereiphylla fragilis	3	0.03	98.72
Odostomia weberi	3	0.03	98.75
Onuphidae	3	0.03	98.78
Phyllodoce arenae	3	0.03	98.81
Piromis roberti	3	0.03	98.84
Polynoidae	3	0.03	98.88
Rhithropanopeus harrisii	3	0.03	98.91
Sphenia sp.	3	0.03	98.94
Synidotea sp.	3	0.03	98.97
Vitrinellidae	3	0.03	99.00
Alpheus armillatus	2	0.02	99.02
Ampithoidae	2	0.02	99.04
Anachis lafresnayi	2	0.02	99.06
Arcidae	2	0.02	99.08
Autolytus sp.	2	0.02	99.10
Bateidae	2	0.02	99.12
Bowmaniella floridana	2	0.02	99.14
Caprellidae	2	0.02	99.16
Ceratonereis irritabilis	2	0.02	99.18
Decapoda	2	0.02	99.20
Euceramus praelongus	2	0.02	99.22
Eunicidae	2	0.02	99.24
Glyceridae	2	0.02	99.26
Hargeria rapax	2	0.02	99.28
Laeonereis culveri	2	0.02	99.30
Lepidonotus variabilis	2	0.02	99.33
Monoculodes sp.	2	0.02	99.35
Nereididae	2	0.02	99.37
Nereis riisei	2	0.02	99.39
Notomastus hemipodus	2	0.02	99.41
Odontosyllis enopla	2	0.02	99.43
Oedicerotidae	2	0.02	99.45
Paraeupolymnia sp. A	2	0.02	99.47
Podocopida	2	0.02	99.49
Scoletoma verrilli	2	0.02	99.51
Synidotea sp. F	2	0.02	99.53
Tagelus plebeius	2	0.02	99.55
Apoprionospio dayi	1	0.01	99.56
Armandia agilis	1	0.01	99.57

Taxa Name	Abundance	% Abundance	% Cumulative Abundance
Caprella scaura	1	0.01	99.58
Carazziella hobsonae	1	0.01	99.59
Chione cancellata	1	0.01	99.60
Columbellidae	1	0.01	99.61
Corbula sp.	1	0.01	99.62
Crassinella lunulata	1	0.01	99.63
Crepidula sp.	1	0.01	99.64
Cyclostremiscus pentagonus	1	0.01	99.65
Diplodonta sp.	1	0.01	99.66
Doridella sp.	1	0.01	99.67
Doridella obscura	1	0.01	99.68
Dorvilleidae	1	0.01	99.69
Epitonium sp.	1	0.01	99.70
Eumida sanguinea	1	0.01	99.71
Fimbriosthenelais minor	1	0.01	99.72
Goniada littorea	1	0.01	99.73
Grubeosyllis clavata	1	0.01	99.74
Haustoriidae	1	0.01	99.75
Ischyroceridae	1	0.01	99.76
Macoma tenta	1	0.01	99.78
Mancocuma stellifera	1	0.01	99.79
Nassariidae	1	0.01	99.80
Nassarius trivittatus	1	0.01	99.81
Nematonereis hebes	1	0.01	99.82
Nephtys bucera	1	0.01	99.83
Nuculidae	1	0.01	99.84
Pagurus sp.	1	0.01	99.85
Pagurus longicarpus	1	0.01	99.86
Petrolisthes politus	1	0.01	99.87
Pinnixa sp.	1	0.01	99.88
Pinnotheridae	1	0.01	99.89
Pista palmata	1	0.01	99.90
Porcellanidae	1	0.01	99.91
Rhepoxynius epistomus	1	0.01	99.92
Scolelepis texana	1	0.01	99.93
Streblosoma hartmanae	1	0.01	99.94
Syllis sp.	1	0.01	99.95
Turridae	1	0.01	99.96
Unciola serrata	1	0.01	99.97
Ungulinidae	1	0.01	99.98
Upogebia affinis	1	0.01	99.99
Veneridae	1	0.01	100.00