3.0 MATERIALS AND METHODS

3.1 Field

3.1.1 Logistical Rationale and Needs

The large spatial scale of this study required design of the field sampling around helicopters (Bell Jet Rangers four-passenger with floats) to make the sampling as efficient and rapid as possible. All stations were located with handheld global positioning system (GPS) equipment (Trimble® Pathfinder Pro) corrected to within ±25 m. A synoptic sample over the entire ecosystem proceeding from south to north was completed in a 6-day period for canals (50 stations) and transects (45 stations) and an 8-day period for the marsh grid (125 stations). To reduce costs, about 60% of the 50 canal sampling stations in each cycle were accessed by a single helicopter and the remainder by jon boat. The helicopter was idled at mid-channel while the canal samples were being collected to maintain position and to prevent damage to the aircraft. The helicopter was shut down at all marsh sampling sites. To sample the marsh transects, a single helicopter was used for all mid-marsh sites; however, some sites near the canals were accessed with an airboat. The marsh grid was sampled with two crews and two helicopters. A two-person sampling team was used in each aircraft and all gear and sample containers were designed to fit in the fourth seat and the aft storage compartment.

3.1.2 Apparatus

The development and application of clean sampling methods was a primary goal of this project. During project initiation the first canal sampling cycle and the marsh transect sampling effort were used to work out a final sampling protocol for the remaining canal and marsh sampling cycles. During these pilot studies the water samples were dipped by submerging the water bottles under the surface of the water until filled. The dip method had several limitations: (1) water samples could not be collected from water that was less deep than the width of the bottle eliminating samples from large areas of shallow marsh, (2) there was no way to prevent large particulate matter from entering with the water, and (3) there was little assurance that a clean sample could be collected by dipping. A hand-operated vacuum water sampling chamber

was developed and used to consistently collect a screened ultra trace level water sample. (Specifications and pictures of sampling equipment are provided in Appendix A.) The intake wand was fitted with a 105μm replaceable Nitex® screen to prevent large particle matter from entering the sample container. The screen was held over the end of the wand by a plastic coated magnetic ring (Gelman magnetic filter funnel with funnel cut off) and easily and quickly replaced between stations. The samples were not filtered to permit quantification of total constituent concentration, which can be ecologically significant. The chamber was made of machined ¼-inch Plexiglas® with an o-ring seal for the lid. All intake and exit lines fitted into the lid were of ¼-inch Teflon® tubing. The chamber was sized to hold a 2-liter Teflon® bottle required for clean low level Hg samples.

The sampling procedure was initiated at each station by placing a 2-liter polypropylene bottle in the chamber and pumping the bottle about 25% full. This water was used to rinse the bottle and discarded. The bottle was then pumped 75% full and this sample was used for TSO_4, TP, TOC, TN, turbidity, and APA samples. The low level Hg sample was taken immediately after by placing the 2-liter Teflon® bottle into the chamber and pumping it full with no headspace. A 2-liter bottle was filled in about 5 minutes with about 380 mm (15 inches) Hg of vacuum. The bottle was labeled, its number recorded, inserted into a Fisher® plastic bag, and placed in a cooler inside a black plastic bag. The device was flushed twice before each clean low level Hg sample was collected at each station when this sampling sequence was followed. During this procedure, the operator was gloved with PVC rubber gloves covered with shoulder length polyethylene gloves and clothed in chest waders and/or a flight suit. Water samples were collected near the helicopter at about 1 foot below the surface when sampling in deep water and at mid-depth when sampling shallow water. Acidification of the Hg samples was made the same day following return to the clean laboratory on the Florida International University (FIU) campus, where 1 ml of trace metal grade HCl per 1000 ml of sample was added to each Hg sample on the same day. Water field blanks (carry along controls) of Hg free deionized water were taken into the field with each crew each day and analyzed for ultra trace level THg before and after transport to the field. Sampling near the helicopter had the potential for sample contamination especially on the canals where the helicopter had to be operated continuously to maintain position and when landing in shallow marsh. Water samples collected at various distances from the helicopter as well as the

field blanks indicated no contamination of the samples was evident. Additional information on quality assurance/quality control (QA/QC) can be found in the data quality objectives (DQO) (Appendix B).

Chlorophyll *a* and particulate samples were collected from the canals using a 140 cc plastic syringe fitted with a 0.45μ glass fiber filter (GF/F) membrane filter cartridge. An effort was made to pass 2 to 3 volumes of the syringe through the filter. The final water volume filtered was recorded and the filter removed with clean forceps and placed into a microfuge tube and capped. The chlorophyll *a* sample was stored on ice in the dark and transported to the laboratory.

A stainless steel petite ponar dredge, previously cleaned and sealed in plastic, was used to obtain canal sediment samples. A sample was used only when the dredge was retrieved full with no apparent disturbance to the surface of the sediment. The sample was placed in a clean glass pan and mixed thoroughly with a plastic spoon. Large pieces of plant material, mollusc shells, and other debris were separated from the fine sediment and discarded. Three 120-milliliter plastic cups were filled approximately 75% full at each station and sealed in Fisher® plastic bags and placed on ice. The dredge was thoroughly rinsed in canal water between stations.

After testing numerous commercially available soil sampling devices a 3-inch diameter clear polycarbonate coring tube (0.125-inch wall thickness) was developed to collect marsh soil samples. This tube diameter was selected because it was large enough to minimize compaction of the soil in the tube and retain the sample in wet conditions. The leading edge was serrated and sharpened to facilitate cutting through dense peat and plant roots. Coring tubes were designed to sample depths to 45 cm and tested during the transect study. Following that effort a determination was made to focus all remaining marsh soil sampling on the top 10 cm. A stainless steel tube top (Appendix A) was developed with a threaded receiver for the threaded lexan® tubes, which were cut to 25 cm in length. The tube top was designed with a flapper valve for the escape of air and water, a foot pad and a receiver for a stainless steel handle were also included. Stainless steel handles in 4-foot lengths could be added with stainless steel attachment pins when the water depth required. This soil sampling device allowed quick assembly and disassembly. The soil cores could be rapidly retrieved, the tube unscrewed, and a clean PVC/rubber plunger used to push the core top to the 10 cm mark on the tube. The excess soil was sliced off with a Teflon® coated spatula

and the remainder of the core placed in the sample. A sealed 1-gallon plastic container was used to transport triplicate cores from each station to the laboratory in a cooler.

Upon arrival at the laboratory at the end of each day, the soil samples were processed by technicians wearing PVC gloves. A clean polypropylene spoon was used to mix and homogenize the soil cores, which were composited in the plastic container. During the mixing, large debris (e.g., large plant roots and sticks) that occurred in the samples were removed. Depending on the sample volume requirements, from five to seven 4-ounce plastic sample containers were filled approximately 75% full, labeled, and tagged. One hundred milliliters of deionized water was mixed with the soil remaining in the 1-gallon plastic container. Temperature, pH, and Eh probes were inserted into the soil slurry and allowed to equilibrate for 5 minutes, after which the temperature, pH, and Eh measurements were made.

An in situ Eh probe was developed by recessing 1 cm square platinum electrodes into the outside of a l¼-inch PVC pipe. The electrodes were attached at intervals of 2.5, 5, 10, 15, and 20 cm from the top. A wire was silver soldered to the inside of each electrode and run through a water tight connector at the top of the pipe to a 5-position switch. An adjustable stainless steel stop plate with friction screws was designed with an extension to protect the water tight fitting and to provide a receptacle for the attachment of a 4-foot stainless steel handle (Appendix A). During development a solution of known Eh was used to test the response of each system. A millivolt meter with an attached $AgCl_2$ reference electrode completed the instrument. The probe was inserted into the soil so that the stop plate was against the soil surface. The millivolt meter was plugged together with the probe with the reference electrode in the water. The system was allowed to equilibrate for 15 minutes, at which time the electrodes were queried sequentially from top to bottom with the switching device and the readings recorded on the field data sheet. The same protocol was repeated at each station. Soil pH and Eh along the marsh transects were measured on site by inserting an Eh electrode into an intact soil core.

A Hydrolab Scout 2 Water Quality Data System (Hydrolab) was used to measure water temperature (°C), DO (mg/L), specific conductivity (mS/cm), pH, and Eh (mV). The data sonde was suspended in the water column at mid-depth and the DO probe was allowed to equilibrate prior to recording the readings on the field data sheet. The Hydrolab calibration procedure defined

in the EPA Science and Ecosystem Support Division (SESD) *Standard Operating Procedure* (SOP) was executed in the laboratory prior to entering and leaving the field each day.

Mosquitofish were collected with a Turtox Indestructible dipnet (800 x 900 mm multifilament nylon net) with a 40-inch wooden handle. The sampler used the net in an aggressive manner in an attempt to capture a complete size range of the fishes in the area near the helicopter. When necessary, both crew members used the same technique to collect the required number of mosquitofish to shorten the time on station. The fish captured with each swipe of the net were handled with latex gloves and placed in a 5x8-inch Fisher® plastic bag and labeled according to station number (place) and documented on the field data sheet. A minimum of 20 fish were collected at each site except when additional fish were collected for isotope or food habits analyses. After the fish were bagged, the bag was placed on ice in a small cooler for transport to the FIU laboratory. In the laboratory the bags of fish were visually checked for completeness and placed immediately in a freezer for storage until analysis for Hg, which occurred within a 1-month holding time. There was concern that the preservation of fish in air evacuated sealed bags may result in the desiccation of these small fish. An experiment was conducted to test for dessication and it was found that weight loss resulting from this method of preservation and freezing did not exceed 5% (B. Loftus personal communication).

3.1.3 Schedule

A typical daily schedule started with the arrival of the support personnel at the FIU laboratory around 6 AM to calibrate the Hydrolabs, pack the sampling equipment and supplies, and disconnect the GPS unit from the charger and pack the instruments. Around 7 AM the field personnel arrived to load equipment and supplies into vans for transport to the helicopter landing zone. The sampling teams secured and prepared personal gear and safety equipment (flight suits and helmets). The helicopter/boat was loaded at 8 AM, and the crew(s) departed for the field. While the field team was collecting samples, the support team serviced and repaired the field equipment, completed analyses from the previous day, labeled/packaged samples, completed chain-of-custody forms, shipped samples to other laboratories, and made up packs of sample containers for the next day. With return of the sampling crew(s) around 5 PM the helicopter was

unloaded and the samples, equipment, and supplies were delivered to the FIU laboratory, where the field data sheets were verified, sample preservatives added, the GPS unit(s) downloaded, and the Hydrolab(s) end-calibrated. The support team remained on duty tracking the samples with computer software (FORMS), labeling, processing sediments, performing bench-top analyses of water samples for turbidity, APA, and sulfide. The support team completed their duties around 10 PM each evening.

3.1.4 Sampling Routine

A field sampling routine was developed that facilitated the efficient collection of water, sediment, and biota from remote sites using a helicopter. Sampling from an airboat or a jon boat was slower and less demanding but followed a similar routine. All tasks were divided among a two-person sampling crew. The crew person in the front seat usually operated the handheld GPS equipment so that it could be compared with the helicopter GPS readings and in case of failure of the handheld unit be used as a substitute. The crew person in the rear seat managed the water sampling gear and all the sample containers, which were stored in Fisher® plastic bags inside two ice chests. The sediment and fish sampling gear was stored in the rear compartment of the helicopter. The sampling stations were selected for each helicopter each day by identifying a group of 8 to 10 stations, which minimized flight time, refueling, and potential interaction with the other helicopter. During each sampling event, stations were sampled from south to north, moving upstream. Flight following was practiced with ENP radio dispatch by each helicopter each day with the preselected stations for the day. To initiate field sampling the pilot was given the coordinates for each sampling station that the team wanted to visit during the day. These coordinates were usually keyed into the helicopter GPS to aid navigation from point to point. The helicopter GPS was used to navigate to within 0.5 km of the sampling site and the handheld GPS was used for final approach and landing on the site. In rare cases if the landing site was unsafe due to extremely tall cattails, sawgrass, or cypress trees, the pilot was directed to move to the nearest safe landing site. Upon landing, the GPS coordinates were recorded on the field data sheet along with the start time and logged electronically for 3 minutes before the unit was turned off.

Immediately following landing, preparations were made to initiate water sampling, which was always carried out first in the sequence to minimize contamination and disturbance of the water column. The application of clean sampling methods were most critical for water and the vacuum bottle sampler was used to begin the sampling sequence at each station. The water sampling sequence resulted in two 125-milliliter polyethylene bottles for TP, APA, TOC, TN, and turbidity; a 500-milliliter HDPE bottle for TSO_4; and a 2-liter Teflon® bottle for Hg. Each Teflon® bottle was etched with a unique number, which was recorded on the field data sheet at the time of sampling. An additional 125-milliliter polyethylene bottle was filled for H_2S by dipping and preserved with two drops of zinc acetate. The number of bottles and types of samples were recorded on the field data sheet. The collection of water samples was followed immediately by placing the Hydrolab sonde at a mid-depth position and recording the temperature, DO, pH, conductivity, and Eh on the field data sheet. This unit is equipped with a stirrer to maintain constant water flow across the membrane.

The following basic information was entered on the field data sheet: the station number, date, helicopter number and pilot, crew members' initials, water field blanks (when taken), duplicate samples (when taken), Eh probe number, Hydrolab number, and camera model. A marquee indicating station number, date, cycle, and film roll number was completed and photographed as one in a series of 35 mm color slides including the marquee, a ground level scene, a soil core, and an oblique photo of the sampling site from an altitude of approximately 100 ft. The film roll number and frame numbers of these images were recorded on the field sheets at each station.

Plant community composition and presence or absence of cattails (*Typha spp*.) and floating periphyton mats were recorded at each of the sampling sites. A visual assessment of the vegetation types and their relative dominance at each site was made by each sampling team while onsite. Two 35 mm photographs were also taken for later review. The dominant and secondary plant communities occurring at each site were identified as well as the community sampled.

In addition, the presence or absence of cattails and floating periphyton mats were made from a secondary review of the two 35 mm photographs taken at each marsh site. The presence of a cattail or floating periphyton mat was enough to indicate presence, as long as the indicator was

visible from both photographs taken at each point. Although no formal plot size or distance criteria were established, in order for a specific plant type to be included in the qualitative habitat assessment, it had to be identifiable in the photographs.

Observations made by the crew included weather, surface water flow, soil type, and vegetation type/fish habitat, which were qualitatively coded on the field data sheet.

The remaining tasks carried out to obtain soil/sediment measurements and tissue samples were organized by each sampling team to maximize speed and efficiency. The in situ Eh probe was inserted into the marsh soil and connected to the reference electrode, switch box, and meter, and the start time was recorded. This probe was allowed to equilibrate for 15 minutes prior to switching across the soil depths and recording the readings on the field data sheet. The surface water and soil depths were measured by using a rod, marked in tenths of feet, which could be lengthened by screwing on additional sections. These readings were recorded on the field data sheet. Three soil cores were collected at each site, and the soil periphyton, which could be separated as a distinct surface layer, was placed in a separate soil periphyton sample container. Only the top 10 cm of the soil column from three cores was retained. When floating periphyton occurred at a site it was collected with gloved hands and placed in three sealed, 4-ounce plastic containers. Fish were collected last with a dipnet, immediately placed in Fisher® plastic bags, and stored in a cooler on ice until placement in a laboratory freezer.

Deviations from this routine were made when sampling the canals and the marsh transects. The primary deviations from this routine when sampling the canals were the omission of the Eh probe and the addition of particle samples, chlorophyll *a* samples, and Hydrolab measurements throughout the water column except for temperature and DO. Sediment samples were collected with a clean stainless steel petite ponar dredge. The primary deviations from this routine for the transects were that water samples were dipped and not screened, soil cores were collected to a depth of 45 cm, and soil Eh and pH were measured onsite in intact soil cores. Water samples were collected by filling water bottles underwater at each site.

Upon completion of sampling at each site, the samples and equipment were packed into the helicopter. The field data sheet was checked for completeness and signed by both members of the sampling team before the helicopter departed for the next site.

3.2 Laboratory Analyses

A flow chart showing the progression of methods development for this project is presented in Figure 3.1. The development of analytical methods for THg in water, soil, and fish was initiated in early 1993 prior to the first sample cycle in September.

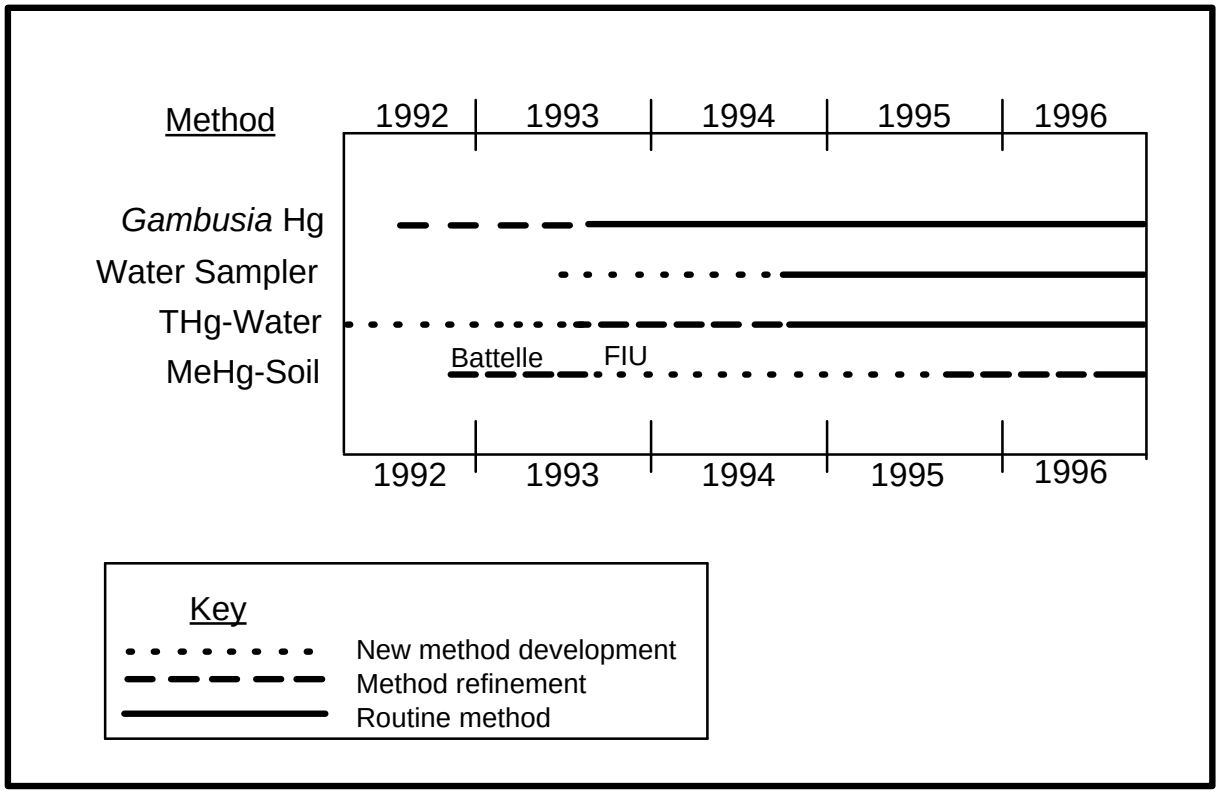

Figure 3.1 Methods development timeline

The measurement of trace level quantities of THg and MeHg in water were required to successfully carry out this project with minimum detection limits (MDLs) at 0.3 and 0.02 ng/L, respectively. At the outset, it was known that there was no standard method available for the analysis of THg and MeHg in any media and most methods used a slower batch process with small sample throughput; however, there were several research methods in use around the world. Because there was no trace level Hg analytical capability in EPA in 1992 to 1993, it was determined that the project would use three laboratories to allow intercalibration and comparison. The FIU-Southeast Environmental Research Program (FIU-SERP) laboratory in Miami was

selected because a trace level Hg laboratory was already under development with US National Park Service (ENP), and the proximity and experience of FIU-SERP in analyzing Everglades samples was a definite advantage. The FIU-SERP laboratory had considerable experience in developing state-of-the-art methods for several other parameters that the project also wanted to use. The Battelle Marine Sciences Laboratory (MSL) in Sequim, WA was chosen to analyze MeHg and THg in water samples, to analyze MeHg in soils and sediments, and to provide QA/QC on split and duplicate samples. The EPA Region 4 Science and Ecosystem Support Division laboratory in Athens, GA began development of the capability to analyze THg in soil, sediment, and fish tissue. Table 3.1 shows the distribution of parameter analyses among the laboratories and the primary and secondary QA/QC responsibilities. Due to the large sample volume generated during the project it was equally necessary to use multiple laboratories to distribute the work load in order to achieve timely completion of sample analysis.

Atomic fluorescence-based methods were developed for measuring ultra trace levels of Hg in environmental (water, soil) and biological (fish tissue) samples (Jones et al. 1995). In addition, methods for preparation of water, soil, and tissue samples were developed. For the analysis of THg in soil, sediment, and fish, the samples were digested with concentrated nitric acid in sealed glass ampules and subsequently autoclaved. Following digestion, the digestate in the sealed ampules could be stored for later analysis to better manage the flow of work. Water samples were digested using standard brominating procedures. A Merlin Plus, PS Analytical atomic fluorescence spectrometer (AFS) system equipped with an autosampler, vapor generator, fluorescence detector, and a PC-based integrator package was used in the determination of THg. Prior to and during development of the vacuum water sampling chamber full-scale deflection was 30 ng/L; however, following development of the chamber full-scale was limited to 10 ng/L. The determination of Hg species in water, without prederivitization, involved adsorbent

Table 3.1 Distribution of parameter analyses for multiple laboratory design.

LABORATORY			
PARAMETER	**FIU-SERP**	**BATTELLE**	**EPA-SESD**
WATER			
THg	Primary	Splits/Duplicates	
MeHg	Splits/Duplicates	Primary	
TSO_4			Primary
Turbidity	Primary		
TOC	Primary		
TP	Primary	Splits/Duplicates	
TN	Primary	Splits/Duplicates	
APA	Primary		
Chlorophyll *a*	Primary		
SOIL/SEDIMENT			
THg	Splits/Duplicates		Primary
MeHg	Primary [1]	Splits/Duplicates	
EtHg	Primary		
TSO_4			Primary
TP	Primary		
Ash Free Dry Weight (AFDW)	Primary		
Bulk Density	Primary		
PERIPHYTON (Floating & Soil)			
THg	Primary		Splits/Duplicates
MeHg	Primary		
EtHg	Primary		
GAMBUSIA			
THgF	Primary		

[1] Initially Battelle following cycle 0

preconcentration of the organomercurials onto sulfhydryl cotton fibers. The organic Hg compounds were eluted with a small volume of acidic potassium bromide (KBr) and copper sulfate ($CuSO_4$) and extracted into dichloromethane. Sediment, soil, and tissue samples were homogenized, and the organomercurials first released from the sample by the combined action of acidic KBr and $CuSO_4$ and extracted into dichloromethane. The initial extracts are subjected to thiosulfate clean-up and the organomercury species are isolated as their chloride derivatives by cupric chloride addition and subsequent extraction into a small volume of dichloromethane. Analysis of organic Hg compounds was accompanied by capillary column chromatography coupled with atomic fluorescence detection.

Following the MSL analysis of the initial canal sediment samples collected in September 1993, it was apparent that the detection levels were not sufficiently low to allow the reporting of other than the MDLs. Since this would not provide the desired information for which this project had been designed, FIU-SERP was charged with the development of a method to determine organic Hg compounds in soil and sediment. A sensitive method for the determination of MeHg, and EtHg in soil and sediment was developed (Alli et al. 1994). The organomercurials are released from the sample matrix by the combined action of acidic KBr and cupric ions and extracted into dichloromethane. The initial extracts are subjected to thiosulfate clean-up, and the organomercury species are isolated as their chloride derivatives by cupric chloride addition and subsequent extraction into a small volume of organic solvent. Capillary gas chromatography coupled with an atomic fluorescence detector system proved a very selective and sensitive technique with excellent separation efficiencies for MeHg and EtHg. The absolute detection limit for both MeHg and EtHg was 0.2 pg.

A new method based on capillary gas chromatography/atomic fluorescence spectrometry (GC/AFS) was developed for the determination of MeHg and EtHg in water samples (Cai et al. 1996). An improved sample preparation methodology was developed, which involved preconcentration of the alkylmercury species from water samples, drawn with a 12-channel peristaltic pump, onto sulfhydryl cotton fibers adsorbent packed in a screening column, elution of MeHg and EtHg with a mixture of acidic KBr and $CuSO_4$ solution, and back-extraction using

methylene chloride. Analysis was performed by capillary GC/AFS with a DB-1 column (Cai et al. In Press a). Some important parameters, including sample pH, presence of anions and cations, concentration of TOC, eluent type, and eluent volume were evaluated. With AFS as a detector, the capillary gas chromatographic technique provides high selectivity, high sensitivity, and a straightforward method for organomercury halide analysis. It eliminates possible spectral interferences to the detector from other sample components and from chemicals used in the sample preparation procedure. The detection limit was 0.01 ng/L in a 1-liter water sample for both MeHg and EtHg. The result for organomercury analysis in a number of natural water samples was comparable to MeHg analysis using other methods with the following advantages. The method following the solid phase extraction procedure based on the sulfhydryl cotton fibers offers a number of advantages over other sample preparation methods, including higher concentration factors, large sample throughput, less use of organic solvents, and improved sample clean-up during the solid phase extraction procedure. This method also allows detection of EtHg, which cannot be determined with ethylation-purge-trap methods, and avoids steam distillation (Cai et al. 1997b). Steam distillation has been shown to generate artificial MeHg (Bloom et al. 1997).

Finally evaluation of some isolation methods for organomercury determination in soil and fish samples by capillary GC/AFS were made (Cai et al. In Press b). Three extraction methods, acidic KBr/$CuSO_4$ isolation-methylene chloride extraction, acidic KBr/$CuSO_4$ isolation-methylene chloride extraction with an alkaline digestion pretreatment, and an extraction method at a milder condition with citrate buffer and dithizone in chloroform, were tested for MeHg and EtHg determination in soils, sediments, and fish samples by the recently developed capillary GC/AFS. The acidic KBr/$CuSO_4$-methylene chloride extraction and the acidic KBr/$CuSO_4$-methylene chloride extraction with an alkaline digestion pretreatment were shown to be the effective methods for soil and sediment analysis and fish sample analysis, respectively. The presence of EtHg species in soils of the Florida Everglades, observed with the acidic KBr/$CuSO_4$ isolation and methylene chloride extraction procedure, was further confirmed with the dithizone complexation/extraction procedure (Cai et al. 1997b). The GC/AFS analytical method offers high

sensitivity and selectivity for the determination of organomercury halides. Since serious limitations have been found with both the distillation and the ethylation procedures used to analyze both MeHg and EtHg species in soils, sediments, and biological samples, the modified acidic isolation/organic solvent extraction combined with the GC/AFS technique provides a useful analytical tool for organomercury speciation and an alternative for the current methods available in the literature. Development and refinement of soil and sediment extraction and analytical methods continued throughout the study until spring 1996 (Figure 3.1).

Unfiltered water samples were used for the determination of TOC, TP, and TN. TOC was measured by acidifying to pH <2 with 3N HC1, purging the sample with CO_2-free air, and analyzing for total carbon using a hot platinum catalyst direct injection analyzer. TP was determined using a dry ashing and acid hydrolysis technique (Solorazano and Sharp 1980). Turbidity was determined by nephelometry using formizan calibration standards. TN was measured by high temperature oxidation in an ANTEK 7000N Total Nitrogen Analyzer, using the methods described by Frankovich and Jones (1998).

Soils and sediments were analyzed for TP, bulk density, and percent organic matter. TP was analyzed using the ashing method described by Solorzano and Sharp (1980). Soil bulk density was determined by drying a known volume at 80° C to a constant weight. Organic matter was determined by loss on ignition at 550° C (Nelson and Sommers 1982).

Chlorophyll *a* was determined using the fluorometric method described by Strickland and Parsons (1972). APA was measured using the method described by Jones (1997).

3.3 QA/QC

Numerous QA/QC exercises in water, soil, sediment, and tissue were conducted among the project laboratories during the study. Differences in methods were expected to produce differences in results, and every effort was made to achieve agreement, whenever possible, even though standard methods could not be required. An internal *Quality of Science Review* was conducted by EPA Office of Research and Development (ORD) National Exposure Research Laboratory (NERL) Environmental Sciences Division (ESD) during the project (1) to identify

quality-related issues and provide recommendations for correction or improvement and (2) to provide the project participants with the necessary tools to enable them to continue to monitor data quality. Onsite visits were made to all active project participants and copies of all preliminary data sets were reviewed. Data analyses included calculation of data quality indicators (e.g., completeness, precision, accuracy); investigations of potential variability due to transportation means, crew, or season; and analyses of issues relating to THg and MeHg in water methods. The review described results of the onsite visits and data analyses, identified particular strengths and weaknesses of the project, and provided recommendations for corrective actions. An adjustment was made in the calculation of the standard curve for THg in water data developed by FIU-SERP using a flow-thru atomic fluorescence system without gold amalgamation to make the data more comparable with those developed by Battelle MSL, which used a cold vapor atomic fluorescence batch method with gold amalgamation. Data package and validation/verification issues have been addressed by EPA Region 4 SESD.

Decision-Based DQOs (Appendix B) were prepared generally following the *Guidance for the Data Quality Objectives Process EPA QA/G-4* (USEPA 1994). This EPA guidance document, however, is not entirely appropriate for research projects. The EPA ORD Quality Assurance Management Staff are in the process of preparing DQO guidance for research projects, but this guidance was not available. This project is a research project that in part, is developing risk-based criteria for decisions because the existing criteria are not appropriate or no criteria exist. Two separate but complementary approaches were used to develop project DQOs: (1) using the EPA QA/G-4 documentation and (2) revising the DQOs originally proposed in the *REMAP Research Plan* (Stober et al. 1993). The DQOs are presented with statements for data representativeness, completeness, comparability, precision, and accuracy for each of the constituents measured by the project (Appendix B). The canal and marsh data were evaluated using the DQO criteria. Evaluation of the canal and marsh data field precision showed that a consistent database was developed by the project with no canal or marsh parameters having outlier in excess of 4% and 2.9%, respectively. The database has a very high degree of internal

consistency, and future monitoring should endeavor to continue this consistency and comparability to minimize the introduction of artifacts into the baseline that has been established.

3.4 Data Analysis

3.4.1 Data Verification and Validation

Data verification and validation analyses were conducted on the data, both for QA/QC and to establish the database for statistical and spatial analyses. This data set, with associated meta data, can be obtained from EPA Region 4 SESD, Athens, GA. Inquiries can be sent to the address on the title page. OA/OC findings are summarized in Appendix C.

A number of statistical analyses were performed on these validated/verified data. These analyses are listed in Table 3.2 and are briefly discussed below.

3.4.2 Descriptive Statistics

Descriptive statistics, including the range, mean, median, standard deviation, and quartiles for each constituent, by media, sampling cycle, and system type, were computed for various subpopulations (WCAs, ENP, area north of Alligator Alley, marsh, canals, etc.). These descriptive statistics provided initial insight into the structure and attributes of these subpopulations in the South Florida Everglades ecosystems. Box and whisker plots also were computed and displayed by constituent, media, and subpopulation to provide a visual image of the subpopulation attributes.

Cumulative distributions also were computed for each constituent, by media, cycle, and subpopulation to characterize the structure of subpopulations and to provide initial insight into any data transformations that might be required for parametric statistical analyses.

Constituent information was sorted by latitude and longitude to determine if there might be north to south or east to west gradients that could provide insight into possible Hg sources or indicate other factors that might be contributing to the elevated fish Hg concentrations measured in the Everglades ecosystem.

Table 3.2 Statistical analyses performed on data.

Analysis	Canal	Marsh	Structures	Transects
Descriptive Statistics	✓	✓	✓	✓
by sampling cycles	✓	✓		
by seasons	✓	✓		
by subpopulations	✓	✓		
by year			✓	
Notched Box and Whisker Pots	✓	✓		✓
by sampling cycle	✓	✓		✓
by season	✓	✓		✓
by subpopulation	✓	✓		
Cumulative Distributions	✓	✓		
by sampling cycle	✓	✓		
by subpopulation	✓	✓		
Scatter Plots	✓	✓	✓	✓
by latitude	✓	✓		
by longitude	✓	✓		
by depth		✓		
Scatter Plot Matrices	✓	✓	✓	✓
untransformed	✓	✓	✓	✓
transformed	✓	✓	✓	
Principle Component Analysis	✓	✓		
untransformed	✓	✓		
transformed	✓	✓		
by season	✓	✓		
transformed and season	✓	✓		
Factor Analysis	✓	✓		
by season	✓	✓		
transformed, by season	✓	✓		

Table 3.2 (Continued).

Analysis	Canal	Marsh	Structures	Transects
Discriminant Analysis				
by subpopulation	✓			
transformed by subpopulation	✓			
transformed	✓			
untransformed	✓			
Cluster Analysis	✓	✓		
untransformed	✓	✓		
transformed	✓	✓		
by season	✓	✓		
Cramer Von Mises	✓	✓		
ANOVA	✓	✓		
by subpopulation	✓	✓		
by subpopulation and season	✓	✓		
Analysis of Covariance		✓		
by cycle		✓		
by subpopulation		✓		
Linear Regression	✓	✓	✓	
untransformed	✓	✓	✓	
transformed	✓	✓	✓	
by subpopulation	✓	✓		
Frequency Tables		✓		

3.4.3 Exploratory Analyses

A number of exploratory analyses were conducted on the data to gain greater insight into the structure and attributes of various subpopulations of interest. These exploratory analyses included scatter plots or multiple bivariate plot matrices, principal component, factor, discriminant and cluster analyses. These analyses identified several factors or principal components that contributed to the distribution of Hg in various media throughout the Everglades.

3.4.4 Inferential Statistics

Once the population and subpopulation attributes were described, statistical tests were performed to test various hypotheses about differences among subpopulation characteristics. These tests included the Cramer von Mises test (Kiefer 1959) for differences among cumulative distributions and analyses of variance and covariance to determine if various constituent combinations were contributing to differences among subpopulations. General linear models also were used to determine the proportion of the variance in fish Hg concentrations accounted for by a suite of other factors and constituent concentrations. Frequency tables were used to evaluate possible differences among the distribution of selected constituents.

3.4.5 Spatial Statistics

Kriging was used to characterize the spatial patterns of constituent concentrations throughout the marsh ecosystems. The kriging predictor of a constituent $Z(s_0)$ at an unsampled site s_0 is equal to the linear combination of the data

$$\hat{Z}(s_0) = \sum_{i=1}^{n} \lambda_i Z(s_i) ,$$

where $Z(s_i)$ is the value of the constituent at the *i-th* sample site s_i. The λ_is are chosen to minimize the mean squared prediction error subject to the constraint that the resulting predictor is unbiased (Cressie 1991). The values of the λ_is are functions of the spatial correlation structure of the data. However, the kriging predictor is not sensitive to misspecification of that correlation structure

(Cressie and Zimmerman 1992). In practice, kriging predictors are obtained at a fine grid of sites (here, every 0.1° latitude and longitude), from which a contour map of predicted values can be obtained. The contour map of predicted constituent concentrations was obtained using Surfer® for Windows, Version 6 (Golden Software, Inc. 1995).

3.4.6 Mass Estimates

Mass estimates for THg and MeHg were calculated for the study area. Hg concentrations and discharges through the structures were measured and used to estimate Hg loads to the WCAs and ENP. Atmospheric loads were estimated by the Florida Atmospheric Mercury Monitoring Program. Periphyton and fish Hg concentrations were measured and biotic densities estimated from the literature. Water and soil Hg concentrations were measured and the mass estimates were based on the spatial weighting factors associated with each probability sample. The methods and results of these mass estimates are discussed more fully in Chapter 9.0.