Appendix 2B-1: Annual Permit Compliance Monitoring Report for Mercury in Downstream Receiving Waters of the Everglades Protection Area

Darren Rumbold, Nicole Niemeyer, Fran Matson, Shane Atkins, Joseph Jean-Jacques, Kevin Nicholas, Christy Owens, Karl Strayer and Brent Warner

SUMMARY

This appendix summarizes data from compliance monitoring of mercury (Hg) influx and bioaccumulation in the downstream receiving waters of the Stormwater Treatment Areas (STAs) for Water Year 2005 (WY2005) (May 1, 2004 through April 30, 2005).

The key findings presented in this appendix are as follows:

1. Difficulties were encountered in rainfall collection for mercury analysis due to the landfall of four hurricanes in 2004 (especially hurricanes Frances and Jeanne that made landfall September 8 and September 26, 2004, respectively). These storms prevented weekly sample collections in two instances, and resulted in temporary malfunction of equipment at the Mercury Deposition Network site, located at Stormwater Treatment Area 1 West (STA-1W). Consequently, preliminary estimates for both volume-weighted concentration and deposition are considered gross underestimation at that site. Volume-weighted concentrations of total mercury (THg) at the Everglades National Park (ENP or Park) Baird Research Center site were similar in WY2005 and WY2004; concentrations declined at the Florida Power and Light's Andytown substation in 2004. Based on an average deposition rate measured at the ENP and Andytown sites, wet-only atmospheric loading of THg to the Everglades Protection Area was estimated at 172 kg (or 0.47 kg per day) in 2004. Although efforts were made to adjust for missing data at the STA-1W site, this deposition estimate is lower than previous years, especially the sudden, and apparently transient, increase in WY2004. However, a seasonal Kendall analysis failed to show any significant long-term trends in the wet-only deposition.
2. The maximum THg concentration observed at non-Everglades Construction Project water control structures was 28 ng/L observed at S-5A during the third quarter of WY2005. This value exceeds the Florida Class III water quality standard of 12 ng THg/L; however, it should be noted that the analytical laboratory reported that the sample contained a significant amount of suspended particulate matter. The maximum water-column methylmercury (MeHg) concentration at a non-ECP structure was 0.63 ng/L, which occurred at S-141 during the first

quarter. Currently, Florida has no Class III numerical water quality standard for MeHg. After more than seven years of monitoring, little indication of statistically significant temporal trends have been found in either THg or MeHg concentration (or percent MeHg) at any of the individual structures.

3. Mosquitofish (*Gambusia holbrooki*) collected from downstream marsh sites had Hg levels ranging from 13 ng/g to 85 ng/g and had an average basinwide concentration of 46 ng/g. This represents a 22 percent increase from the basinwide mean concentration in WY2004.

4. Sunfish (*Lepomis* spp.) collected from downstream sites had Hg levels ranging from 7 ng/g to 1,500 ng/g. The basinwide average concentration in sunfish was 160 ng/g, representing a 5 percent decrease from the previous year. Although resident fish in the northern ENP (i.e., site L-67F1) continue to have the highest Hg burdens, the pattern of progressively increasing Hg in sunfish over the past few years at both CA3F1 and Holey Land WMA is of concern.

5. Fillets from individual largemouth bass (*Micropterus salmoides*) collected from downstream sites had tissue-Hg concentrations ranging from 89 ng/g to 2,800 ng/g. Site-specific, age-standardized concentrations (estimated for a three-year-old bass) ranged from 230 ng/g to 1,190 ng/g. In WY2005, levels remained stable or declined in fish at several sites, including site CA315, the former MeHg "hotspot" at Water Conservation Area 3. The factor(s) responsible for this decline are presently uncertain, but do not appear to be linked to fluctuations in atmospheric deposition. Bass from two sites, CA3F1 and Holey Land Wildlife Management Area, have shown progressively increasing Hg levels over the past few years. Based on the U.S. Fish and Wildlife Service and the U.S. Environmental Protection Agency guidance on Hg concentrations in fish, localized populations of fish-eating avian and mammalian wildlife continue to be at some risk from adverse effects due to mercury exposure, depending on the foraging area.

6. Due to unfavorable conditions, great egrets (*Ardea alba*) initiated fewer than normal nests in 2005; nest initiation also tended to be later in the season. Consequently, the few feather samples that we were able to collect for Hg analysis will be reported on in the next annual report.

INTRODUCTION

This appendix is the annual permit compliance report for Water Year 2005 (WY2005) (May 1, 2004 through April 30, 2005), summarizing results of monitoring mercury in the downstream receiving waters of the Everglades Protection Area (EPA). This report satisfies the mercury-related reporting requirements of the Florida Department of Environmental Protection (FDEP) Everglades Forever Act (EFA) permits [Chapter 373.4592, Florida Statutes (F.S.)], including permits for Stormwater Treatment Areas 1 West, 2, 3/4, 5, and 6 (STA-1W, STA-2, STA-3/4, STA-5, and STA-6) (Nos. 503074709, 0126704, 192895, 0131842, and 2629183090, respectively). This report includes the monitoring results in Water Year 2005 (WY2005) (May 1, 2004 through April 30, 2005). The results of monitoring mercury within the STAs are presented separately in Appendix 4-4 of the *2006 South Florida Environmental Report – Volume I* (2006 SFER).

Following this introduction, this report consists of three main sections including (1) background, (2) summary of the Mercury Monitoring and Reporting Program, and (3) monitoring results. The background section briefly summarizes the operation of the STAs and discusses their possible impact on South Florida's mercury problem. The next section summarizes sampling and reporting requirements of the Mercury Monitoring Program. Monitoring results are then summarized and discussed. Recent results from the Mercury Monitoring and Reporting Program describe significant spatial distributions and, in some instances, among-year differences in mercury concentrations.

BACKGROUND

In 1994, the Florida legislature enacted the EFA (Chapter 373.4592, F.S.) that established long-term water quality goals for the restoration and protection of the Everglades. To achieve these goals, the South Florida Water Management District (SFWMD or District) implemented the Everglades Construction Plan (ECP). A crucial element of the ECP was the construction of six wetlands, termed STAs, to reduce phosphorus loading in runoff from the Everglades Agricultural Area (EAA). These STAs were to be built on formerly cultivated lands within the EAA and total over 20,000 hectares. The downstream receiving waters to be restored and protected by the ECP include the SFWMD's water management canals of the Central and Southern Florida (C&SF) Project and the interior marshes of the EPA. The EPA comprises several defined regions: the Arthur R. Marshall Loxahatchee National Wildlife Refuge, which contains Water Conservation Area 1 (WCA-1); Water Conservation Areas 2A and 2B (WCA-2A and 2B); Water Conservation Areas 3A and 3B (WCA-3A and 3B); and Everglades National Park (Park or ENP).

However, concerns were raised that in reducing downstream eutrophication, this restoration effort might inadvertently worsen the Everglades mercury problem (Mercury Technical Committee, 1991). Widespread elevated concentrations of mercury were first discovered in freshwater fish from the Everglades in 1989 (Ware et al., 1990). Mercury is a persistent, bioaccumulative, toxic pollutant that can build up in the food chain to levels harmful to human and ecosystem health. Based on mercury levels observed in 1989, state fish consumption advisories were issued for select species and locations [Florida Department of Health and Rehabilitative Services and Florida Game and Fresh Water Fish Commission (currently known as the Florida Fish and Wildlife Conservation Commission, or FWC), March 6, 1989]. Subsequently, elevated concentrations of mercury have also been found in predators, such as raccoons, alligators, Florida panthers, and wading birds (Fink et al., 1999).

A key to understanding the Everglades mercury problem is recognizing that it is primarily a methylmercury (MeHg) problem, not an inorganic mercury or elemental mercury problem. MeHg is the more toxic and bioaccumulative form of mercury. Elsewhere, industrial discharge or mine runoff (e.g., chlor-alkali plant in Lavaca Bay, Texas, Idrija Mercury Mine in Slovenia, or New Idria Mine in California) can contain total mercury (THg) concentrations much greater (in some areas three-hundredfold higher) than found in the Everglades but, at the same time, have lower MeHg concentrations. In the Everglades, atmospheric loading has been found to be the dominant, proximate source of inorganic mercury, with the ultimate source likely being coal-fired utility boilers (far-field) and municipal and medical waste incinerators (for review, see Atkeson and Parks, 2002). After deposition, a portion of this inorganic mercury is then converted to MeHg by sulfate-reducing bacteria (SRB) in the sediments of aquatic systems. A significant part of the local mercury problem is that this methylation process is extraordinarily effective in the Everglades, possibly due to the availability of sulfate (for review, see Gilmour and Krabbenhoft, 2001; Renner, 2001; Bates et al., 2002).

To provide assurance that the ECP was not exacerbating the mercury problem, construction and operating permits for the STAs, issued by the FDEP, required that the SFWMD monitor the levels of THg and MeHg in various abiotic (e.g., water and sediment) and biotic (e.g., fish and bird tissues) media, both within the STAs (for details, see Appendix 4-4 of this volume) and within the downstream receiving waters.

SUMMARY OF THE MERCURY MONITORING AND REPORTING PROGRAM

PRE-OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

Levels of THg and MeHg in various compartments (i.e., media) of the downstream receiving waters collected prior to the operation of the first STA define the baseline conditions from which to evaluate the mercury-related changes, if any, associated with the STA operation. The pre-ECP mercury baseline conditions are defined in the Everglades Mercury Background Report, which summarizes all the relevant mercury studies conducted in the Everglades through July 1997, during the construction of, but prior to, the operation of the first STA. Originally prepared for submittal in February 1998, it has now been revised to include the most recent data released by the U.S. Environmental Protection Agency (USEPA) and the U.S. Geological Survey (USGS) and was submitted in February 1999 (FTN Associates, 1999).

OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

The downstream system is monitored to track changes in mercury concentrations over space and time in response to the changes in hydrology and water quality associated with the ECP (for site locations, see **Figures 1** through **4**).

MERCURY DEPOSITION NETWORK

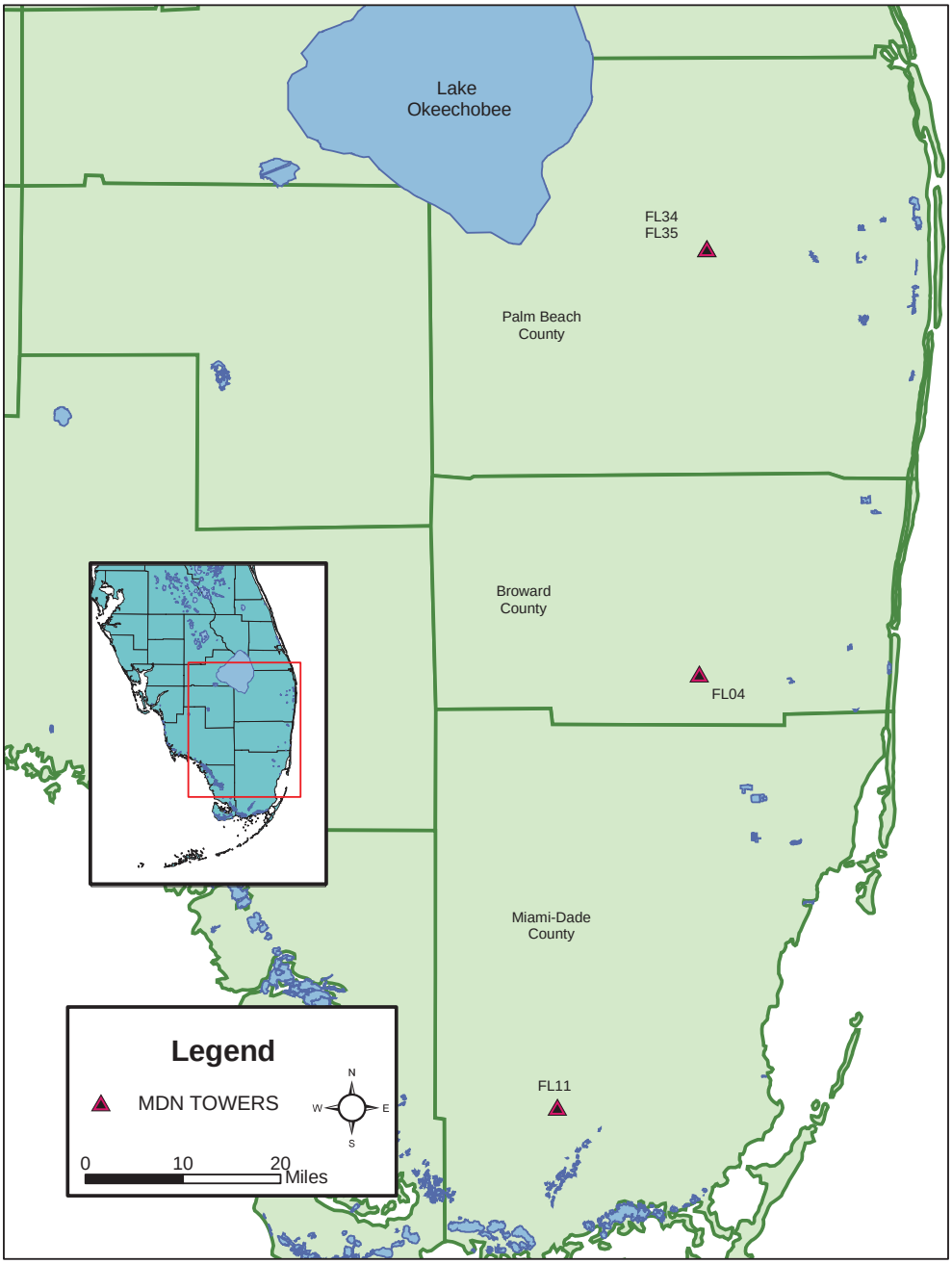

Figure 1. Map showing mercury deposition monitoring sites.

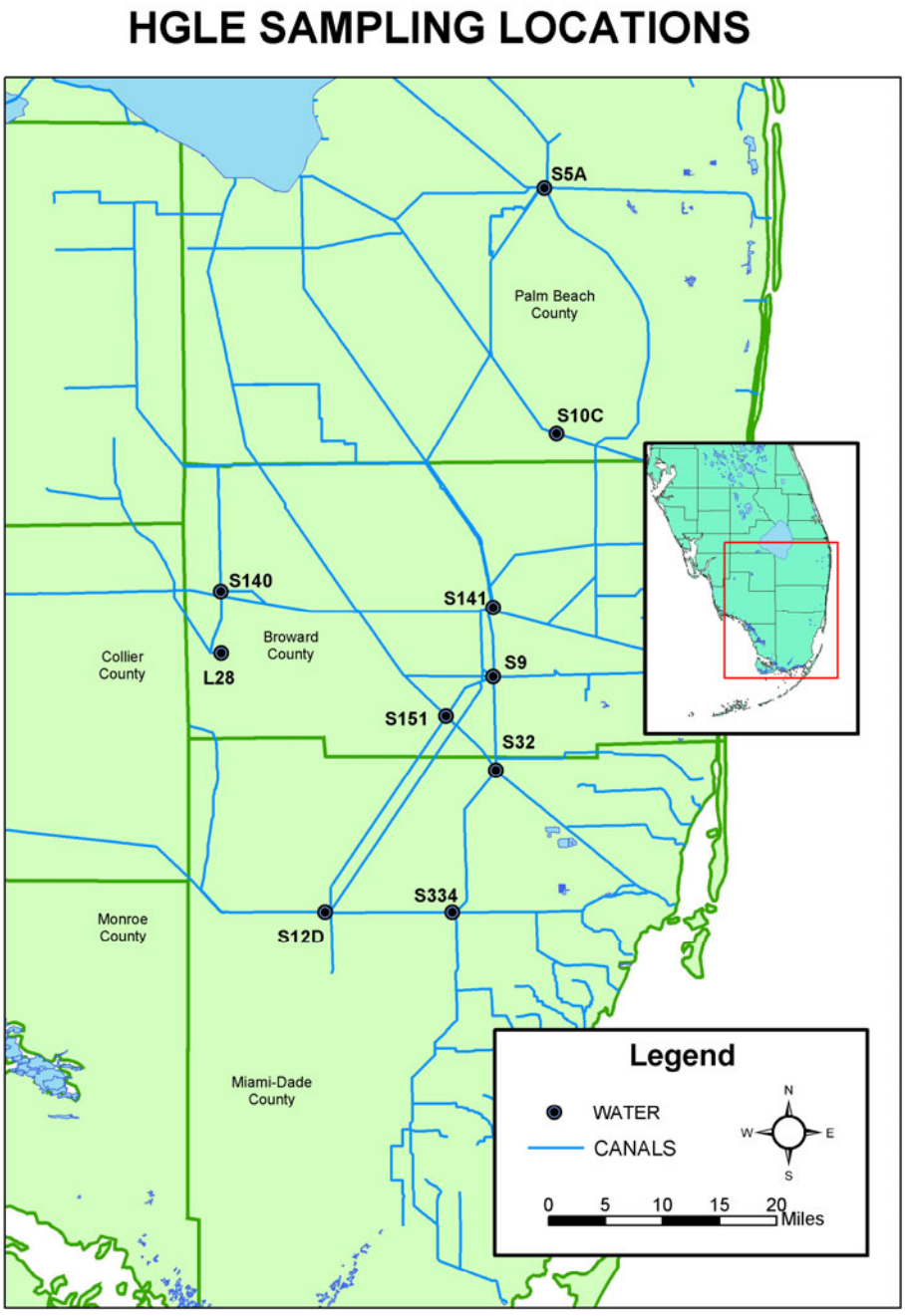

Figure 2. Map showing non-ECP structures where unfiltered surface water is collected quarterly to monitor concentrations of total mercury (THg) and methylmercury (MeHg).

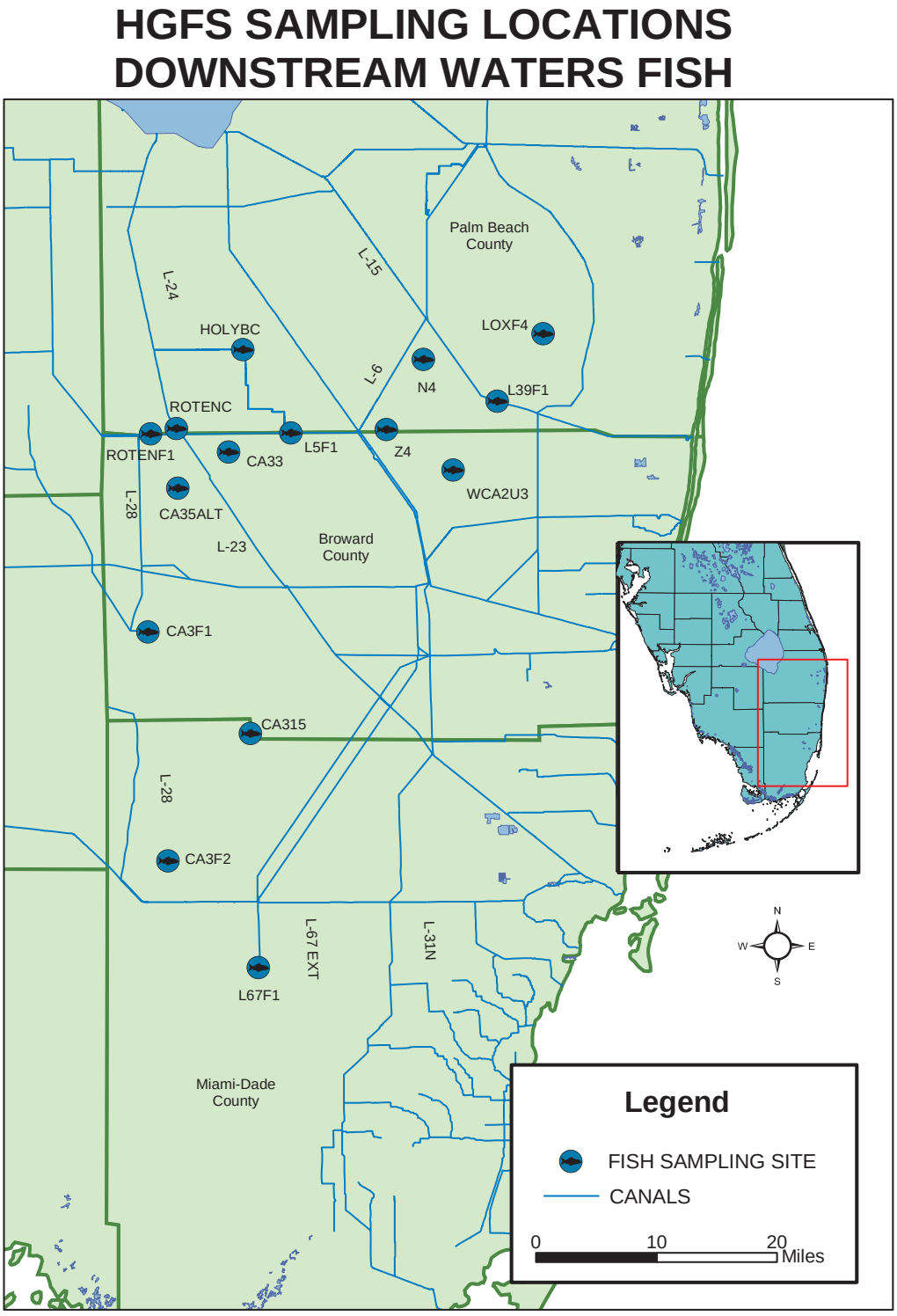

Figure 3. Map showing collection sites for monitoring Hg levels in mosquitofish, sunfish, and largemouth bass.

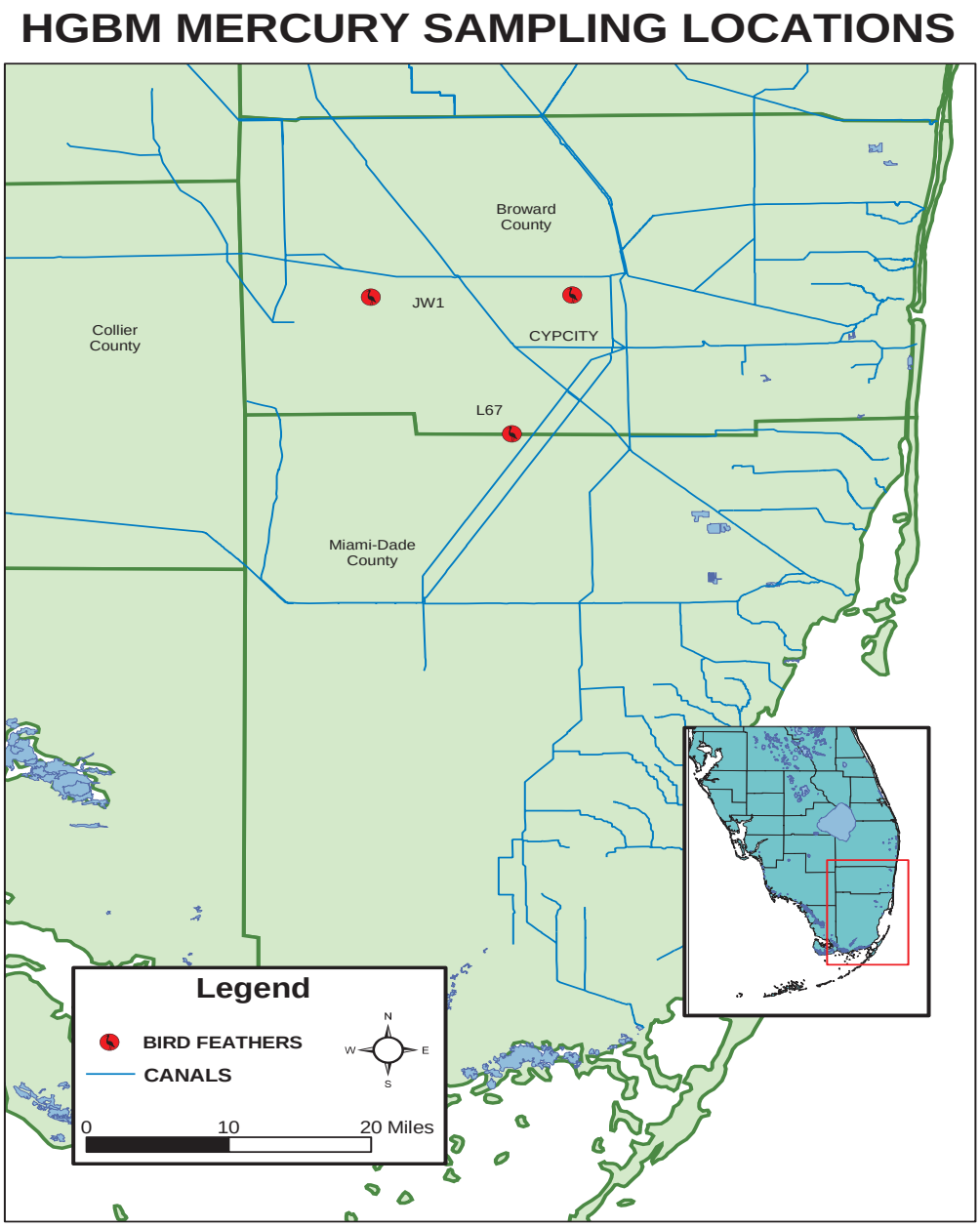

Figure 4. Map showing colonies where great egret nestling feathers are collected.

Rainfall

From 1992–1996, the District, the FDEP, the USEPA, and a consortium of southeastern U.S. power companies sponsored the Florida Atmospheric Mercury Study (FAMS). The FAMS results, in comparison with monitoring of surface water inputs to the Everglades, showed that greater than 95 percent of the annual mercury budget came from rainfall. As such, it was clear that the major source of mercury to the Everglades was from the atmosphere. Accordingly, the District continues to monitor atmospheric wet deposition of THg to the Everglades by participating in the National Atmospheric Deposition Program's (NADP) Mercury Deposition Network (MDN). Following MDN protocols, bulk rainfall samples were collected weekly at the top of 48-foot towers located at the Everglades Nutrient Removal (ENR) Project, at the Andytown substation of Florida Power and Light (I-75/U.S. 27), and the ENP (for map, see **Figure 1**). These samples were analyzed for THg.

Surface Water

Unfiltered grab samples of surface water were collected quarterly using an ultraclean technique upstream of structures S-5A, S-9, S-10C, S-12D, S-140, S-141, S-151, S-190/L-28 interceptor; although not identified in the Non-ECP permit Section 11 a.i., samples were also collected at S-32 and S-334 (see **Figure 2**). These samples were analyzed for THg and MeHg.

Preyfish

Using a dip-net, a grab sample of between 100 and 250 mosquitofish (*Gambusia* sp.) was collected during a single sampling event at 12 downstream interior marsh sites (see **Figure 3**). Fishes were homogenized, the homogenate was subsampled in triplicate, and each subsample was analyzed for THg. (Note: On March 5, 2002, the FDEP approved a reduction in the number of replicate analyses of the homogenate from five to three; correspondence from F. Nearhoof, FDEP.) This species was selected as a representative indicator of short-term, localized changes in water quality because of its small range, short lifespan, and widespread occurrence in the Everglades. Mosquitofish become sexually mature in approximately 3 weeks and have an average lifespan of only 4 to 5 months (though some individual females may live up to 1.5 years); the lifespan of males is shorter than females (Haake and Dean, 1983; Haynes and Cashner, 1995; Cabral and Marques, 1999).

Secondary Predator Fish

Using electroshocking techniques, up to 20 sunfish (*Lepomis* sp.) were collected at 12 downstream interior marsh sites (see **Figure 3**). Each whole fish was analyzed for THg. Because of their widespread occurrence, and because they are a preferred prey for a number of fish-eating Everglades species, sunfish were selected as an indicator of mercury exposure to wading birds and other fish-eating wildlife. Sunfish are thought to have an average lifespan of 4 to 7 years in the wild.

Top-predator Fish

Using electroshocking techniques, up to 20 largemouth bass (*Micropterus salmoides*) were also collected at these 12 downstream interior marsh sites (see **Figure 3**), and fillets analyzed for THg. Largemouth bass, which are also long-lived (oldest bass collected as part of this effort was

9 years old), were selected both as an indicator of potential human exposure to mercury and because this species has been monitored at several Everglades sites since 1989.

It should be recognized that tissue-concentrations in each of the three monitored fish species will reflect ambient MeHg levels, i.e., integrate exposure as a function of a combination of factors including body size, age, rate of population turnover, and trophic position. Mosquitofish should respond rapidly to changing ambient MeHg concentrations due to their small size, lower trophic status, short life span, and rapid population turnover. Alternatively, owing to their specific life history characteristics, sunfish and bass should take a greater amount of time to respond, in terms of tissue concentrations, to changes in ambient MeHg availability. Most importantly, they represent exposure at higher trophic levels (TLs) with a requisite time lag for trophic exchange. Furthermore, the focus here on a three-year-old bass, while appropriate to assess exposure to fishermen, complicates interpretation because its tissue concentration will reflect integration over a three-year period. The key is to use these species-related differences to better assess MeHg availability within the system.

It is important to note that virtually all (i.e., greater than 85 percent) of the mercury in muscle tissue of fish is present in the methylated form (Grieb et al., 1990; Bloom, 1992; SFWMD, unpublished data). Therefore, the analysis of fish tissue for THg, which is a more straightforward and less costly procedure than for MeHg, can be interpreted as being equivalent to the analysis of MeHg.

Feathers

To monitor temporal trends in Hg bioaccumulation in fish-eating wildlife, the District collects feathers from great egret (*Ardea alba*) nestlings and compares the results to results from similar collections made in 1994 and 1995 by Frederick et al. (1997; later published by Sepulveda et al., 1999). In accordance with USACE permit 199404532, Condition 8b.2, the results of the 1994 and 1995 collections were found to be representative of background mercury concentrations in Everglades wading birds (FTN Associates, 1999). The survey by Frederick et al. (1997) involved collecting and analyzing THg in feathers of the great egret nestlings at various Everglades colonies. The District's monitoring program has focused on two egret colonies, designated as JW1 and L67, which are located in WCA-3A (**Figure 4**). These two colonies consistently showed the highest THg concentrations during background studies (Frederick et al., 1997; FTN Associates, 1999; Sepulveda et al., 1999). However, nesting at the JW1 colony has been erratic in recent years and consequently, samples have been collected from another nearby colony – designated Cypress City (**Figure 4**). Feathers are collected (for THg analysis) from the oldest nestling in 10 nests in each of the two different nesting colonies, under appropriate state and federal permits. It should be noted that this is a modification from the sampling scheme initially proposed, which would have involved collecting molted feathers from post-breeding adults at or in the immediate vicinity of nests or from feathers found at STAs. This modified sampling design is more consistent with protocols used in the collection of background data (Frederick et al., 1997). In previous years, the District also collected egret eggs from these colonies to support validation of exposure models and formal risk assessments. Because it was not mandated by permit and because it was not deemed a high priority, egg collections were discontinued in 2004.

In addition to the monitoring program described above, in accordance with Condition 4.iv of the Mercury Monitoring Program, the District is required to "report changes in wading bird habitat and foraging patterns using data collected in ongoing studies conducted by the permittee and other agencies."

Further details regarding rationales for sampling scheme, procedures, and data reporting requirements can be found in the District's Everglades Mercury Monitoring Plan revised in March 1999 (Appendix 1 of the Quality Assurance Protection Plan, June 7, 1999).

QUALITY ASSESSMENT FOR THE MERCURY MONITORING PROGRAM

The following section is a quality assessment of the District's Mercury Monitoring Program during WY2005 and, where appropriate, evaluates the data quality in terms of accuracy, precision, and completeness. This assessment is based on data quality objectives contained in the District's Quality Assurance Project Plan (QAPP) for the Mercury Monitoring and Reporting Program which was approved on issuance of the permit by the FDEP on June 7, 1999.

Quality assurance (QA) and quality control (QC) are integral parts of all monitoring programs. A stringent QA/QC program is especially critical when dealing with ultra-trace concentrations of analytes in natural and human-impacted environments. Quality assurance includes design, planning, and management activities conducted prior to implementation of the project to ensure that the appropriate kinds and quantities of data will be collected with the required representativeness, accuracy, precision, reliability, and completeness. The goals of QA are to ensure the following: (1) standard collection, processing, and analysis techniques will be applied consistently and correctly; (2) the number of lost, damaged, and uncollected samples will be minimized; (3) the integrity of the data will be maintained and documented from sample collection to entry into the data record; and (4) data are usable based on project objectives. During WY2004, the level of QA monitoring was increased. This enhanced process, in conjunction with a more timely feedback mechanism to communicate any problems to the field sampling teams, laboratories, QA program personnel, and data validators, helped in improving the overall quality of the monitoring program.

QC measures are incorporated during the sample collection and laboratory analysis to evaluate the quality of the data. QC measures give an indication of measurement error and bias (or accuracy and precision). Aside from using these results as an indication of data quality, an effective QA program must utilize these QC results to determine areas of improvement and implement corrective measures. QC measures include both internal and external checks. Typical internal QC checks include replicate measurements, internal test samples, method validation, blanks, and use of standard reference materials. Typical external QC checks include split and blind studies, independent performance audits, and periodic proficiency examinations. Because mercury-related degradation of water quality is being defined in this project relative to baseline data that was generated by one or more laboratories, data comparability is a primary concern. It is important to establish and maintain comparability of performance and results among participating laboratories, assessing the reporting units and calculations, database management processes, and interpretative procedures. This comparability of laboratory performance must be ensured if the overall goals of the project are to be realized.

Laboratory Quality Control

Data for this program were generated by FDEP and Frontier Geosciences, Inc. (FGS) laboratories (FDEP being the primary lab and FGS the secondary), both of which are certified by the Florida Department of Health under the National Environmental Laboratory Accreditation Program (NELAP). The following methods were utilized when analyzing samples for total

mercury (THg) and methylmercury (MeHg) during WY2005: USEPA Method 1631E (Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry), USEPA Draft Method 1630 (Methylmercury in Water and Tissues by Distillation, Extraction, Aqueous Phase Ethylation, Purge and Trap, Isothermal GC Separation, Cold Vapor Atomic Fluorescence Spectrometry), USEPA Method 245.5 (Mercury in Sediment by Cold Vapor AAS), USEPA Method 245.6 (Mercury in Tissues by Cold Vapor AAS), and USEPA Method 245.7 (Mercury-CVA Fluorescence spectrometry), all of which are performance-based standards employing the appropriate levels of QA/QC required by National Environmental Laboratory Accredidation Conference (NELAC), the specific reference method, and the mercury program. Methods used by both FDEP and FGS had some level of variance from the approved reference method, but both laboratories had satisfied the requirements to show acceptability of these variances and had sought the proper approvals from FDEP and NELAC-accrediting authorities.

Field Quality Control Samples

A total of 410 field QC samples, including field kit prep blanks (FKPB), equipment blanks – both laboratory-cleaned equipment blanks (EB) and field-cleaned equipment blanks (FCEB), replicate samples (RS) and split samples (SS), were collected for both THg and MeHg (both filtered and unfiltered) surface water samples at STA-1W, STA-2, STA-3/4, STA-5, STA-6, non-ECP structures, and at pre-operational STA-1E during WY2005. An FKPB is a sample of the deionized distilled water (DDW) sent as blank water for field QC that remains at the lab to monitor low-level background inorganic Hg contamination of the laboratory DDW system, which can vary over time. An EB is collected at the beginning of every sampling event, and an FCEB is collected at the end of the event. Because field blanks (FBs) added little value to the assessment of data quality and because it was no longer a requirement, FDEP FBs were eliminated in WY2003. Field QC check samples represented approximately 32 percent of the 1,291 water samples collected during this reporting period. The results of the field QC blanks are summarized in **Table 1**.

Analytical and Field Sampling Precision

Field replicates are samples that have been collected simultaneously or in rapid succession from the same site. Laboratory replicates are aliquots of the same sample that are prepared and analyzed within the same run.

WATER SAMPLES

To assess the precision of field collection and analysis, 23 replicate samples collected at STA-1W, STA-2, STA-3/4, STA-5, STA-6, and non-ECP structures were processed during the course of WY2005. **Table 2** reflects the results of the sample analyses.

MOSQUITOFISH COMPOSITE SAMPLES

To monitor spatial and temporal patterns in mercury residues in small-bodied fishes, individual mosquitofish (100–250 individual fish) were collected at various locations in the STAs, ECP, and non-ECP marshes. These individuals were then composited for each site. Composite sampling can increase sensitivity by increasing the amount of material available for analysis, reduce inter-sample variance effects, and dramatically reduce analytical costs. However, there are disadvantages to composite sampling. Subsampling from a composite introduces uncertainty if homogenization is incomplete. Since 1999, the District has used a Polytron®

homogenizer to homogenate composited mosquitofish. Until late 2001, the homogenate was subsampled in quintuplicate, and each subsample analyzed for THg. Based on the apparent degree of homogenization as evidenced by the low relative standard deviation (RSD) among aliquots reported in the 2002 Everglades Consolidated Report, the District revised its Standard Operation Procedure (SOP) after consultation with and approval from the FDEP, reducing subsampling of the homogenate from five to three. Laboratory replicates of mosquitofish were processed by the analytical laboratories and analyzed for THg. For WY2005, the mean RSD in THg concentrations among the 96 composite triplicate aliquots was 4.8 percent (**Table 2**).

Table 1. Frequency of occurrence and mean concentration (ng/L) of THg and MeHg results from filtered and unfiltered FQC blanks from STA-1, STA-2, STA-3/4, STA-5, STA-6, and non-ECP structures/area surface water samples. Detection limits are 0.1 ng THg/L and 0.022 ng MeHg/L.

	THg							MeHg					
FQC[1]	n[2]	Collection Frequency %	n >MDL	Mean ng/L[3]	n V[4] Flagged	% Flagged		n[2]	Collection Frequency %	n >MDL	Mean ng/L[3]	n V[4] Flagged	% Flagged
FKPB	53	3.8	2	0.275	0	0		49	2.0	1	0.040	0	0
EB	83	10.8	9	0.476	4	6.0		79	11.4	9	0.031	1	3.8
FCEB	75	9.3	7	0.356	3	6.7		71	12.7	9	0.030	1	2.8

[1]FKPB-Field kit preparation blank, EB-Lab-cleaned equipment blank, FCEB-Field-cleaned equipment blank collected at the end of the sampling event.
[2]Total number (n) of surface water samples collected during WY2005 was 368 THg and 356 MeHg.
[3]Mean concentration of contaminated QC samples
[4]Analyte was detected in the blank.

Table 2. Precision among replicate unfiltered surface water samples and mosquitofish collected at STA-1, STA-2, STA-3/4, STA-5, STA-6, and non-ECP structures.

		Precision (% RSD)			
Analyte	**n**	**Minimum**	**Maximum**	**Mean**	**Median**
Surface Water THg	37	0	69.7	12.4	6.3
Surface Water MeHg	36	0	31.6*	6.5	4.7
Mosquitofish THg	96	0	12.5	4.8	4.3

*Sample result less than PQL-associated data not flagged. *Sample result less than PQL-associated data not flagged.

Another disadvantage to composite sampling is that the same amount of information is not generated as when samples are analyzed individually. Because samples are physically averaged, no variance estimate for the population is generated and consequently, uncertainty is introduced regarding the representativeness of the sample in describing the population. This also hampers statistical comparisons. To assess the representativeness of composite samples, five field duplicate (FD) mosquitofish composites were collected during WY2005 (i.e., a second set of 100–250 individuals were collected at the sites and composited as a second sample). Unlike abiotic media that may change little over the time period of replicate sample collection, dip-netting mosquitofish likely disperses the local population. Consequently, the resampled population may not represent a true replicate of the first sample. The mean relative percent difference (RPD) between aliquot means (of FD composite samples) was 56 percent, ranging from 3–93 percent. This variability seems elevated from previous years and is under investigation.

Interlaboratory Comparability Studies

To ensure further comparability (i.e., reproducibility) between ongoing mercury sampling initiatives, split samples of surface water, fish, and sediment are routinely submitted on an annual basis to a second laboratory for independent analysis of THg and MeHg.

SURFACE WATER

No surface water splits were done in WY2005. However, FDEP performed satisfactorily in a recent round-robin (i.e., inter-laboratory comparison study) involving 11 laboratories analyzing ambient surface water samples from Florida (for details, see Niu and Tintle, 2005). On a scale of 0 to 5, with 5 as the best, FDEP ranked 3.67 for both THg and MeHg determination; FGS ranked 3.33 for THg and 4.33 for MeHg. It should be noted that Battelle Marine Science Laboratory, which was recently contracted by the District as the secondary mercury laboratory, ranked 4.33 for THg and 4.0 for MeHg.

FISH

Five mosquitofish composites collected during WY2005 were sent to FGS for independent analysis. THg concentration (average of triplicate aliquots) ranged from 0.007 mg/kg to 0.037 mg/kg. The RPD between aliquot means was 27.6 percent and 200 percent. Although one paired sampled was problematic (as evident by the 200 percent RPD), a signed rank test found no consistent bias in the small dataset ($W = 15$, $p = 0.63$).

One hundred and forty six large-bodied fish species (i.e., whole sunfish homogenates and fillets of largemouth bass) collected during WY2005 were also sent to the secondary laboratory (FGS, Inc.) for independent analysis. The analytical range of concentration for THg was from 0.01 mg/kg to 1.2 mg/kg. A signed rank test found significant differences between the two labs for Hg levels reported in paired fish ($W = 5735$, $p < 0.0001$); with FGS biased slightly high (**Figure 5**). It should also be noted that discrepancies were found in the values in hard-copy and electronic reports from FGS; this decrepancy is under investigation.

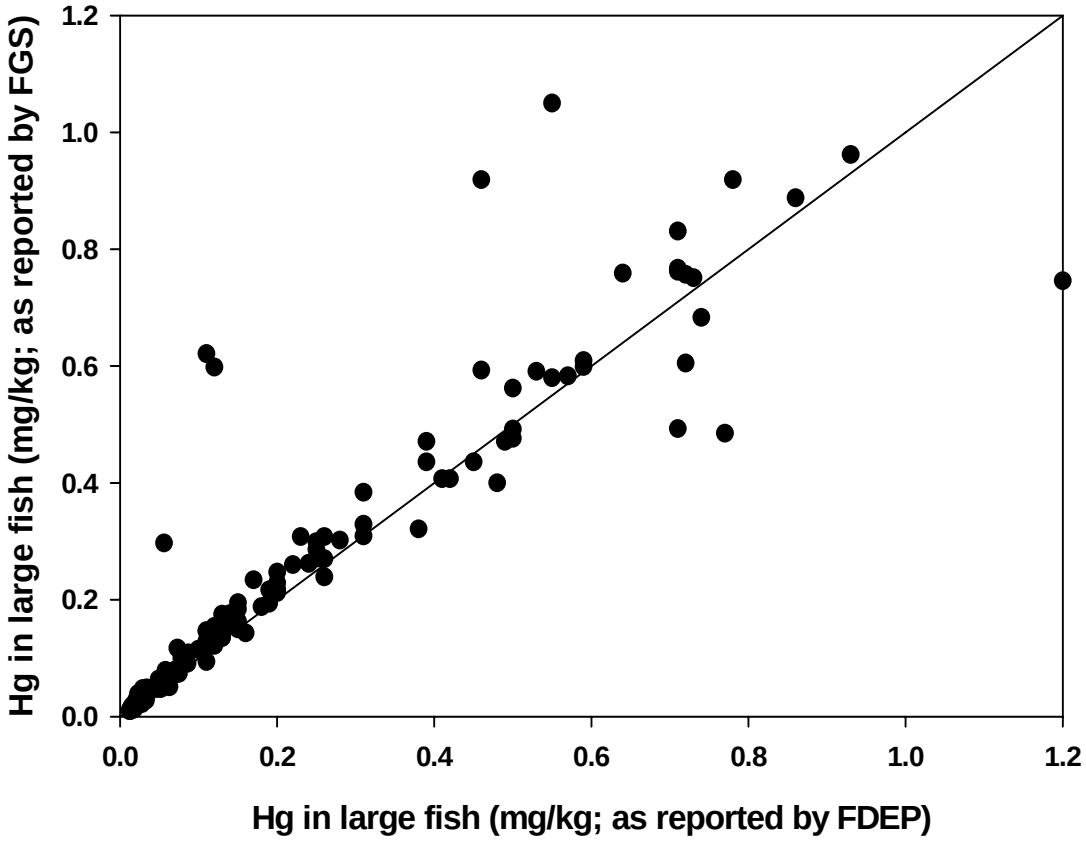

Figure 5. Interlaboratory comparison of THg determination in tissues of large-bodied fish.

SEDIMENT

FGS has been the District's primary laboratory for the analysis of sediments for mercury over the past nine years, principally due to its lower method detection limit (MDL) for methylmercury (MeHg). During WY2005, an inter-laboratory comparison study was carried out to evaluate FDEP's recent efforts to modify its analytical methods and reduce its MDL for MeHg in sediments. Prior to reviewing the results of this study, it should be recognized that, unlike the established (and in some cases, codified) protocols for determining THg or MeHg in water (identified above), considerable variability exists in the analytical methods for leaching (dissolution) and extraction of MeHg from sediments. These methods include but are not limited to: (1) distillation, (2) KOH (alkaline) leaching and CH_2Cl_2 (methylene chloride) extraction, (3) KOH leaching and methanol extraction, (4) $H_2SO_4/KBr/CuSO_4$ or (5) $KBr/CuSO_4$ leaching and CH_2Cl_2 extraction, or (6) $HNO_3/CuSO_4$ leaching and CH_2Cl_2 extraction. It should also be noted that there has been considerable debate in the published literature recently regarding potential artifact formation when employing these various methods, i.e., transformation of Hg^{2+} to MeHg (Bloom et al., 1997; Quevauviller and Horvat, 1999; Liang et al., 2004). Uncertainties regarding possible widespread inaccuracy in MeHg determination has led some to even suggest that certification of all current reference materials (i.e., CRMs) for sediments be revoked (Bloom et al., 1999). Alternatively, Liang et al. (2004) report artifact formation was not a problem at concentrations less than 2,000 ng THg/g, but did find poor recoveries of MeHg in a number of the current methods. Consequently, selecting a laboratory for MeHg determination in sediments may not be a simple process.

Thirty-two sediment samples collected from the EAA Project in late 2004 to early 2005 (i.e., Cross and Bolles canal, bioaccumulation tests) were split and sent to both FGS and FDEP for determination of THg and MeHg. Although reported values varied between labs (RPD as high as 93 percent), this was not unexpected for heterogeneous matrix such as sediment. There was no statistically significant (consistent) bias in THg concentrations in the paired splits (paired t-test; $df = 31$, $t = -1.113$, $p = 0.27$). The average difference in reported value for THg between the two labs was 0.005375 mg/kg. Results of inter-laboratory comparisons in MeHg determinations were very different from that of THg. On average the value reported by FGS was 153 percent higher than FDEP's reported value; this ranged as high as 722 percent (i.e., 0.37 vs. 0.045 ng MeHg/g). This consistent bias was statistically significant (Signed Rank Test; $W = -518$, $T+ = 5$, $T-=-523$, $p < 0.001$). Although less critical than the bias issue, it should be noted that FDEP reported 8 of the 32 sediment samples (25 percent) as below their MDL. With only two labs participating in the study, it is not possible to determine which is more accurate. It is also interesting to note that Bloom et al. (1997) reported the KOH/Methanol protocol used by FDEP to have a high potential for artifact formation, especially over the $KBr/CuSO_4/CH_2Cl_2$ protocol used by FGS. If their assessment was correct, FDEP should then be biased high for MeHg not low as compared to FGS.

In May 2005, another interlaboratory comparison was initiated with three labs participating to assess both THg and MeHg analysis of sediments; results will be reported next year.

Statistical Methods

Temporal trends in atmospheric THg deposition and water column THg and MeHg concentrations were evaluated using the seasonal Kendall test (SAS; for macro, see USEPA, 1993), which is a generalization of the Mann-Kendall sum test for trend detection (Gilbert, 1987). The test is applied to datasets exhibiting seasonality, and may be used even though there are missing, tied, or non-detect values. The validity of the test does not depend on the data being

normally distributed. However, use of this analysis presupposes the presence of large multiyear, multi-season datasets. It is argued that five years is a minimum dataset for proper use of both the test and standard statistical tables; consequently, the application of this test on quarterly data, some of which were unusable do to fatal qualifiers, should be approached cautiously, and results should be viewed as approximations only.

Monitoring Hg concentrations in aquatic animals provides several advantages. However, interpretability of residue levels in animals can sometimes prove problematic due to the confounding influences of the age or species of the collected animal. For comparative purposes, special procedures are used to normalize the data. Standardization to size, age, or lipid content is a common practice (Wren and MacCrimmon, 1986; Hakanson, 1980). To be consistent with the reporting protocol used by the FWC (Lange et al., 1998, 1999), mercury concentrations in largemouth bass were standardized to an expected mean concentration in three-year-old fish (EHg3) at a given site by regressing mercury on age (for details, see Lange et al., 1999). It should be noted that to adjust for the month of collection, otolith ages were first converted to decimal ages using protocols developed by Lange et al. (1999). Because sunfish were not aged, age normalization was not available. Instead, arithmetic means were reported. However, efforts were made to estimate a least square mean (LSM) THg concentration based on the weight of the fish. Additionally, the distribution of the different species of *Lepomis,* including warmouth (*L. gulosus)*, spotted sunfish (*L. punctatus*), bluegill (*L. macrochirus*), and redear sunfish (*L. microlophus*), collected during electroshocking was also considered to be a potential confounding influence on THg concentrations prior to each comparison. To be consistent with the reporting protocol of Frederick et al. (1997; see also Sepulveda et al., 1999), THg concentrations in nestling feathers were similarly standardized for each site and were expressed as LSM for chicks with a 7.1 cm bill.

Where appropriate, an analysis of covariance (ANCOVA; SAS GLM procedure) was used to evaluate spatial and temporal differences in mercury concentrations, with age (largemouth bass), weight (sunfish), or bill size (egret nestlings) as a covariate. However, the use of ANCOVA is predicated on several critical assumptions (for review, see ZAR, 1996), including that regressions are simple linear functions and are statistically significant (i.e., non-zero slopes); that the covariate is a random, fixed variable; that both the dependent variable and residuals are independent and normally distributed; and that slopes of regressions are homogeneous (parallel). Where these assumptions were not met, standard analysis of variance (ANOVA) or Student's t-test (SigmaStat, Jandel Corporation, San Rafael, California) was used; possible covariates were considered separately. The assumptions of normality and equal variance were tested by the Kolmorogov-Smirnov and Levene Median tests, respectively. Datasets that either lacked homogeneity of variance or departed from normal distribution were natural-log transformed and were reanalyzed. If transformed data met the assumptions, then they were used in ANOVA. If the assumptions were not met, then the raw datasets were evaluated using non-parametric Mann-Whitney Rank sum tests. If the multigroup null hypothesis was rejected, then the groups were compared using either Tukey HSD or Dunn's method.

MONITORING RESULTS

RAINFALL: NATIONAL ATMOSPHERIC DEPOSITION PROGRAM, MERCURY DEPOSITION NETWORK

Samples of rainfall were collected weekly under the protocols of the NADP MDN at the ENR Project (i.e., STA-1W), Florida Power and Light's Andytown substation, and the Baird Research Center in ENP (**Figure 1**). For more information on MDN and to retrieve raw data, refer to the NADP's web site, http://nadp.sws.uiuc.edu/mdn/ (available as of July 21, 2005). It should be noted that difficulties were encountered in 2004 due to the landfall of four hurricanes in Florida (especially hurricanes Frances and Jeanne that made landfall on September 8 and 26, respectively). These storms prevented sample collection in two instances and resulted in temporary malfunction of equipment at the ENR site. Consequently, preliminary estimates for both volume-weighted concentration and deposition are considered a gross underestimation at that site.

With this caveat in mind, atmospheric deposition of THg to South Florida continues to be highly variable both spatially and temporally (**Table 3** and **Figures 6** and **7**). As observed in the past, THg concentrations in precipitation were substantially higher during the summer months (**Figure 6**), possibly due to seasonal tall convective thunderclouds that can scavenge particulate Hg, and water soluble reactive gaseous mercury (RGM) from the middle and upper troposphere. This is consistent with observations of Guentzel (1997) during the FAMS. Because both THg concentrations and rainfall volumes generally increase during the summer, THg wet deposition typically peaks in mid-summer (**Figure 6**).

Annual volume-weighted THg concentrations differed among the three sites in 2004 (**Table 3**). Although volume-weighted concentrations were similar at the southern-most site (ENP) in 2004 and 2003, both Andytown and ENR showed a marked decline in 2004 (**Table 3, Figure 7**). With the exception of an unusually elevated concentration at a New Mexico station (27 ng/L in 2003), Florida typically has some of the highest THg concentrations in the MDN (refer to http://nadp.sws.uiuc.edu/mdn/maps/).

A seasonal Kendall analyses (of ranks) revealed no significant trends in monthly median THg concentrations at ENR (1997-2004; n = 84 months; Tau = -0.008; p = 0.96), Andytown (1998–2004; n = 77 months; Tau = -0.033; p = 0.77) or ENP sites (1996–2004; n = 101 months; Tau = 0.07; p = 0.37; S. Hill, SFWMD, personal communication). The finding of no trend was consistent with a recent report by Nilles (2004), which found no trends in volume-weight monthly averages from the three sites in South Florida (i.e., residuals from regression of concentration on precipitation to adjust for "washout").

Wet deposition (wet-only flux), which is a function of both concentration and rainfall, differed among sites in 2004 (**Table 3** and **Figure 7**). The much lower deposition at the ENR site in 2004, relative to other two sites, was a direct result of equipment malfunction and, as a consequence, an underestimation of rainfall by 50 cm (based on results from the nearby District tipping-bucket rain gauge).

Owing to a combination of elevated concentration and the high annual rainfall in South Florida, wet THg deposition flux to the Everglades is substantially greater than most other regions of the MDN (http://nadp.sws.uiuc.edu/mdn/maps). Although deposition was highly variable, seasonal Kendall analysis again failed to show any long-term trends in the monthly

deposition at either ENR (n = 86 months, Tau = -0.068; p = 0.48), Andytown (n = 80, Tau = -0.035; p = 0.75) or ENP (n = 102, Tau = -0.06; p = 0.45; S. Hill, SFWMD, personal communication).

Table 3. THg concentration data (ng/L; wet-only) from the compliance sites of the MDN in calendar year 2004. Note: Annual point estimates are based on calendar year.

Week ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
1/6/2004	N/A	N/A	N/A
1/13/2004	N/A	N/A	N/A
1/21/2004	3.66	N/A	4.82
1/27/2004	34.45	N/A	52.96
2/3/2004	5.71	7.42	8.40
2/10/2004	N/A	2.22	N/A
2/17/2004	14.41	10.50	7.31
2/24/2004	N/A	N/A	48.82
3/2/2004	N/A	4.34	9.72
3/9/2004	N/A	N/A	N/A
3/16/2004	5.07	9.99	7.45
3/23/2004	N/A	N/A	N/A
3/30/2004	13.03	6.51	4.02
4/6/2004	N/A	N/A	N/A
4/13/2004	15.93	18.90	27.01
4/20/2004	6.90	5.08	8.33
4/27/2004	N/A	N/A	N/A
5/4/2004	6.81	8.41	11.54
5/12/2004	N/A	N/A	16.43
5/18/2004	4.98	5.64	6.33
5/25/2004	10.61	2.73	N/A
6/1/2004	34.15	N/A	N/A
6/8/2004	49.02	38.19	34.79
6/15/2004	22.50	27.21	21.02
6/22/2004	23.49	16.75	33.82
6/29/2004	N/A	49.13	N/A
7/6/2004	33.24	N/A	24.42
7/13/2004	20.50	41.85	24.76
7/20/2004	22.24	24.07	16.39
7/27/2004	11.49	16.44	18.23
8/3/2004	13.12	14.74	15.26
8/10/2004	12.54	15.15	7.97
8/17/2004	24.36	42.27	15.83
8/24/2004	26.04	31.75	20.25
8/31/2004	19.81	26.63	22.44
9/7/2004	N/A	N/A	9.07
9/14/2004	N/A	4.47	19.82
9/21/2004	N/A	10.09	15.30
9/29/2004	N/A	5.24	4.92
10/5/2004	8.30	10.90	15.40
10/12/2004	15.10	14.30	6.00
10/19/2004	34.70	13.60	11.10
10/26/2004	23.30	7.40	14.40
11/2/2004	8.40	5.50	9.60

Table 3. Continued.

Week ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
11/9/2004	N/A	15.00	N/A
11/16/2004	4.20	10.90	4.90
11/23/2004	N/A	N/A	N/A
11/30/2004	19.70	13.80	8.70
12/7/2004	N/A	N/A	N/A
12/14/2004	49.20	11.30	N/A
12/20/2004	11.50	66.10	22.50
12/28/2004	15.30	N/A	23.10
Volume-wt. concentration (ng/L)			
1996*			14.1
1997*	18.7	NA	14.7
1998*	11.4	13.8	12.7
1999*	10.8	12.3	11.6
2000*	13.7	15.8	13.6
2001*	13.9	13.2	13.1
2002*	12.3	14.2	12.1
2003*	16.1	16.4	16.4
2004[b]	13.7[a]	15.2	16.5
Deposition Annual (µg/m2)			
1996*			17.2
1997*	32.4	NA	27.2
1998*	26.1	20.1	20.3
1999*	12.1	17.5	17.7
2000*	14.3	18.1	20.0
2001*	21.0	21.1	18.0
2002*	10.3[a]	18.7	18.2
2003*	17.8	28.5	26.8
2004[b]	a	17.7	20.4

* Adapted from NADP / MDN Program Office http://www.frontiergeosciences.com/MDN_Data/

a. Rain gauge malfunction; in 2004, several trips missed due to 4 hurricanes.

b. Preliminary data; final data set may use seasonal averages to estimate annual concentration and deposition where Quality Rating of a given value is C. N/A – not available due to mechanical problems with collector or failure to meet QC criteria.

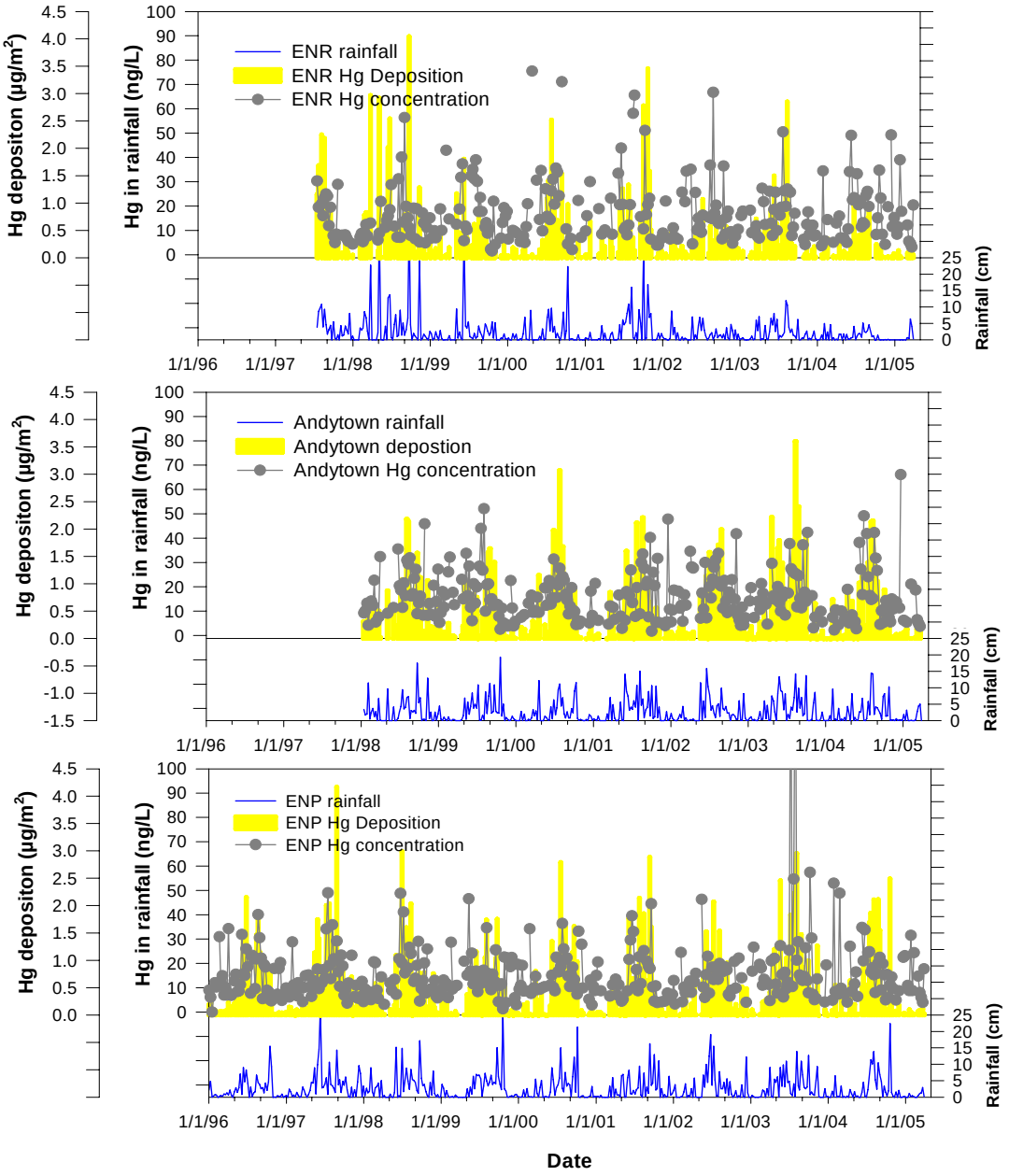

Figure 6. Time series of rainfall, rainfall Hg concentrations, and wet Hg deposition at the ENR Project, Andytown, and ENP Baird Research Center, as reported by the MDN.

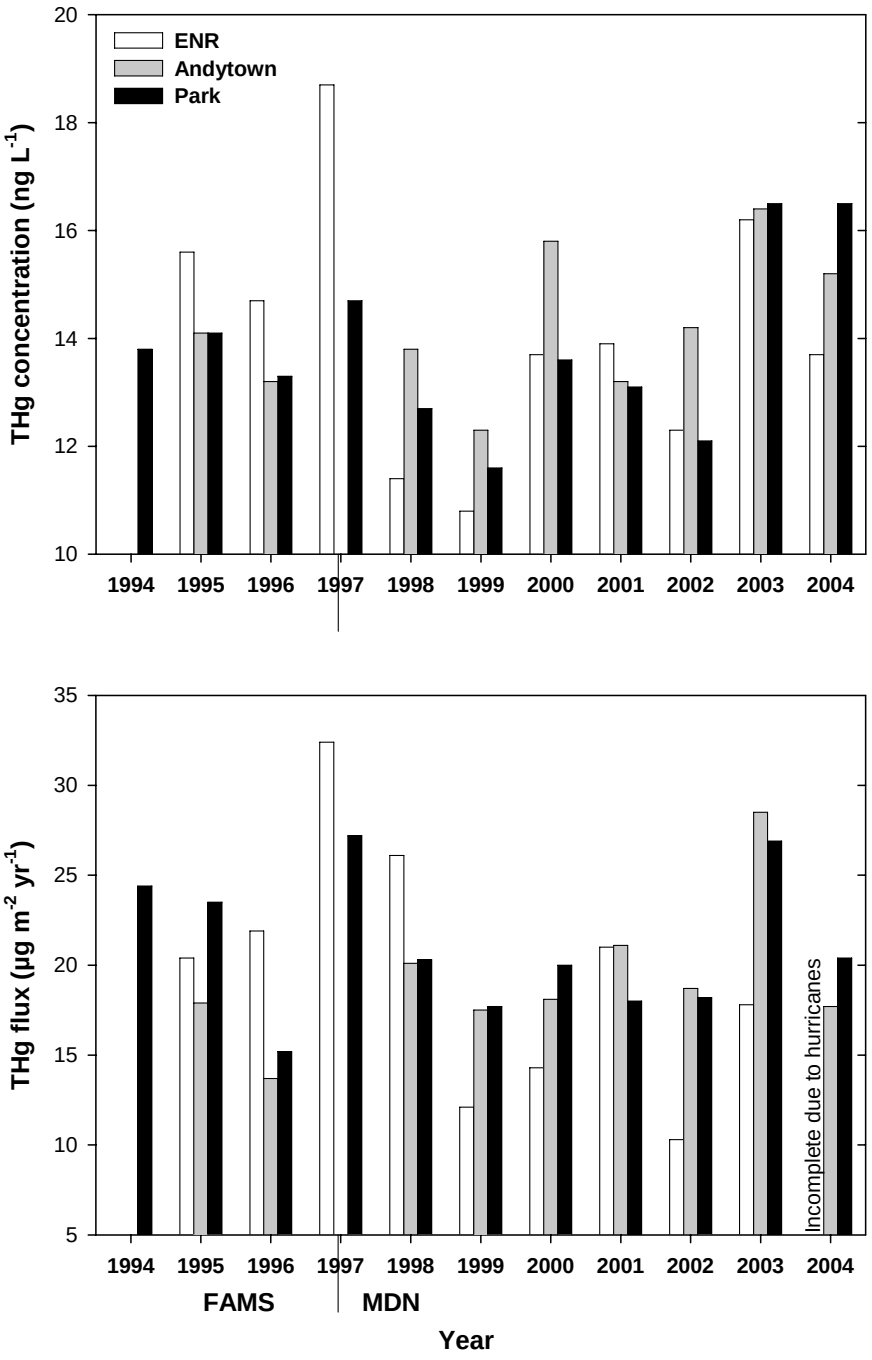

Figure 7. Time series of annual volume-weighted concentration (top) and annual THg flux (bottom) at the three MDN stations (FAMs data from Guentzel et al., 2001).

Based on an average deposition rate measured at Andytown and ENP sites, wet-only atmospheric loading of THg to the EPA (9.01×10^9 m^2) was estimated at 172 kg per year (or 0.47 kg per day) in 2004. Although efforts were made to adjust for missing data at the ENR site, this deposition estimate is lower than previous years (**Table 4**). Nevertheless, the results reported here for wet deposition of THg, along with results of monitoring water-column concentrations at various water control structures (**Table 5**), continue to provide compelling evidence that the major source of mercury to the Everglades is from atmospheric deposition (**Table 4**). This is consistent with previous assessments by both the FDEP (T. Atkeson, available online at http://www.dep.state.fl.us) and the USEPA (USEPA, 1998; Stober et al., 2001).

It should be noted that, while the focus here is on wet-only deposition, dry deposition likely adds significantly (30–60 percent of wet deposited) to the overall atmospheric load (FDEP, 2003).

Table 4. Comparison of atmospheric to surface water loading to the EPA.

Calendar Year	Atmospheric Deposition (kg Hg yr^{-1})	EAA Water Discharge (kg Hg yr^{-1})
1994[a]	238	2
1995[a]	206	3-4
2003	161–258[b]	5.9[c]
2004	172	3.2[c]

[a.] USEPA (2001, as cited by FDEP, 2003) annual deposition derived from Florida Atmospheric Mercury Study (FAMS), 1993–1996; surface water loading derived from biweekly monitoring of 'into' structures discharging from the EAA into the Everglades Protection Area.
[b.] Rumbold (2005)
[c.] Sum of loads at S-5A, S-6, S-7, S-8 for WY2003 and WY2004.

Table 5. Concentrations of THg and MeHg (ng/L) in non-ECP structure surface waters in Water Year 2005 (WY2005).

Structure	Quarter	THg ng/L	remark **	WQS*	MeHg ng/L	remark **	% MeHg
L28	1st May – Jul	1.10		<WQS	0.11		10%
	2nd Aug – Oct	3.30	A	<WQS	0.40		12%
	3rd Nov – Jan	0.94		<WQS	0.11		12%
	4th Feb - April	0.65	A	<WQS	0.11		17%
	Median last 4 qt.	1.02			0.11		12%
	Median POR	1.48			0.11		11%
S10C	1st May – Jul	0.80		<WQS	0.16		20%
	2nd Aug – Oct	2.00		<WQS	0.23		12%
	3rd Nov – Jan	0.62		<WQS	0.14		23%
	4th Feb - April	0.30	I	<WQS	0.11		37%
	Median last 4 qt.	0.71			0.15		21%
	Median POR	0.87			0.105		11%
S12D	1st May – Jul	1.60		<WQS	0.27		17%
	2nd Aug – Oct		J3	<WQS	0.24		
	3rd Nov – Jan	0.55	A	<WQS	0.22		40%
	4th Feb - April	0.62		<WQS	0.11		18%
	Median last 4 qt.	0.62			0.23		18%
	Median POR	0.98			0.150		15%
S140	1st May – Jul	0.91		<WQS	0.07	I	8%
	2nd Aug – Oct	2.50		<WQS	0.38		15%
	3rd Nov – Jan	0.77		<WQS	0.08	I	10%
	4th Feb - April	0.50		<WQS	0.13		26%
	Median last 4 qt.	0.84			0.10		13%
	Median POR	1.07			0.13		11%
S141	1st May – Jul		J3	<WQS	0.63		
	2nd Aug – Oct	2.60		<WQS	0.28		11%
	3rd Nov – Jan	0.76		<WQS	0.19		25%
	4th Feb - April	0.46	A	<WQS	0.099		22%
	Median last 4 qt.	0.76			0.24		23%
	Median POR	1.07			0.17		15%
S151	1st May – Jul		J3	<WQS	0.14		
	2nd Aug – Oct	5.00		<WQS	0.21		4%
	3rd Nov – Jan	1.10	A	<WQS	0.24		22%
	4th Feb - April	0.56		<WQS	0.15		27%
	Median last 4 qt.	1.10			0.18		22%
	Median POR	0.91			0.14		14%
S32	1st May – Jul		J3	<WQS	0.095		
	2nd Aug – Oct	1.90	A	<WQS	0.16		5%
	3rd Nov – Jan	0.94		<WQS	0.16		20%
	4th Feb - April	0.40		<WQS	0.07	I	16%
	Median last 4 qt.	0.94			0.13		17%
	Median POR	0.89			0.12		14%

Table 5. Continued.

Structure	Quarter	THg ng/L	remark**	WQS*	MeHg ng/L	remark**	% MeHg
S334	1st May – Jul	1.10		<WQS	0.087	I	8%
	2nd Aug – Oct		J3	<WQS	0.120		
	3rd Nov – Jan	0.44		<WQS	0.089		20%
	4th Feb - April	0.58		<WQS	0.150		26%
	Median last 4 qt.	0.58			0.104		20%
	Median POR	0.865			0.111		15%
S5A	1st May – Jul	2.00		<WQS	0.063	I	3%
	2nd Aug – Oct	28.00		>WQS	0.086	I	0%
	3rd Nov – Jan	1.90		>WQS	0.062	I	3%
	4th Feb - April	2.10		<WQS	0.240		11%
	Median last 4 qt.	2.05			0.074		3%
	Median POR	2.05			0.110		6%
S9	1st May – Jul		J3	<WQS	0.060	I	
	2nd Aug – Oct	2.50		<WQS	0.085	I	6%
	3rd Nov – Jan	0.26	I	<WQS	0.041	I	32%
	4th Feb - April	0.26	I	<WQS	0.026	I	19%
	Median last 4 qt.	0.26			0.056		12%
	Median POR	0.72			0.058		8%
	Median 05 1st	1.10	(6)¶		0.102	(10)	13%
	Median 05-2nd	2.55	(8)		0.220	(10)	11%
	Median 05-3rd	0.76	(10)		0.125	(10)	19%
	Median 05-4th	0.53	(10)		0.110	(10)	20%
	Cum. Median 1st Q	1.10	(55)¶		0.140	(60)	12%
	Cum. Median 2nd Q	1.60	(54)		0.180	(61)	11%
	Cum. Median 3rd Q	0.91	(70)		0.092	(87)	10%
	Cum. Median 4th Q	0.90	(77)		0.095	(65)	14%

*Class III Water Quality Standard of 12 ng THg/L

**For qualifier definitions, see FDEP rule 62-160: "A" - averaged value; "U" - undetected, value is the MDL; "I" - below PQL; "J" - estimated value, the reported value failed to meet established QC criteria; "J3" -estimated value, poor precision, "V" - analyte detected in both the sample and the associated method blank. Flagged values were not used in calculating medians.

¶ Value in parenthesis, i.e., (n), is number of unqualified values used to calculate median

SURFACE WATER AT NON-ECP STRUCTURES

Table 5 and **Figures 8** and **9** summarize monitoring results of unfiltered THg and MeHg in surface water samples collected quarterly at non-ECP structures (for map of locations, see **Figure 2**). The maximum water-column THg concentration observed during WY2005 was 28 ng/L that occurred at S-5A during the third quarter (**Figure 8**). This value exceeds the Florida Class III water quality standard of 12 ng THg/L; however, it should be noted that the analytical laboratory reported that the sample contained a significant amount of suspended particulate matter. Invariably, elevated water-column THg concentrations, above the WQS, are associated with high TSS. Likely as a result of frequent spikes in TSS, site S-5A had greater THg concentrations (median was 2.05 ng THg/L) compared to all other sites except L28 (median 1.48 ng THg/L), when the entire period of record is examined (Kruskal-Wallis ANOVA on ranks; $H = 49.3$, $df = 9$, $P < 0.0001$ – Dunn's method of pairwise multiple comparisons); no other pairwise comparison between sites was significant.

The maximum water-column MeHg concentration observed during WY2005 at a non-ECP structure was 0.63 ng/L that occurred at S-141 during the first quarter of WY2005 (**Table 5** and **Figure 9**). Currently, Florida has no Class III numerical water quality standard for MeHg. When the entire period of record is examined for MeHg, the most obvious spatial trend was that site S-9 typically had the lowest concentration compared to all other sites except S-32; this spatial pattern was statistically significant ($H = 32.2$, $df = 9$, $P < 0.0001$ – Dunn's method of pairwise multiple comparisons). No other pairwise comparisons were significant.

After more than seven years of monitoring, a seasonal Kendall's Tau test finds little indication of statistically significant temporal trends in either THg or MeHg concentration (or percent MeHg) at any of the individual structures. Calculated Tau values, which were based on four seasons, i.e., quarterly samples ($n \leq 29$), ranged from -0.39 to +0.29 for THg and from -0.17 to +0.33 for MeHg (a negative Tau indicates a decreasing trend, whereas a positive Tau an increasing trend). In general, P values (both with and without autocorrelation correction) were not significant ($P > 0.05$); the only exception being THg at S-32 (Tau = -0.34) with P values of 0.04 (without correction for autocorrelation) and 0.12 (with autocorrelation correction; assessment by S. Hill, SFWMD).

Further, when data were pooled for all sites, neither median THg or MeHg concentration in 2005 differed significantly in pairwise comparisons with other years ($P > 0.05$). As observed in previous reports, concentrations of both THg and MeHg were generally highest during the months of August–October (i.e., second quarter) of WY2005. When data were pooled for all sites over all years, median THg concentration was significantly greater in the second quarter compared to either the third or fourth quarter ($H = 37.4$, $df = 3$, $P < 0.0001$; Dunn's test $P < 0.05$) but not the first quarter ($P > 0.05$); THg concentration also tended to be greater in the first quarter (May–July) as compared to the fourth quarter ($P < 0.05$). Similarly, median MeHg concentration was also significantly greater in the second quarter compared to the third, fourth, and/or first quarter ($H = 34.3$, $df = 3$, $P < 0.0001$; Dunn's test $P < 0.05$); first quarter median MeHg concentration was again found to be greater than fourth quarter.

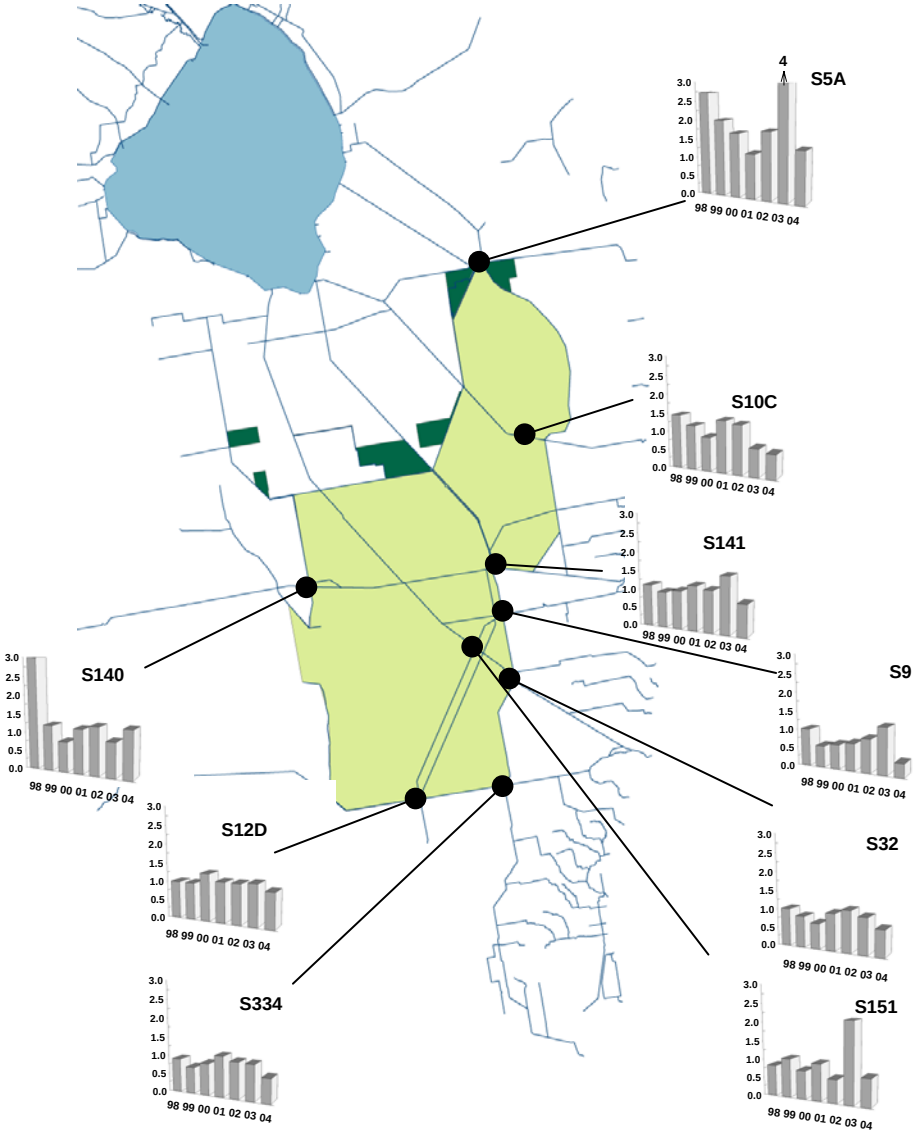

Figure 8. Annual median THg concentrations for period of record at stations sampled under Project Code HGLE.

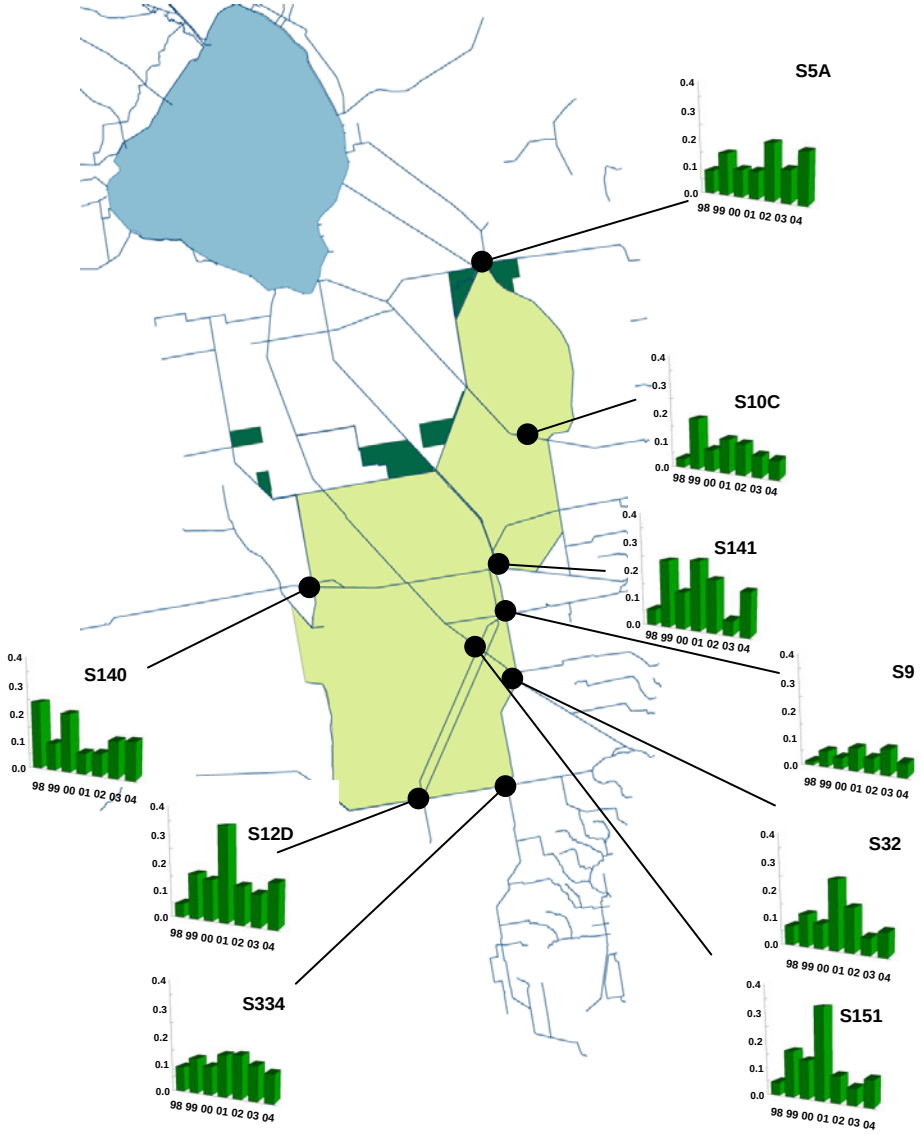

Figure 9. Annual median MeHg concentrations for period of record at stations sampled under Project Code HGLE.

FISH FROM ECP AND NON-ECP INTERIOR MARSHES

Results from monitoring downstream interior marsh mosquitofish (*Gambusia holbrooki*), sunfish (*Lepomis* spp.), and largemouth bass (*Micropterus salmoides*) are summarized in **Tables 6** through **8**, respectively. It should be noted that raw data for individual fish can be found at www.sfwmd.gov/org/ema/dbhydro/index.html. Fish collections were targeted at 12 downstream marsh sites in the interior of the WCAs and ENP (**Figure 3**). Three of these sites (LOXF4 or WCA-1-GFC4, CA2U3 or WCA-2A-U3, CA315 or WCA-3A-15) have been monitored by the FWC since 1993. Where fish could not be collected from a targeted marsh site (i.e., due to inaccessibility, poor habitat or both), collections defaulted to nearby marshes or, in some cases, canals (if source water were similar) where fish were more plentiful (approval for these alternate sites was received from the FDEP on March 5, 2002; see correspondence from F. Nearhoof, FDEP). To preserve long-term datasets that are crucial for temporal trend assessment, reverting back to original target site will be done with care and will involve sampling at both the alternate and the original site for some period (i.e., to assess spatial differences). Accordingly, sampling will revert back to the original targeted site only after it has been established that long-term hydrology and habitat restoration has occurred (i.e., to insure chances of finding fish year-to-year are high). Although this may take a number of years at certain sites (e.g., sites WCA-2-F1, WCA-3-3, WCA-3-5), it will prevent alternating collections between the two sites and disruption of data continuity.

Fishes collected in 2004 showed both spatial and temporal patterns in tissue Hg concentrations. In keeping with the primary objective of this monitoring program, the focus will be on temporal changes in mercury concentration in fish tissues to assess possible adverse effects from the construction of the ECP and the operation of the STAs. Nevertheless, spatial patterns of tissue Hg concentrations are important, particularly where there has been a variation from background conditions (i.e., pre-ECP conditions established by the FWC). Therefore, spatial patterns will be reviewed in detail only where there have been changes over time (i.e., interaction between treatment effects).

Table 6. Mean concentrations of THg in mosquitofish composites (*Gambusia* sp.) (ng/g wet weight) collected in 2004 from downstream sites. Value represents a mean of 3 analyses.

Location	THg (ng/g)	Between-yr. change (%)	Cum. Aver (ng/g)
LOX4	48	NA	
CA2 F1 (L39F1)	13	117%	
CA27 Alt (Z4)	57	-2%	
CA27 Alt (N4)	85	18%	
Holey Land (North canal)	27	-43%	
Rotenberger Alt. (RotenF1)	11	-39%	
Rotenberger rim canal (RotenC)	39	NA	
CA2U3	83	63%	
CA33	52	NA	
CA35alt2	78	121%	
Non-ECP North (CA3F1; end of L-28)	14	-30%	
CA315	50	16%	
Non ECP South (CA3F2)	35	-10%	
L67F1	50	9%	
annual mean	46	22%	

NA = data not available.
Grandmean for POR (1998-04; aliquot means pooled across time and space): n=95, 84 ng/g; 50^{th} and 90^{th} percentile for POR is 63 ng/g and 188 ng/g, respectively.

Table 7. Mean concentrations (±1 SD; ng/g wet weight) of THg in sunfish (*Lepomis* spp.) collected in 2004 from marshes within the EPA downstream of the STAs.

Target location	Sampling Location	Mean THg ng/g (±1SD, n)	Between-yr. change (%)	Grandmean of annual means
WCA1-LOX3	LOXF4	98 (±36, 20)	1%	132
WCA-2A F1	L39F1	47 (±43, 20)	-48%	72
WCA-2A 2-7	Z4*	NA	NA	148
	N4*	NA	NA	168
Holey Land	Holey Land	215 (±81, 20)	10%	124
Rotenberger	RotenC (canal)	118 (±35,20)	-34%	185
WCA-2A U3	CA2U3	164 (±66, 20)	-13%	150
WCA-3A 3	3A-3	154 (±76, 20)	NA	154
WCA-3A 5	Alt. 2 site	116 (±64, 20)	-30%	187
Non-ECP North	CA3F1	176 (±107, 18)	40%	130
WCA-3A 15	CA315	151 (±70, 20)	--44%	294
Non-ECP South	CA3F2	86 (±24, 20)	19%	135
ENP P33 Marsh	L67F1	437 (±331, 20)	14%	469
Average		160	-5%	

* Unable to collect 20 fish from each site.
NA = data not available due to the absence of fish at the site.
Grandmean of site means (pooled across space and time) for POR (1998-03) ±95%CI: n=81, 186±30; 50th and 95th percentile site mean concentration was 145 and 442 ng/g, respectively.

Table 8. Standardized (EHg3) and arithmetic mean concentrations of THg in largemouth bass fillets (*Micropterus salmoides*) (ng/g wet weight) collected in 2004 from ECP and non-ECP interior marsh sites.

Target Location	Sampling Location	EHg3 ± 95th CI (mean ±1SD, n) ng/g wet	Between-yr. Change (%)	Cum. average EHg3
CA1-LOX3	LOX4	NC (1) (310±110, 20)	NA	501
CA2-F1	L39F1	230±50 (230±140, 19)	-23%	274
CA2-7	Z4	NA	NA	448
Holeyland	HOLYBC	650±40 (650±190, 20)	12%	414
Rotenberger	ROTENC	NC (2) (210±40, 5)	NA	847
CA2-U3	CA2U3	589±97 (500±210, 20)	-22%	676
CA3-3	CA33	NA	NA	
Non-ECP North	CA3F1	747±60 (530±430, 20)	11%	537
CA3-15	CA3-15	650±60 (490±310, 20)	2%	833
Non-ECP South	CA3F2	NC (1) (300±160, 8)	NA	436
ENP-P33	L67F1	1,190±130 (1,190±660, 20)	-24%	1,308

NC - not calculated for: (1) insignificant slope or (2) if poor age distribution. NA - not available
Annual average EHg3 = 670 ng/g
Grandmean of site EHg3 for POR +95%CI: n = 46, 625 ±99 ng/g

Mosquitofish

Hg levels in mosquitofish (*Gambusia holbrooki*) collected from marsh sites in 2004 ranged from 13 ng/g at site L39F1 to 85 ng/g at site N2 (**Table 6** and **Figure 10**). The annual basinwide average concentration in mosquitofish collected in 2004 was 46 ng/g (**Table 6)** (for locations, see **Figure 3**), which represents a 22 percent increase from the 2003 basinwide mean concentration. The 50th and 90th percentile tissue-Hg concentrations in mosquitofish (i.e., aliquot means) for the period of record (1998–2004, n = 95) were 63 ng/g and 188 ng/g, respectively.

In 2004, THg levels in mosquitofish declined at five sites and increased at six sites compared to the previous year (**Table 6**). When sites sampled in three or more years were assessed using a two-way ANOVA, both among-year differences (df = 6, 61; F = 31.9; p < 0.001) and locational differences (df = 14, 61; F = 6.3; p < 0.001) were statistically significant (ANOVA;); it should be noted that due to missing data, interaction was not assessed in this two-way ANOVA. The most dramatic (and statistically significant) differences were spikes in Hg levels that occurred 1999 (**Figure 3**). As discussed in previous reports, mercury levels increased dramatically in mosquitofish in 1999 following a drydown and reflooding, decreased substantially in 2000 and then rebounded (increased) in 2001 (**Figure 10**). Pairwise comparisons revealed Hg levels in 2004 also differed from the higher levels observed in 1998 and 2001 (Tukey HSD, p < 0.05).

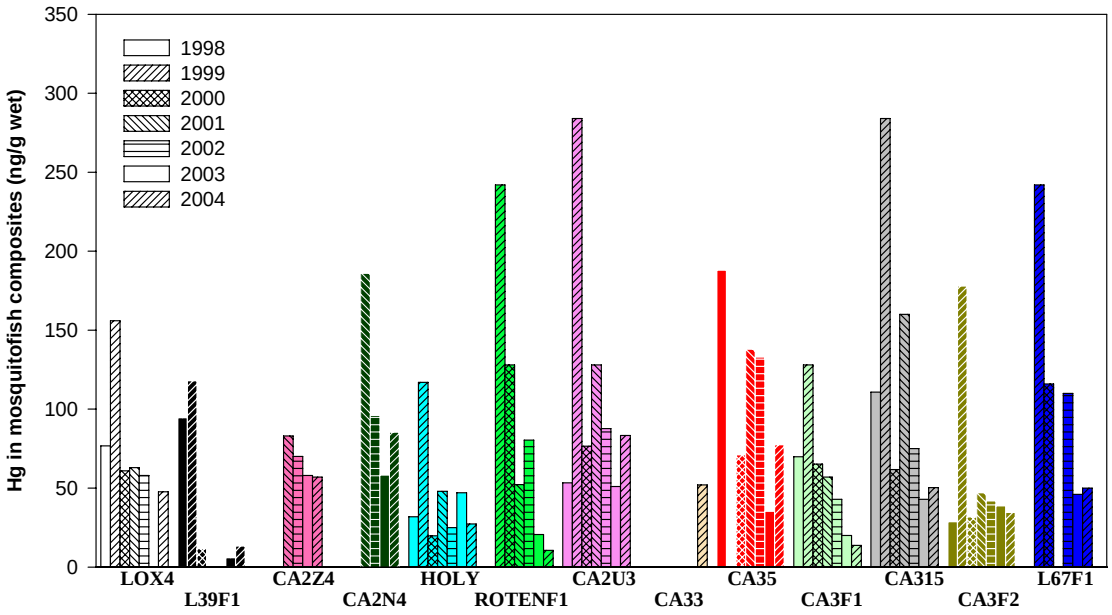

Figure 10. Hg concentrations in mosquitofish (*Gambusia* sp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2004). Not all sites were sampled in all years (for details, see Table 6).

Sunfish

Hg levels in sunfish (*Lepomis* spp.) collected from downstream sites in 2004 (n = 218) ranged from a low of 7 ng/g in a redear sunfish (*L. microlophus*) from site L39F1 to as high as 1,500 ng/g in a bluegill (*L. macrochirus*) from L67F1. The grandmean of site means was 160 ng/g in 2004, which represents a 5 percent decrease from the previous year. However, as discussed below, caution should be exercised when interpreting basinwide concentrations.

Hg content in sunfish differed over both space and time. However, results must be interpreted with caution due to differences in sizes and species of collected sunfish. Although there are statistical methods to address confounding factors, such as age or weight, addressing species differences is more problematic, particularly when convolved with size differences. As discussed in previous reports, attempts to use ANCOVA to evaluate patterns of mercury concentrations in sunfish using weight as a covariate were often unavailable because weight-concentration relationships were inconsistent (i.e., slopes were either not significant or were not parallel each year). The lack of a strong concentration-size relationship likely resulted from interspecies differences (i.e., among the different *Lepomis* spp.) in growth and bioaccumulation factors, which are likely a function of diet. As observed over the past six years, when data were pooled across sites, fish species was a significant factor in tissue Hg concentration in 2004 (Kruskal-Wallis ANOVA on Ranks, df = 3, H = 16.1, $p < 0.001$); Hg levels were lower in *L. microlophus* (redear, median 96 ng/g) than each of the other three species (Dunn's method, $p < 0.05$), e.g., *L. macrochirus* (bluegill, median = 130 ng/g), *L. punctatus* (spotted sunfish, median = 150 ng/g), *L. gulosus* (warmouth, median = 155 ng/g); no other paired comparison between species was significant ($p > 0.05$). Interestingly, the redear were also larger on average than the other three species (Kruskal-Wallis ANOVA on Ranks, df = 3, H = 49.6, $p < 0.001$; Dunn's Method $p < 0.05$); hence, this among-species difference was not related to size (typically larger, older fish have higher Hg) but due to diet; the redear, also known as the shellcracker, is known to have a preferred diet, which included snails, different than the other three species.

In 2004, sunfish continued to show significant spatial patterns in Hg levels (**Figure 11**, df = 10, H = 105.8, $p < 0.001$). As observed in previous years, resident sunfish at site L67F1 had significantly greater mercury burdens (Dunn's Method, $p < 0.05$) than fishes from a number of other sites (e.g., CA3F2, L39F1, LOX4, ROTEN, CA35Alt, CA33, and CA315). Fish from the Holey Land WMA had the second highest median concentration that differed from the four other sites (e.g., CA3F2, L39F1, LOX4, CA35alt). However, fish caught from the Holey Land WMA in 2004 were also larger (median weight was 144 g) than fish from many other sites (e.g., L39F1, LOX4, CA35Alt, L67F1; Dunn's method ($p < 0.05$) and this size-related difference (as a surrogate for age) could account for the higher Hg levels (**Figure 11**).

In 2004, sunfish also continued to show clear temporal variability in Hg burdens. Although sunfish at most sites contained Hg levels similar or lower than levels observed in 2003, fish from four sites (e.g., Holey Land, CA3F1, CA3F2, and L67F1) showed an increase in 2004 (**Table 7** and **Figure 11**). Between-year (i.e., from 2003 to 2004) percent change in Hg levels at individual sites ranged from a 48 percent decrease at site L39F1 to a 40 percent increase at site CA3F1. In several instances, these between-year differences were statistically significant.

Sunfish collected at L39F1 had significantly lower Hg levels in 2004 as compared to 1998 (df = 6, H = 20.5, $p < 0.002$; Dunn's Method $p < 0.05$); neither fish size nor species composition of sample appeared to account for the temporal variability in Hg levels. No other between-year comparison was significant at site L39F1.

Fish collected from the Rotenberger WMA also exhibited between-year differences in mercury levels ($df = 2$, $H = 37.4$, $p < 0.001$). Although arithmetic average concentrations were higher in 2003 (**Table 7**), owing to several fish with elevated levels (i.e., skewed the distribution and mean), the median concentration was significantly higher in 2004 (Dunn's Method $p < 0.05$); the 2004 median was significantly lower than in 2002 ($p < 0.05$). As discussed in previous reports, two different sites have been sampled within the Rotenberger WMA (i.e., ROTENF1, a marsh site, was sampled in 2002, and ROTENC, a canal site, was sampled in 2003 and 2004). Thus, care must be taken when making among-year comparisons.

Hg levels declined in sunfish at CA35 for the second year in a row (**Table 7** and **Figure 11**); among-year differences were significant ($df = 4$, $H = 24.9$, $p < 0.001$) with levels in 2004 differing from 2000, 2001, and 2002 ($p < 0.05$), but not 2003 ($p > 0.05$).

Except for a short-lived spike in 2002, Hg levels have also shown a steady decline in sunfish from CA315 since 1999 (**Table 7** and **Figure 11**; and prior to this, based on FFWCC long-term datasets). As discussed below, declines were also evident in bass at CA315 over the past few years.

As reported last year, Hg levels were elevated in sunfish at site L5F1 in 2002 and 2003 as compared to four previous years. In 2004, sampling at the L5F1 site was replaced by sampling at two nearby sites (on either side of L5F1) to serve as outflow monitoring stations for the newly operational STA-3/4 (i.e., ST34DCW and ST34DCE; two sites were sampled rather than one, to assess whether a plug in the canal for construction purposes may have created two different outflows with differing fish populations). Additionally, as part of the downstream monitoring program, efforts were made to collect fish from the original marsh site targeted in northeastern WCA-3A, CA3A3; this attempt was successful, at least in terms of sunfish. When data from these three new sites were compared to data from samples collected from site L5F1 during the previous year, statistical differences were evident ($df = 3$, $H = 36.9$, $p < 0.001$); CA33 fish contained significantly higher Hg levels than the other three sites (median values were 130 ng/g at CA33, 109 ng/g at ST34DCW, 72 ng/g at ST34DCE, and 120 ng/g at L5F1). However, there were also significant among-site differences in size of fish and species of lepomid collected. Although site CA33 had the smallest fish (median weight was 20 g), the sample of 20 sunfish included six warmouth and two spotted sunfish but no redear (i.e., the remainder were bluegill); none of the other sites included warmouth. Further, although ST34DCE contained the largest fish on average (median weight was 67 g), 16 of the 20 fish sampled were redear sunfish. The sample from site ST34DCW contained only 8 redear. Based on the among-species differences in Hg levels discussed in the preceding section, it is likely that the differences in composition of species of lepomids collected from the various sites confounded any among-site differences in ambient Hg conditions (especially between sites ST34DCW and ST34DCE). This conclusion was strengthened when an assessment of the bluegill sunfish collected from these sites revealed no significant among-site differences in Hg levels ($df = 3$, $H = 0.45$, $p = 0.93$).

Although the Hg burden in sunfish increased at CA3F2 in 2004 (increased 19 percent over 2003, **Table 7, Figure 11**), 2004 levels remained significant lower ($df = 6$, $H = 45.5$, $p < 0.001$; Dunn's Method, $p < 0.05$) than both 1998 and 1999 levels.

Hg levels have varied little in sunfish at L67F1 over the past three years as compared to spikes in Hg observed in fish from this site in 1999 and 2001. Sunfish collected at L67F1 in 1999 contained some of the highest concentrations of mercury ever observed in Everglades *Lepomis*. A 45 gm bluegill (137 mm), for example, was found to have 3,300 ng THg /g (3.3 ppm). Although fish at L67F1 continue to have the highest burdens, the pattern of progressively increasing Hg in

sunfish over the past few years at both CA3F1 and Holey Land WMA is also of considerable concern.

Hg levels increased 40 percent in sunfish at CA3F1 in 2004; these levels were significantly higher than the low concentrations observed in 2001 (df = 6, H = 15.8, $p < 0.015$; Dunn's Method, $p < 0.05$), but no other between-year comparison was statistically significant. Alone this may not be cause for concern; however, as discussed below, Hg levels have also increased over the past few years in bass at this site. Sunfish at the Holey Land site have also shown increases over the past few years; in 2004, levels increased another 10 percent. Between-year comparisons in Hg levels in Holey Land sunfish were statistically significant for 2004 (median = 210 ng/g) and 2000 (median = 59 ng/g), 2004 and 1999 (median = 38 ng/g), and 2004 and 1998 (median = 30 ng/g). Although, as previously reported (Rumbold, 2005), among-year differences in species collected may be responsible for some of the observed between-year differences, when combined with results from bass at this site (discussed below), this trend of increasing Hg in fish at this site appears genuine.

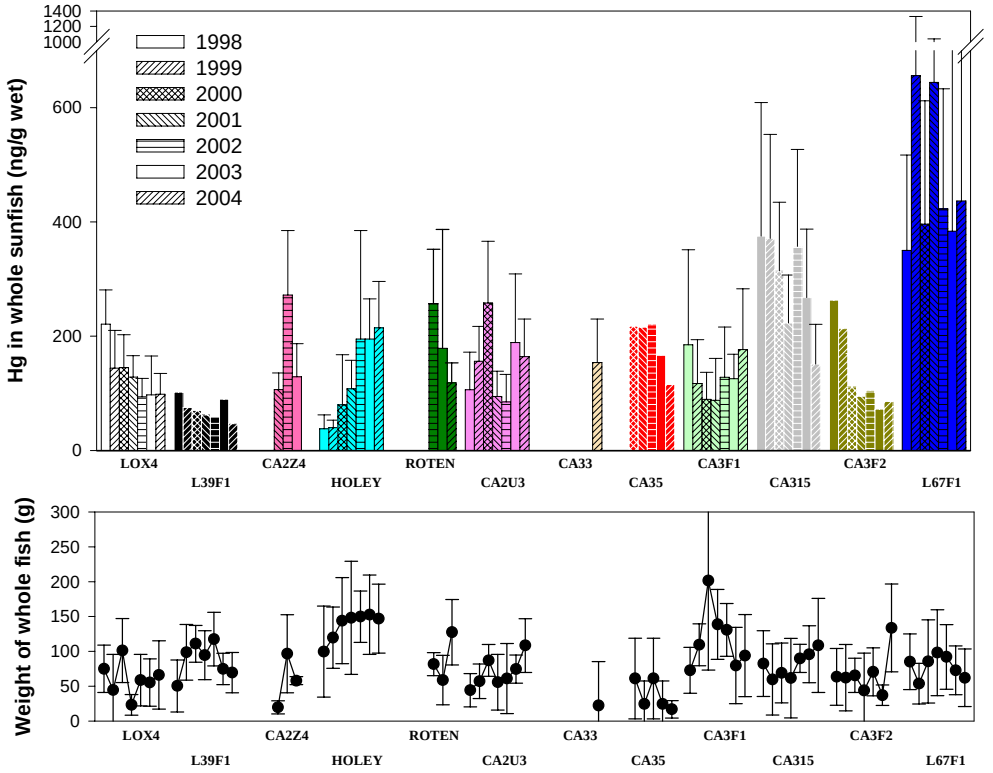

Figure 11. THg concentration (top) and weights (bottom) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2004).

Largemouth Bass

A total of 152 largemouth bass (*Micropterus salmoides*) were collected at nine downstream sites from October–November 2004. Despite the best efforts of the FWC (who were contracted to electrofish these sites), bass could not be collected from sites Z4, N4, CA33, or CA35Alt. The bass that were collected had tissue Hg concentrations ranging from a low of 89 ng/g in a fish from site L39F1 to 2,800 ng/g in a fish from L67F1; these two sites typically represent the minimum and maximum for Hg levels in Everglades fish. Site-specific, age-standardized concentrations (expected in a three-year-old bass, EHg3) ranged from 230 ng/g at site L39F1 to 1,190 ng/g at site L67F1 (**Table 8** and **Figure 12**). Calculation of EHg3 was not appropriate at sites LOX4, CA3F2, and ROTENC, either because the tissue Hg-age relationship was not significant (first two sites) or because of small sample size (latter site). The grandmean of site-specific EHg3 values was 670 ng/g in 2004 (based on the six sites where it was appropriate to calculate an EHg3), which represents a 7 percent decrease over the grandmean estimated for 2003.

Largemouth bass exhibited spatial patterns in tissue Hg concentrations similar to those observed in sunfish, with higher levels generally being found at the southern sites (**Table 8, Figure 12**). These relationships are best illustrated when levels in young bass (less than 1.8 years old) are compared to levels in bluegill (**Figure 13)**. Because of a statistically significant interaction between location and age ($F = 23.9$, $df = 5, 107$; $p < 0.001$), ANCOVA could not be used to assess differences in LSM Hg levels among all sites.

Hg levels in bass at CA315 satisfied the requirements for ANCOVA and were found to differ among years (e.g., 1999, 2002, 2003, 2004; $F = 14.3$; $df = 3, 78$; $p < 0.001$), with levels in 2004 lower than levels observed in both 2002 and 1999 (Tukey HSD, $p > 0.05$), but similar to levels in 2003 ($p = 0.71$). This decrease in Hg over the past few years has added significance because site CA315 had been recognized as the MeHg "hotspot" in the Everglades. Initially, declines in Hg level in fish at this and other sites, along with declines in birds were attributed to decreased Hg emissions and deposition (Atkeson and Axelrad, 2004). However, as reported last year, researchers at USGS (D. Krabbenhoft and W. Orem, personal communication, 2004) have reported a concomitant decline in sulfate concentrations at this site and argue that, lacking this critical electron acceptor, sulfate-reducing bacteria were inhibited from methylating Hg. In other words, the decline was a result of changes in water quality rather than decreased atmospheric deposition. At this time, it is uncertain whether other sites exhibiting declines in Hg in resident fish populations are also experiencing similar declines in sulfate. It is also uncertain as to why sulfate concentrations have decreased at site CA315 (i.e., whether loading has decreased from the source or just been rerouted). This debate over the principle driver controlling MeHg production and bioaccumulation was further fueled last year by the marked increase in deposition (for details see Rumbold, 2005) that raised questions regarding long-term trends in local emissions and deposition. Further puzzling is the fact that Hg levels in fish (i.e., mosquitofish last year or large-bodied fish this year) have not shown any consistent, widespread fluctuations that might be linked to the sudden increased deposition of THg in 2003 (**Figure 13**).

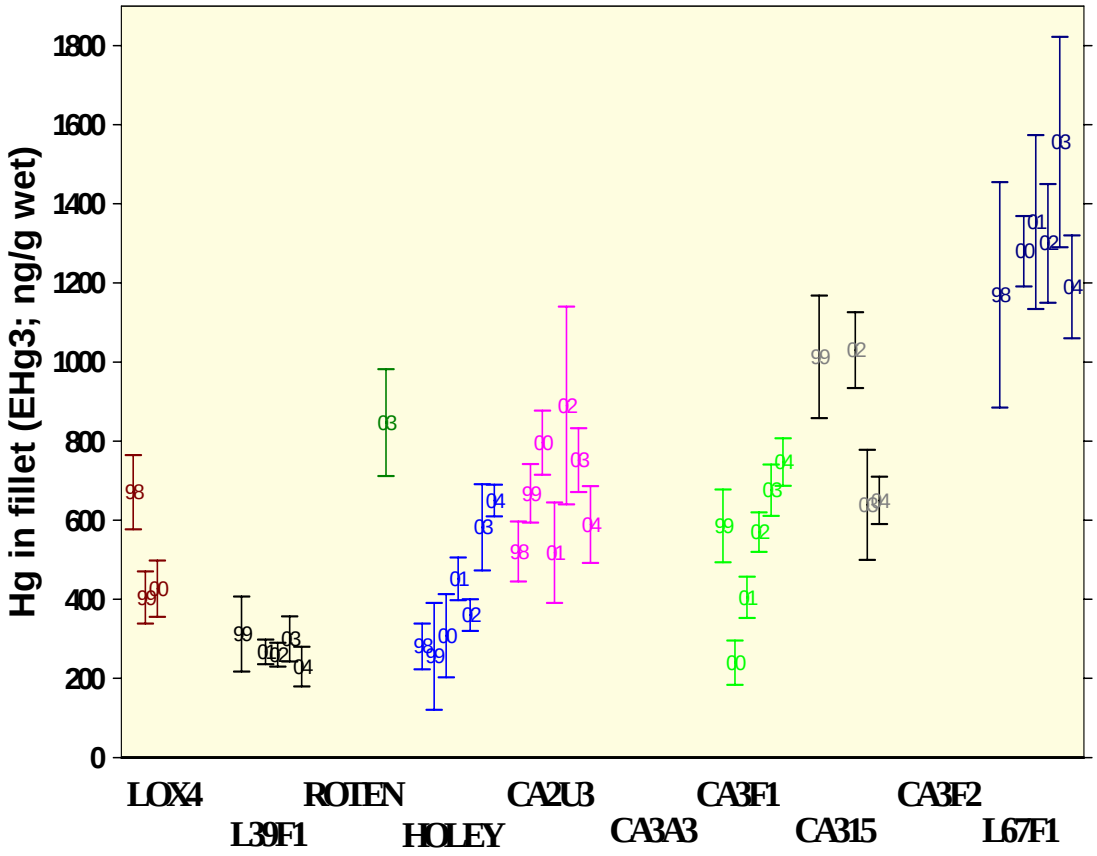

Figure 12. Age standardized (Class III year) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at downstream sites for period of record (i.e., 1998–2004). EHg3 was not calculated if regressions were not significant or if age distributions were narrow (see **Table 8**).

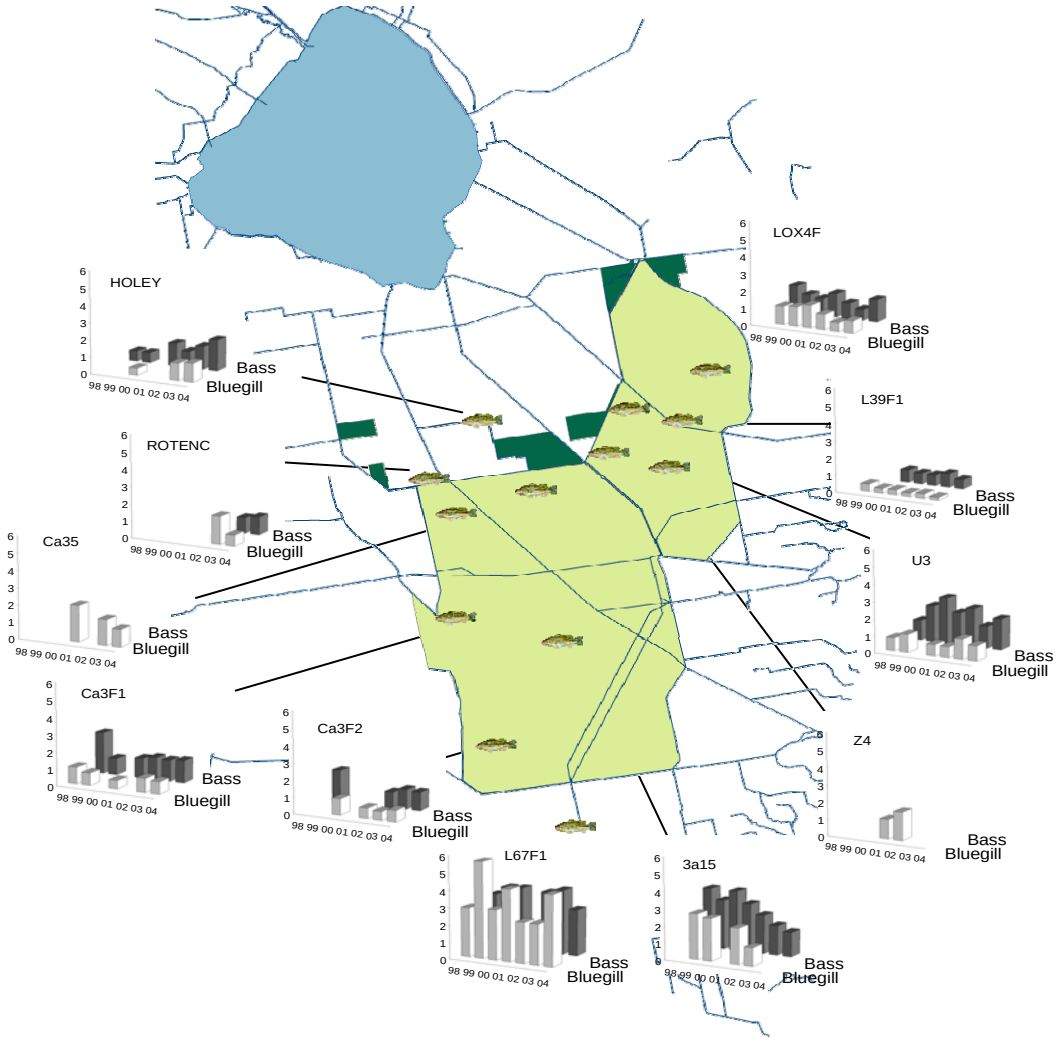

Figure 13. Spatial and temporal patterns in Hg levels in young largemouth bass (i.e., less than 1.8 years old) and bluegill sunfish (4–7 inches in length). Hg levels in fish were further normalized by dividing concentration in a given fish by its total length. Hence, the units of the y-axis is are mg/kg/m (c.f. Brumbaugh et al., 2001). Note: few large fish have been collected from site CA2N4 in NW WCA-2 and from site CA33 in NE WCA-3.

Hg levels have varied considerably over the monitoring period in both bass and sunfish at L67F1 (**Figures 11** through **13**). As reported by Rumbold (2005), Hg levels increased in bass at L67F1 in 2003. In 2004, Hg levels in bass declined by 24 percent. The between-year deference was statistically significant (ANCOVA, $F = 2.5$, $df = 5, 111$; $p = 0.03$; Tukey HSD, $p < 0.04$); 2004 levels did not differ from levels in other years ($p < 0.05$).

ANCOVA was not available to assess temporal differences in Hg levels in bass from sites CA2U3 and CA3F1 because of an interaction between year and bass age ($F = 5.7$, $df = 6, 124$, $p < 0.001$; $F = 15.8$, $df = 5, 107$, $p < 0.001$; respectively), i.e., significant between-year variability in slopes of regressions of Hg on age. Nevertheless, temporal patterns in Hg levels are evident by visual inspection of **Figure 12**. Levels peaked in bass at CA2U3 in 2002 and now are on a decline. Alternatively, Hg levels appear to have increased progressively over time in fish at CA3F1. Hg levels also appear to be increasing progressively over time in bass at the Holey Land; although the 12 percent increase in the 2004 EHg3 was not statistically different from 2003 levels (Tukey post-hoc comparison of LSM mean, $p = 0.65$). Nevertheless, 2004 levels differed from all previous years (ANCOVA, $F = 23.0$; $df = 6, 131$; $p < 0.001$; Tukey comparisons $p < 0.05$). In the 2005 SFER – Volume I (Rumbold 2005), it was speculated that conditions were becoming more favorable for methylation in the Holey Land. While this may be true, the observed trend could also be a result of increasing complexity in the food web (following hydroperiod changes), thus providing additional steps for biomagnifications. In either case, the resulting Hg burdens are reaching, or have reached, levels that may pose a threat to fish-eating wildlife (see below).

PREDATOR PROTECTION CRITERIA

Levels of mercury in fish tissues can also be put into perspective and evaluated with respect to mercury risk to wildlife. The USFWS has proposed a predator protection criterion of 100 ng/g THg in prey species (Eisler, 1987). In the Mercury Study Report to the U.S. Congress, the USEPA proposed 77 ng/g and 346 ng/g for TL 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997).

In 2004, mosquitofish (considered to be at TL 2–3, depending on age; Loftus et al., 1998) at sites N4, CA2U3 and CA35alt exceeded the USEPA criterion but not the USFWS criterion. Mosquitofish from all other sites were well below both criteria (**Table 6**). Alternatively, sunfish, which are at TL 3 (*L. gulosus* at TL 4; Loftus et al., 1998), exceeded one or both predator protection criteria at all but one site (i.e., L39F1, which had an average Hg level of 47 ng/g; **Table 7)**. As discussed previously (Rumbold 2005), this finding is significant because sunfish represent the preferred prey item of many fish-eating species in the Everglades. Whole-body concentrations of Hg in largemouth bass (where whole-body THg concentration = 0.695 x fillet THg; Lange et al., 1998), approached or exceeded the guidance value for TL 4 fish at four out of nine sites (e.g., CA3F1, HOLYBC, L67F1, and CA2U3).

Based on these findings, it appears that certain Everglades populations of piscivorous avian and mammalian wildlife continue to be at risk of adverse effects from mercury exposure depending on where they forage. This conclusion is consistent with an update of the probabilistic risk assessment done recently that focused on STA-2, but included other reference areas (see Appendix 4-6 of this volume).

WADING BIRD FEATHERS FROM ECP INTERIOR MARSHES

In an attempt to optimize the mercury monitoring program, feather collection was coordinated through the District's Everglades Research Division in 2005. It was felt that the avian ecology group could collect the feathers for mercury analysis while in the colony doing research or monitoring for other reasons. This group attempted to locate active egret nests and collect feathers on nine separate occasions (May 13, 19, 24, and 26; and June 3, 6, 9, 14, and 22) at the Alley North (selected to replace the Cypress City colony due to increased size) and L67 Colonies (see field notes by Erynn Call, SFWMD). Regrettably, unusually poor nest initiation by the egrets in 2005 resulted in the location of only three active nests containing nestlings of an appropriate age. Feathers collected from these nestlings were shipped to the FDEP Chemistry Lab in July; as a consequence, results will be reported in next year's annual report.

WADING BIRD HABITAT AND FORAGING PATTERNS

Critical environmental factors that determine the suitability of an area for foraging and nesting wading birds, e.g., water depth, vegetation density, and densities and size distribution of the preferred prey population, have been reviewed in previous reports (see for example Rumbold and Rawlik, 2000). In accordance with Condition 4.iv of the Mercury Monitoring Program, the District conducted a literature search for published and unpublished studies or monitoring programs in WY2005 that may describe possible changes in wading bird habitat and foraging patterns within the Everglades basin and, as a consequence, their potential exposure to mercury (utilizing the Electronic Databases for State Employees; http://dlis.dos.state.fl.us/cgi-bin/services/index.cfm). Studies and monitoring programs identified during this search are discussed below.

From February through June of each year, researchers for the USACE carry out systematic reconnaissance flights (SRFs) for wading bird activity in the WCAs and Big Cypress National Preserve; results of the 2005 SRFs were not available at the date of this report. Various individuals or agencies also made systematic aerial and ground surveys of nesting wading birds in South Florida during the 2005 breeding season; however, these reports were not final at the date of this report (for details, see Chapter 6 of the 2006 SFER – Volume I; also see Cook, in prep).

LITERATURE CITED

Atkeson, T. and D. Axelrod. 2004. Chapter 2B: Mercury Monitoring, Research and Environmental Assessment. In: 2004 Everglades Consolidated Report, South Florida Water Management District. West Palm Beach, FL. Online at http://www.sfwmd.gov/sfer/previous_ecr.html.

Atkeson, T. and P. Parks. 2002. Mercury Monitoring, Research and Environmental Assessment. Chapter 2B in 2002 Everglades Consolidated Report. South Florida Water Management District; West Palm Beach, FL.

Bates, A.E., W.H. Orem, J.W. Harvey and E.C. Spiker. 2002. Tracing Sources of Sulfur in the Florida Everglades. *Journal of Environmental Quality*, 31: 287-299.

Bloom, N.S. 1992. On the Chemical Form of Mercury in Edible Fish and Marine Invertebrates. *Can. J. Fish. Aquat. Sci.*, 49: 1010-1017.

Bloom, N.S., J.A. Colman and L. Barber. 1997. Artifact Formation of Methylmercury during Aqueous Distillation and Alternative Techniques for the Extraction of Methylmercury for Environmental Samples. *Fres. J. Chem,* 358: 371-377.

Cabral, J.A. and J.C. Marques. 1999. Life History, Population Dynamics and Production of Eastern Mosquitofish, Gambusia holbrooki (Pisces, Poeciliidae), in Rice Fields of the Lower Mondego River Valley, Western Portugal. Acta Oecologica, 20: 607-620.

Cook, M., ed.. In prep. South Florida Wading Bird Report. Vol. 11. Unpublished report. South Florida Water Management District. West Palm Beach, FL.

Eisler, R. 1987. Mercury Hazards to Fish, Wildlife and Invertebrates: A Synoptic Review. *U.S. Fish Wildl. Serv. Biol. Rep*., 85(1.10).

FDEP. 2003. Integrating Atmospheric Mercury Deposition with Aquatic Cycling in South Florida: An Approach for Conducting a Total Maximum Daily Load Analysis for an Atmospherically Derived Pollutant. Florida Department of Environmental Protection. Online at ftp://ftp.dep.state.fl.us/pub/labs/assessment/mercury/tmdlreport03.pdf (September 30, 2004).

Fink, L.E., D.G. Rumbold and P. Rawlik. 1999. Chapter 7: The Everglades Mercury Problem. G. Redfield, ed. In: 1999 Everglades Interim Report. South Florida Water Management District, West Palm Beach, FL.

Frederick, P.C., M.G. Spalding, M.S. Sepulveda, G.E. Williams Jr., S.M. Lorazel and D.A. Samuelson. 1997. Effects of Elevated Mercury on Reproductive Success of Long-Legged Wading Birds in the Everglades. Final Report. Prepared by the University of Florida for the Florida Department of Environmental Protection, Tallahassee, FL.

FTN Associates. 1999. Everglades Mercury Baseline Report for the Everglades Construction Project under Permit No. 199404532. Prepared for the South Florida Water Management District, West Palm Beach, FL.

Gilbert, R.O. 1987. Statistical Methods for Environmental Pollution Monitoring. Van Nostrand Reinhold, New York, NY.

Gilmour, C.C. and D.P. Krabbenhoft. 2001. Appendix 7-4: Status of Methylmercury Production Studies. G. Redfield, ed. In: 2001 Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL.

Grieb, T.M., C.T. Driscoll, S.P. Gloss, C.L. Schofield, G.L. Bowie and D.B. Porcella. 1990. Factors Affecting Mercury Accumulation in Fish in the Upper Michigan Peninsula. *Environ. Toxicol. Chem.,* 9: 919-930.

Guentzel, J. 1997. The Atmospheric Sources, Transport and Deposition of Mercury in Florida. Ph.D. Thesis. Florida State University, Tallahassee, FL.

Guentzel, J.L., W.M. Landing, G.A. Gill and C.D. Pollman. 2001. Processes Influencing Rainfall Deposition of Mercury in Florida. *Env. Sci. Technol*., 35: 863-873.

Haake, P.W. and J.M. Dean. 1983. Age and Growth of Four Everglades Fishes Using Otolith Techniques. Report to the National Park Service, Everglades National Park, Homestead, FL. May 1983.

Hakanson, L. 1980. The Quantification Impact of pH, Bioproduction and Hg-Contamination on the Hg Content of Fish (Pike). *Environ. Pollut., (Series B)*, 1: 285-304.

Haynes, J. and Cashner, C., 1995. Life History and Population Dynamics of the Western Mosquitofish: A Comparison of Natural and Introduced Populations. *J. Fish Biol.,* 46: 1026-1041.

Krabbenhoft and Orem, pers. commun., USGS meeting in Reston, VA. August 17–18, 2004.

Lange, T.R., D.A. Richard and H.E. Royals. 1998. Trophic Relationships of Mercury Bioaccumulation in Fish from the Florida Everglades. Annual Report. Florida Game and Fresh Water Fish Commission, Fisheries Research Laboratory, Eustis, FL. Prepared for the Florida Department of Environmental Protection, Tallahassee, FL.

Lange, T.R., D.A. Richard and H.E. Royals. 1999. Trophic Relationships of Mercury Bioaccumulation in Fish from the Florida Everglades. Annual Report. Florida Game and Fresh Water Fish Commission, Fisheries Research Laboratory, Eustis, FL. Prepared for the Florida Department of Environmental Protection, Tallahassee, FL.

Liang, L., M. Horvat, F. Feng, L. Shang, H. Li and P. Pang. 2004. Re-evaluation of Distillation and Comparison with HNO_3 Leaching/Solvent Extraction for Isolation of Methylmercury Compounds from Sediment/Soil Samples. *Applied Organometallic Chemistry*, 18: 264-270.

Loftus, W.F., J.C. Trexler and R.D. Jones. 1998. Mercury Transfer through the Everglades Aquatic Food Web. Final Report submitted to the Florida Department of Environmental Protection, Tallahassee, FL.

Mercury Technical Committee. 1991. Interim Report to the Florida Governor's Mercury in Fish and Wildlife Task Force and Florida Department of Environmental Regulation. Center for Biomedical and Toxicological Research, Florida State University, Tallahassee, FL. 60 pp.

Nilles, M. 2004. The Mercury Deposition Network (MDN) – National Status and Trends. Presented at the U.S. Geological Survey 2004 Mercury Workshop (August 17–18, 2004) Reston, VA.

Niu X.F. and A. Tintle. 2005. Statistical Analysis and Summary of the HgRR6 Mercury Round Robin Data. Report to the Florida Department of Environmental Protection, Tallahassee, FL. April–May 2004. Online at http://www.dep.state.fl.us/labs/everglades/index.htm (April–May 2005).

Renner, R. 2001. Everglades Mercury Debate. *Environmental Science and Technology*, 35: 59A-60A.

Rumbold, D.G. 2005. Appendix 2B-1: Annual Permit Compliance Monitoring Report for Mercury in Downstream Receiving Waters of the Everglades Protection Area. G. Redfield, ed. In: *2005 South Florida Environmental Report,* South Florida Water Management District, West Palm Beach, FL.

Rumbold, D.G. and P. Rawlik. 2000. Appendix 7-2: Annual Permit Compliance Monitoring Report for Mercury in Stormwater Treatment Areas and Downstream Receiving Waters. G. Redfield, ed. In: 2000 Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL.

Quevauviller, P. and M. Horvat. 1999. Artifact Formation of Methylmercury in Sediments. *Anal. Chem.*, 71: 155-156A.

Sepulveda, M., P.C. Frederick, M.S. Spalding and G.E. Williams Jr. 1999. Mercury Contamination in Free-Ranging Great Egret Nestlings (*Ardea albus*) from Southern Florida, U.S.A. *Environ. Tox. Chem.,* 18: 985-992.

SFWMD. 1999. Quality Assurance Project Plan: Mercury Monitoring in Stormwater Treatment Areas and Downstream Receiving Waters. Approved by Department of Environmental Protection, Quality Assurance Section. May 1999. South Florida Water Management District, West Palm Beach, FL.

Stober, Q.J., K. Thornton, R. Jones, J. Richards, C. Ivey, R. Welch, M. Madden, J. Trexler, E. Gaiser, D. Scheidt and S. Rathbun. 2001. South Florida Ecosystem Assessment: Phase I/II – Everglades Stressor Interactions: Hydropatterns, Eutrophication, Habitat Alteration, and Mercury Contamination (Summary). EPA–904-R-01-002. USEPA Region 4 Science & Ecosystem Support Division, Water Management Division, and Office of Research and Development, Athens, GA.

USEPA. 1993. Statistical Methods for the Analysis of Lake Water Quality Trends. EPA-841-R-93-003. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

USEPA. 1997. Mercury Study Report to Congress. Vol. VI: An Ecological Assessment for Anthropogenic Mercury Emissions in the United States. EPA-452/R-97-008. U.S. Environmental Protection Agency, Washington, D.C.

USEPA. 1998. South Florida Ecosystem Assessment. Volume 1. Phase I. Monitoring for Adaptive Management: Implications for Ecosystem Restoration. Final Technical Report. EPA-904-R-98-002. U.S. Environmental Protection Agency Region 4 and Office of Research and Development. Athens, GA.

Ware, F.J., H. Royals and T. Lange. 1990. Mercury Contamination in Florida Largemouth Bass. *Proc. Annual Conference of the Southeast Assoc. of Fish Wildlife Agencies*, 44: 5-12.

Wren, C.D. and H.R. MacCrimmon. 1986. Comparative Bioaccumulation of Mercury in Two Adjacent Freshwater Ecosystems. *Water Research*, 20: 763-769.

Zar, J.H. 1996. *Biostatistical Analysis* (3rd edition). Prentice-Hall, Upper Saddle River, NJ.