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

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SUMMARY

This appendix summarizes data from compliance monitoring of mercury influx and bioaccumulation in the downstream receiving waters of the Stormwater Treatment Areas (STAs) for Water Year 2004 (WY2004) (May 1, 2003 through April 30, 2004).

The key findings presented in this appendix are as follows:

1. Annual volume-weighted total mercury (THg) concentrations in rainfall were similar at all three monitoring stations (e.g., stations at the Everglades Nutrient Removal (ENR) Project, Florida Power and Light's Andytown substation, and Everglades National Park's Baird Research Center) and were elevated as compared to previous years. This among-year difference was statistically significant when 2003 concentrations were compared to concentrations in 1998, 1999, and 2002. Wet deposition (flux), which is a function of both concentration and rainfall, differed among sites in 2003. The lower deposition at the ENR Project in 2003, relative to the other two sites, was likely a result of less rainfall. Based on measured deposition, wet atmospheric loading of THg to the Everglades Protection Area (EPA) was estimated to range from 161 to 258 kilogram per year (kg per yr); the upper range exceeding loading estimates for 1994 (238 kg per yr) and for 1995 (206 kg per yr). Owing to a combination of elevated concentrations and the high annual rainfall in South Florida, wet THg deposition to the Everglades remains substantially greater than most other regions monitored by the National Atmospheric Deposition Program's (NADP) Mercury Deposition Network (MDN).

2. The maximum THg concentration observed at the 12 non-Everglades Construction Project (non-ECP) water control structures was 8.7 nanograms per liter (ng/L) observed at S-5A during the third quarter of WY2004. As such, there were no exceedances of the Florida Class III water quality standard for THg (12 ng/L) at the non-ECP structures. The maximum methylmercury (MeHg) concentration observed during WY2004 at a non-ECP structure was 0.63 ng/L, which occurred at L-28 during the third quarter. Currently, Florida has no Class III numerical water quality standard for MeHg. In general, median concentrations of THg observed at individual structures during the past four quarters were similar or lower than medians observed for the period of record. Median concentrations of MeHg observed during WY2004 were also

similar to cumulative medians. Seasonal Kendall analyses found little indication of statistically significant trends in either THg or MeHg concentration at any of the sites.

3. Mosquitofish (*Gambusia holbrooki*) collected from downstream marsh sites had Hg levels ranging from 6 to 72 nanograms per gram (ng/g) and had an average basin-wide concentration of 38 ng/g. This represents a 51-percent decrease from the 2002 basin-wide mean concentration. Owing to its small size and short lifespan, this sentinel species responds rapidly to short-term changes in ambient MeHg concentrations. This decline in Hg levels in mosquitofish appears inconsistent with increased atmospheric THg loading last year (discussed above), given what is known about the availability of new Hg (in rain) for methylation. This inconsistency cannot be explained at present but may be associated with the single annual collection of mosquitofish, which was completed over a relatively short time span in September–October 2003.

4. Sunfish (*Lepomis* spp.) collected from downstream sites had Hg levels ranging from 14 ng/g to 1,300 ng/g. Sunfish caught at sites L67F1, CA315, and the Holey Land Wildlife Management Area (WMA) had elevated levels compared to other sites. Alternatively, sunfish at sites CA3F2, L39F1, and LOX4 tended to have lower than average levels. With the exception of fish at CA2U3 and L39F1, which showed increased Hg bioaccumulation, average Hg levels at most sites were similar or lower than levels observed in 2002. The grand mean (of site means) was 168 ng/g, which represents a 14-percent decrease from the previous year.

5. Fillets from individual largemouth bass (*Micropterus salmoides*) collected from downstream sites had tissue-Hg concentrations ranging from 56 ng/g to 2,500 ng/g. Site-specific, age-standardized concentrations (estimated for a three-year-old bass) ranged from 300 ng/g to 1,556 ng/g. Bass from site L67F1 were once again found to have significantly greater THg concentrations than fish from most other sites. The basin-wide average THg concentration estimated for a three-year-old bass was 724 ng/g, which represents an 11-percent increase over the 2002 concentration.

6. Based on the U.S. Fish and Wildlife Service (USFWS) and the U.S. Environmental Protection Agency (USEPA) 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.

7. Nestling feathers from great egrets (*Ardea alba*) collected from two colonies in WCA-3 contained Hg at concentrations ranging from 0.27 to 4.8 micrograms per gram (µg/g), with an overall mean concentration (two colonies pooled) of 1.4 ± 1.1 µg/g. Standardized feather-Hg concentration was found to be much reduced compared to standardized concentrations observed in the mid 1990s and compared to levels observed in 1999 and 2000. Based on literature-derived effects thresholds, the egret nestlings at these two colonies did not appear to be at risk of toxicological effects from MeHg in early 2004.

INTRODUCTION

This appendix is the annual permit compliance monitoring report for mercury in the downstream receiving waters of the Everglades Protection Area (EPA). This report summarizes 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 2004 (WY2004) (May 1, 2003 through April 30, 2004). For this year's reporting, the results of mercury monitoring within the STAs are presented separately in Appendix 4-4 of the *2005 South Florida Environmental Report – Volume I* (2005 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 (P) 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 the 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

The Mercury Monitoring and Reporting Program summarized below is described in detail in the Mercury Monitoring and Reporting Plan for the Everglades Construction Project (ECP), the Central and Southern Florida Project, and the Everglades Protection Area, which the District submitted to the Florida Department of Environmental Protection (FDEP), the U.S. Environmental Protection Agency (USEPA), and the U.S. Army Corps of Engineers (USACE) in compliance with the requirements of the aforementioned permits. The details of the procedures to be used in ensuring the quality of and accountability for the data generated in this monitoring program are set forth 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. The FDEP approved the QAPP revisions on June 7, 1999.

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 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**).

Rainfall

From 1992 to 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 Everglades National Park (ENP or Park) (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-10C, S-140, S-9, S-32, S-151, S-141, S-190/L-28 interceptor, S-334, and S-12D (see **Figure 2**). These samples were analyzed for THg and MeHg. These sites bracket the WCAs or are major points of inflow or outflow. Monitoring of these sites is intended to capture the effect of seasonal changes in the relative contributions of rainfall and stormwater runoff contributing to water quality entering the EPA.

Preyfish

A grab sample of between 100 and 250 mosquitofish (*Gambusia* sp.) was collected throughout the year using a dip-net at 12 downstream interior marsh sites (see **Figure 3**). Subsequently, the fish 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.

Secondary Predator Fish

Up to 20 sunfish (*Lepomis* sp.) were collected (via electroshocking) 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.

Top-predator Fish

Up to 20 largemouth bass (*Micropterus salmoides*) were collected (via electroshocking) at 12 downstream interior marsh sites (see **Figure 3**), and fillets analyzed for THg. Largemouth bass 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 this year, eggs were not collected 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 Everglades Mercury Monitoring Plan revised in March 1999 (Appendix 1 of QAPP, June 7, 1999).

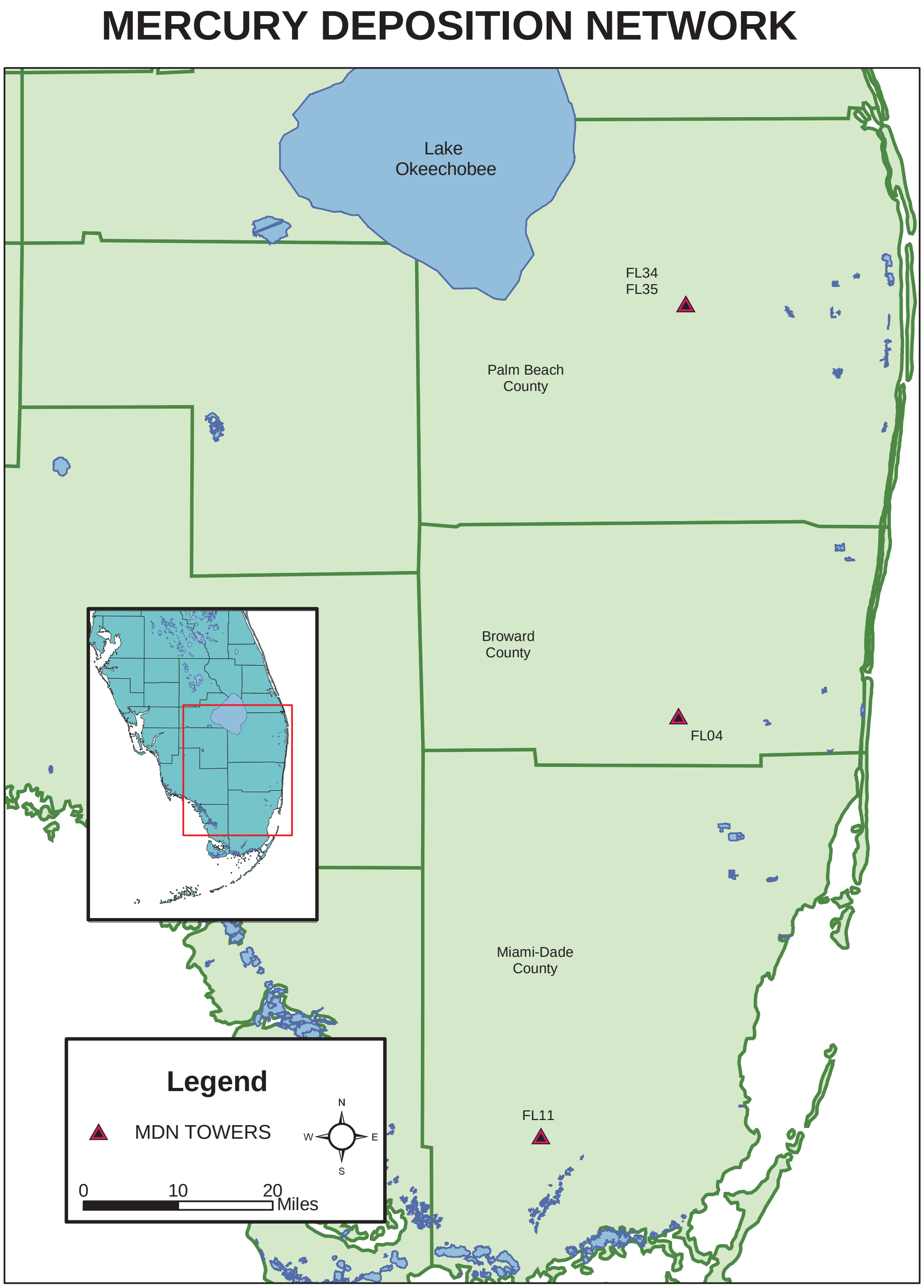

Figure 1. Map showing mercury deposition monitoring sites.

HGLE SAMPLING LOCATIONS

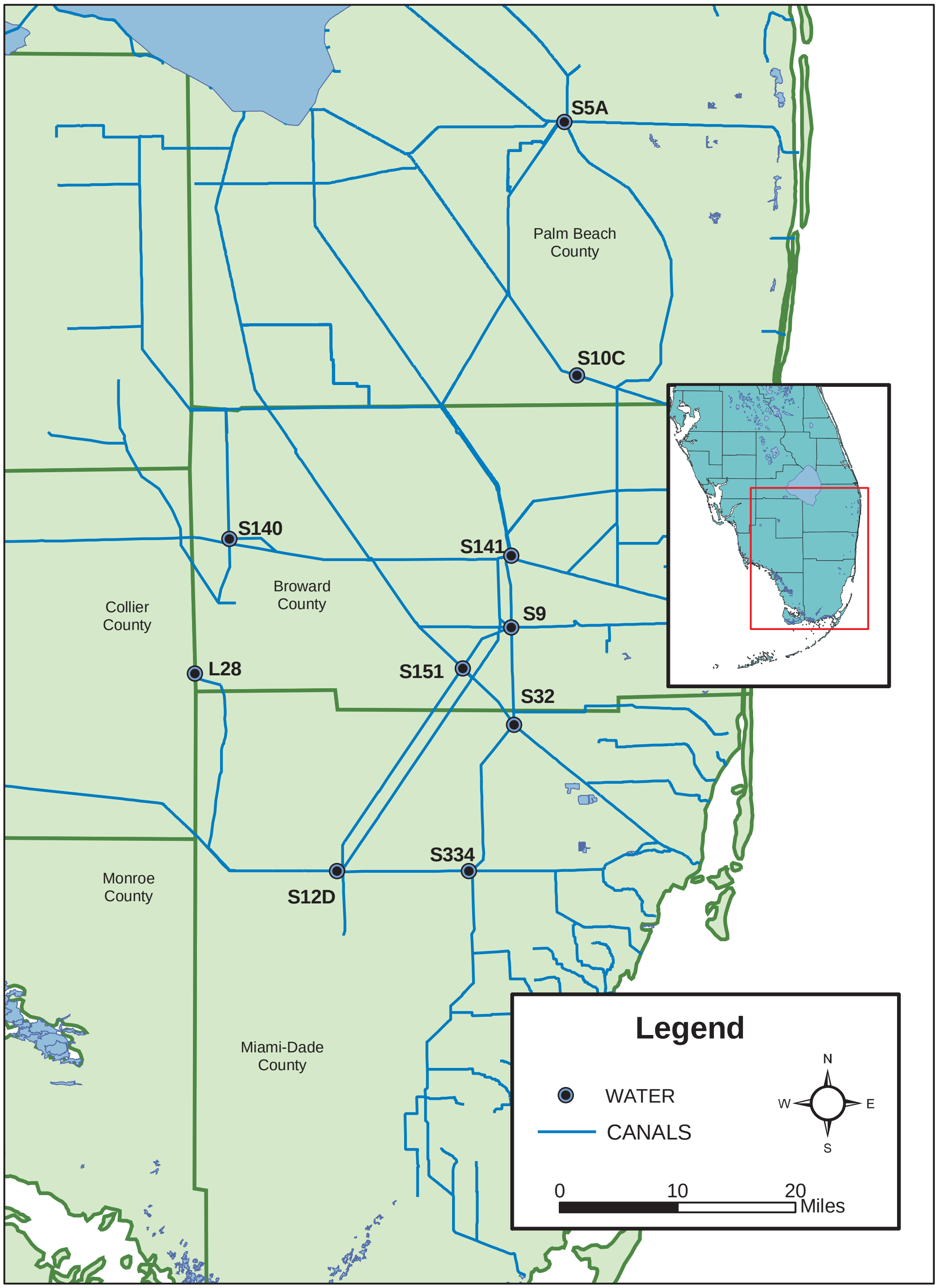

Figure 2. Map showing non-ECP structures where unfiltered surface water is collected quarterly to monitor concentrations of total mercury (THg) and methylmercury (MeHg).

HGFS SAMPLING LOCATIONS
DOWNSTREAM WATERS FISH

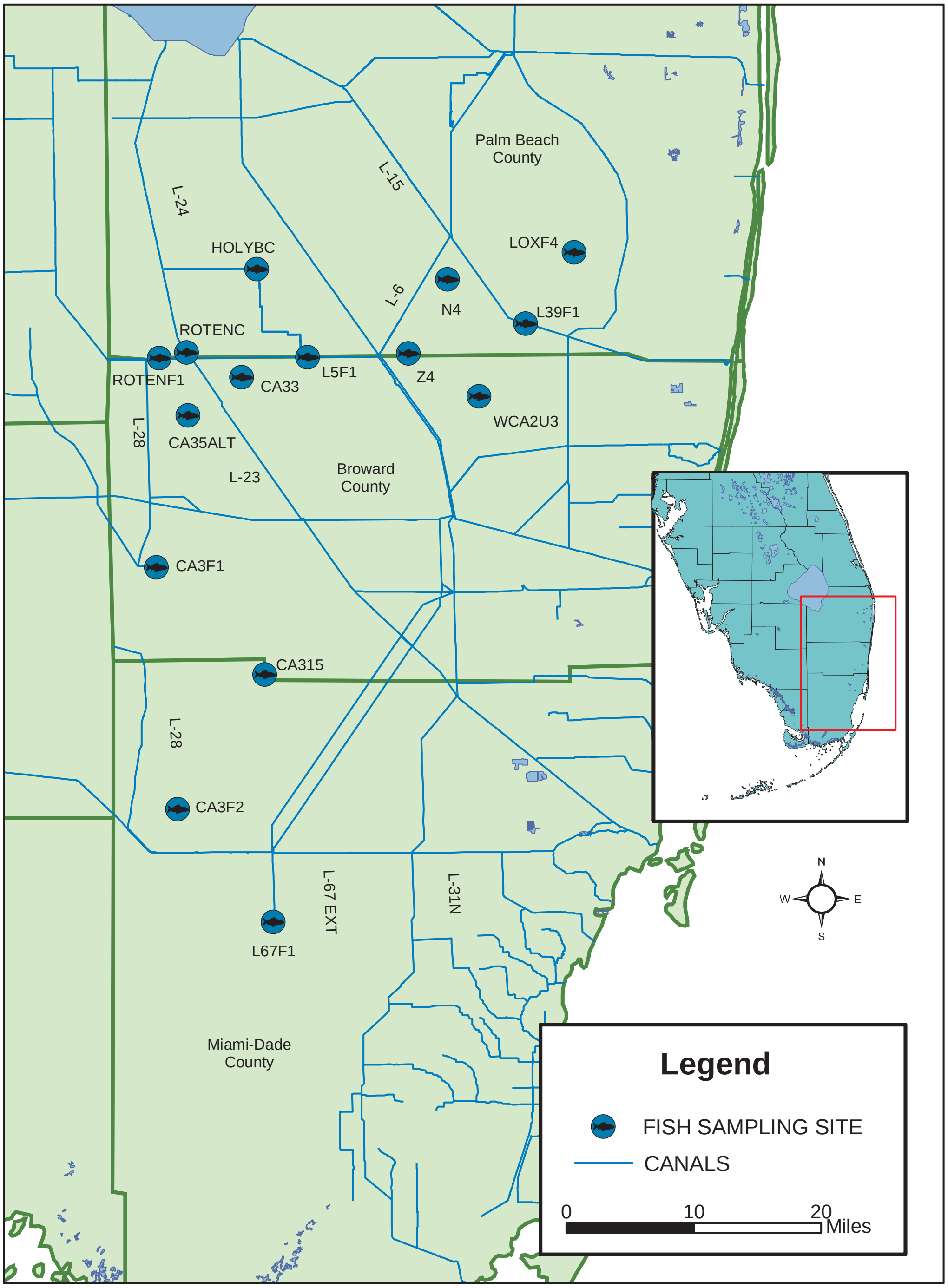

Figure 3. Map showing collection sites for monitoring Hg levels in mosquitofish, sunfish, and largemouth bass.

HGBM MERCURY SAMPLING LOCATIONS

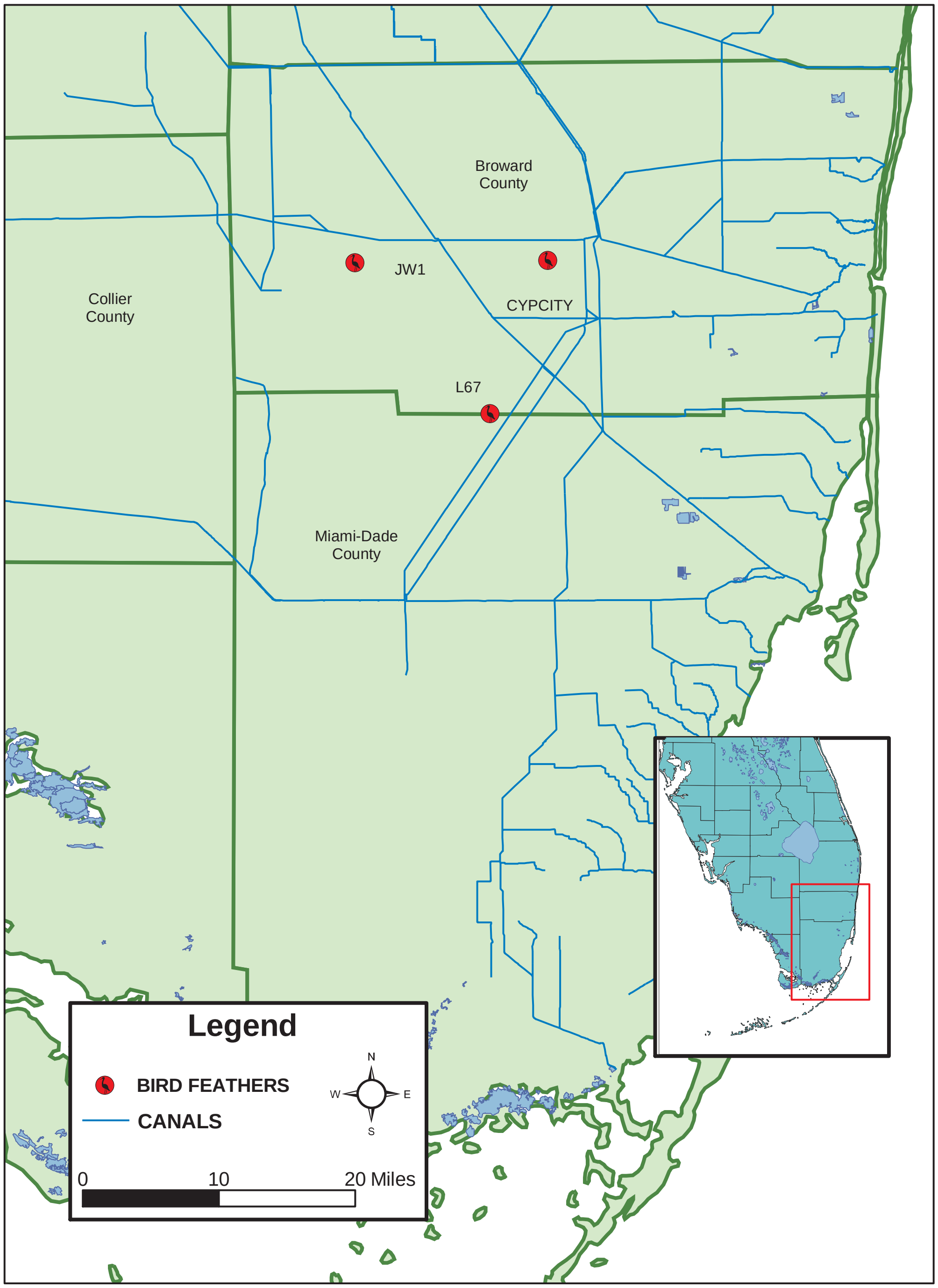

Figure 4. Map showing colonies where great egret nestling feathers are collected.

QUALITY ASSESSMENT FOR THE MERCURY MONITORING PROGRAM

(Contributed by Richard Uhler, Battelle)

The following section is a quality assessment of the District's Mercury Monitoring Program during WY2004 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 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, 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 THg and total methyl mercury during WY2004: 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 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 948 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, and STA-6, and non-ECP structures during WY2004. 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 (FB) 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 44 percent of the 2,171 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 WY2004. **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 sub-sampled in quintuplicate, and each sub-sample 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 Procedures (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 WY2004, the mean relative standard deviation (RSD), in THg concentrations, among the 157 composite triplicate aliquots was six percent (**Table 2**).

Table 1. Frequency of occurrence and mean concentration (ng/L) of THg and TMHg 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 TMHg/L.

	THg							TMHg					
FQC[1]	n[2]	Collection Frequency %	n >MDL	Mean ng/L[3]	V[4] Flagged	% Flagged		n[2]	Collection Frequency %	n >MDL	Mean ng/L[3]	V[4] Flagged	% Flagged
FKPB	73	6	3	0.311	0	0		80	6	4	0.034	0	0
EB	138	11	34	0.327	7	5		147	11	17	0.037	2	1
EB Filtered	30	3	3	0.327	0	0		30	3	3	0.063	1	3
FCEB	115	9	18	0.283	01	0		123	9	10	0.072	0	0
FCEB Filtered	1	0	0	NA	0	0		1	0	0	NA	0	0

[1]FKPB-field kit preparation blank, EB-lab cleaned equipment blank, and FCEB-field cleaned equipment blank collected at the end of the sampling event.

[2]Total number (n) of surface water samples collected during the water year was 510 THg, 110 THg dissolved, 493 TMHg, and 110 TMHg dissolved.

[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 STA1, STA2, STA-3/4, STA-5, STA-6, and non-ECP structures.

		Precision (% RSD)			
Analyte	**n**	**Minimum**	**Maximum**	**Mean**	**Median**
Surface Water THG	31	0	43	9	5
Surface Water TMHG	31	0	87*	14	8
Mosquitofish THG	157	0	17	6	5

*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, four field duplicate (FD) mosquitofish composites were collected during WY2004 (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 re-sampled population may not represent a true replicate of the first sample. The mean % RSD in THg concentrations among the four FD mosquitofish composite aliquots was 23 percent (minimum = 7 percent, maximum = 57 percent, median = 17 percent).

Interlaboratory Comparability Studies

To ensure further comparability (i.e., reproducibility) between ongoing mercury sampling initiatives, split samples of surface water were submitted to a second laboratory, Frontier Geosciences (FGS), for independent analysis of THg and MeHg, as were fish and sediment samples for the independent analysis of THg.

Water

A total of 23 water samples were collected during WY2004 and sent to FGS for independent analysis. The analytical range of concentration for THg was 0.02 ng/L (MDL) to 2.20 ng/L, with an average concentration of 1.215 ng/L for FDEP, and 1.198 ng/L for FGS. Ultra-trace THg concentrations in surface water splits exhibited considerable variance from the expected 1-to-1 line (**Figure 5**); the data were not significantly correlated (Pearson Product Moment correlation, $r = 0.31$, $p = 0.15$). However, this spread was not a result of a statistically significant (consistent) bias by one the labs (paired t-test, $t = -1.685$, $df = 22$, $p = 0.106$). The source of this variability is unknown at present. However, it should be noted that FDEP was ranked 4 and FGS ranked 4.33 (based on a 5-point scoring scale) in a recent round-robin for THg determination in water involving 10 national and international laboratories (Niu and Tintle, 2004; for previous round-robin results, see Niu and Tintle, 2003).

Ultra-trace MeHg concentrations in the 23 water splits exhibited an analytical range of concentration from 0.027 ng/L to 0.400 ng/L, with an average concentration of 0.119 ng/L for FDEP, and 0.106 ng/L for FGS. MeHg (=TMHg) results from the two labs exhibited little spread from the expected 1-to-1 line (**Figure 6**); and thus were highly correlated ($r = 0.936$, $p < 0.001$). No consistent bias was observed in MeHg determination ($t = 0.89$, $df = 22$, $p = 0.38$). In the previously discussed round-robin, FDEP ranked 4.33 and FGS ranked 5 for MeHg determination (Niu and Tintle, 2004)

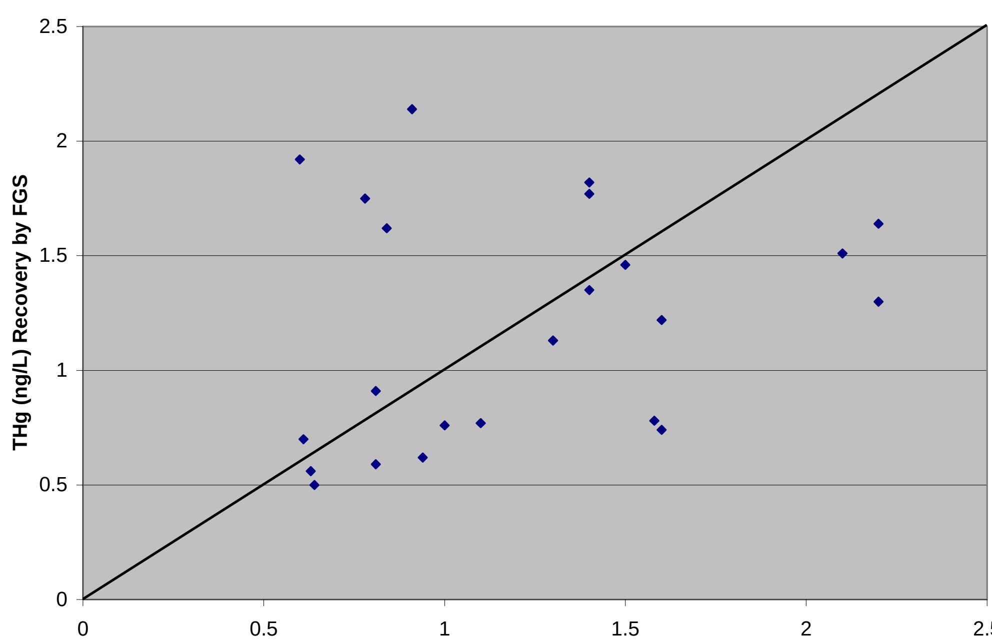

Figure 5. Interlaboratory comparison of THg determination in surface water.

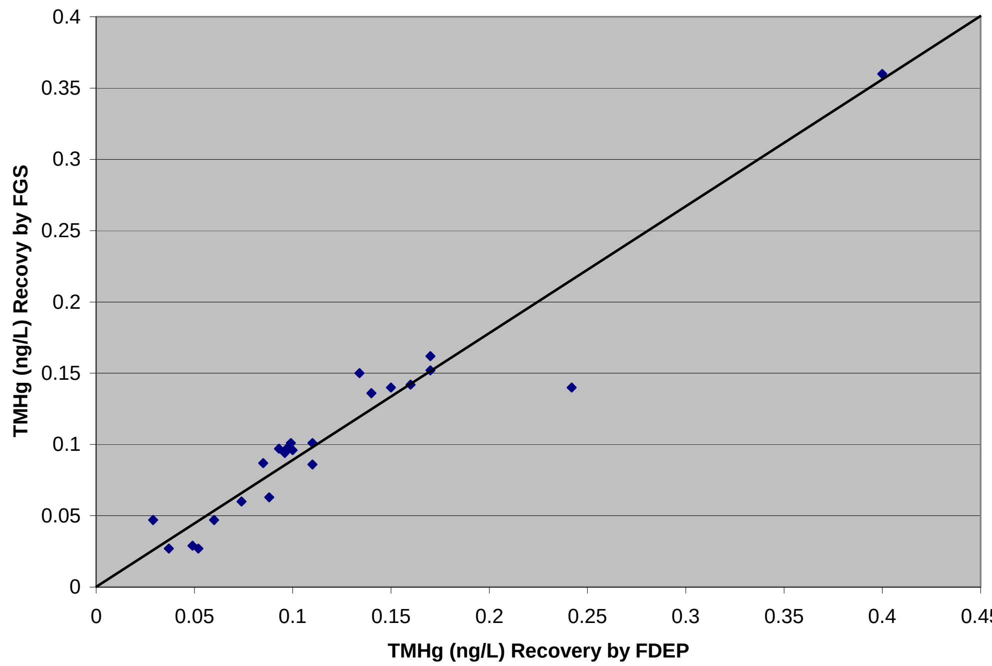

Figure 6. Interlaboratory comparison of MeHg determination in surface water.

Fish

Two mosquitofish composites collected during WY2004 were sent to FGS for independent analysis. THg concentration (average of triplicate aliquots) ranged from 0.003 to 0.023 mg/kg. The RPD between aliquots means was six percent and 67 percent (note that the latter was based on mosquitofish with very low levels: 0.003 mg/kg as reported by FDEP and 0.005 mg/kg by FGS).

A total of 77 large-bodied fish species (i.e., whole sunfish homogenates and fillets of largemouth bass) collected during WY2004 were also sent to the secondary laboratory (FGS) for independent analysis. The analytical range of concentration for THg was from 0.02 mg/kg to 0.58 mg/kg. Distributions of the two datasets were nearly identical with medians of 0.11 mg/kg (FDEP) and 0.12 mg/kg (FGS) (25^{th} percentiles were 0.058 and 0.061 mg/kg; 75^{th} percentiles were 0.223 and 0.227). Concentration of THg in the splits (Pearson Product Moment correlation, $r = 0.992$, $p < 0.001$) were highly correlated (**Figure 7**). Nevertheless, the two laboratories were found to be significantly different in a paired t-test ($t = -3.807$ with 76 df, $p \leq 0.001$); FGS reported consistently higher concentrations (**Figure 7**). However, the available data from only two labs does not allow us determination of which lab produced more accurate results.

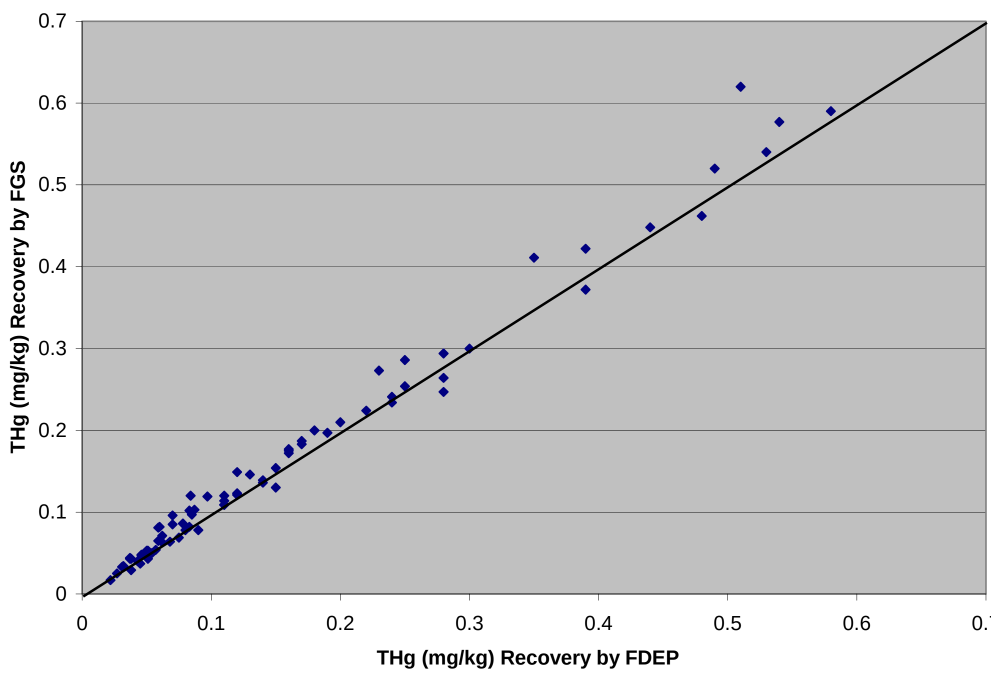

Figure 7. Interlaboratory comparison of THg determination in tissues of large-bodied fish.

Sediment

Unlike other media, sediment samples have been routinely sent to FGS as the primary laboratory due to analytical capabilities and method detection limits for MeHg. Accordingly, in the case of sediment, two split samples were sent to FDEP for independent analysis for THg. The analytical range of concentration for THg was from 0.043 mg/kg to 0.068 mg/kg (43–68 ng/g); RPDs were 1.5 and 24.4 percent, respectively.

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 data sets exhibiting seasonality, and may be used even though there are missing, tied, or nondetect 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 multi-year, multi-season data sets. It is argued that five years is a minimum data set 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). Sunfish were not aged; consequently, 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, CA) 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. Data sets 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 data sets 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 bulk rainfall were collected weekly under the protocols of the NADP MDN at the STA-1W (i.e., ENR Project), the 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 Website at http://nadp.sws.uiuc.edu/mdn/ (available as of July 21, 2004).

As presented in **Table 3** and **Figures 8** and **9**, atmospheric deposition of THg to South Florida continues to be highly variable both spatially and temporally. As observed in the past, THg concentrations in precipitation were substantially higher during the summer months (**Figure 8**), 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, the latter by a factor of 2 to 3, THg wet deposition typically increases fivefold to eightfold during the wet season (**Figure 8**). With the exception of a few stations in the Great Lakes region and an unusually elevated concentration at a New Mexico station (26.4 ng/L in 2002), Florida has some of the highest THg concentrations in the MDN (refer to http://nadp.sws.uiuc.edu/mdn/maps/).

Annual volume-weighted THg concentrations were similar at all three sites in 2003 (**Table 3**) and were elevated as compared to previous years (**Figure 9**). A simple two-way ANOVA (assessing year and location) of annual volume-weighted mean concentrations from FAMS (Guentzel et al., 2001) and MDN revealed a statistically significant difference among years ($F = 6.14$; $df = 9, 15$; $p = 0.001$), but not among sites ($F = 1.7$; $df = 2, 15$; $p = 0.2$); assumed no interaction between factors (necessary due to lack of replication). Post-hoc, pair-wise comparisons (Tukey Test) showed LSM concentration in 1997 (16.9 ng/L) to differ from the LSM concentrations in both 1998 (12.6 ng/L) and 1999 (11.6 ng/L). Similarly, the LSM mean concentration in 2003 (16.4 ng/L; note, 2003 based on preliminary data) also differed from lows in 1998 and 1999, but also 2002 (12.9 ng/L). No other between-year comparison was significant. A seasonal Kendall analyses revealed no significant trends in monthly median THg concentrations (based solely on MDN Final data set for 1997-2002) at ENR ($n = 57$ months, $Tau = 0.046$; $p = 0.76$), Andytown ($n = 58$ months, $Tau = -0.232$; $p = 0.06$) or ENP sites ($n = 83$ months, $Tau = -0.126$; $p = 0.17$; 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-weighted monthly averages from the three sites in South Florida (i.e., residuals from regression of concentration on precipitation to adjust for "washout").

These results were inconsistent with a previous analysis by Rumbold and Fink (2003) that found significant declining trends in concentrations, rainfall or deposition at select sites. However, as cautioned by the authors of that report, only four years of data were available at that time, and most statisticians argue that five years is a minimum data set for proper use of seasonal Kendal analysis.

Table 3. Volume-weighted, biweekly mean bulk rainfall THg concentration data (ng/L) from the compliance sites of the MDN in WY2004. Note: Annual point estimates are based on calendar year.

Week ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
5/6/2003	12.2	15.6	9.3
5/20/2003	26.1	20.1	27.3
6/3/2003	9.9	12.0	8.5
6/17/2003	20.1	16.3	11.1
7/1/2003	10.0	8.3	18.8
7/15/2003	17.5	22.6	132.7
7/29/2003	33.5	26.9	60.3
8/12/2003	23.2	25.4	22.7
8/26/2003	26.4	22.0	13.7
9/9/2003	8.9	12.6	13.5
9/23/2003	21.7	24.8	19.0
10/7/2003	9.9	14.1	57.4
10/21/2003	12.6	0.0	22.1
11/4/2003	4.6	3.8	5.5
11/18/2003	12.6	7.5	13.7
12/2/2003	8.4	0.0	0.0
12/16/2003	9.0	6.3	4.1
12/30/2003	8.1	0.0	19.3
1/13/2004	0.0	0.0	0.0
1/27/2004	5.7	5.1	5.4
2/10/2004	5.7	7.3	8.4
2/24/2004	14.4	10.5	11.0
3/9/2004	9.3	4.3	9.7
3/23/2004	7.6	12.1	7.5
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[1]	16.2	16.4	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**	18.7	18.2
2003[1]	17.8	28.5	26.9

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

1 Preliminary data; final data set may use seasonal averages to estimate annual concentration and deposition where Quality Rating of a given value is C.

** Problem with capture efficiency of MDN-collector; MDN Managers provided supplement data - rainfall and, thus, deposition estimate is suspect.

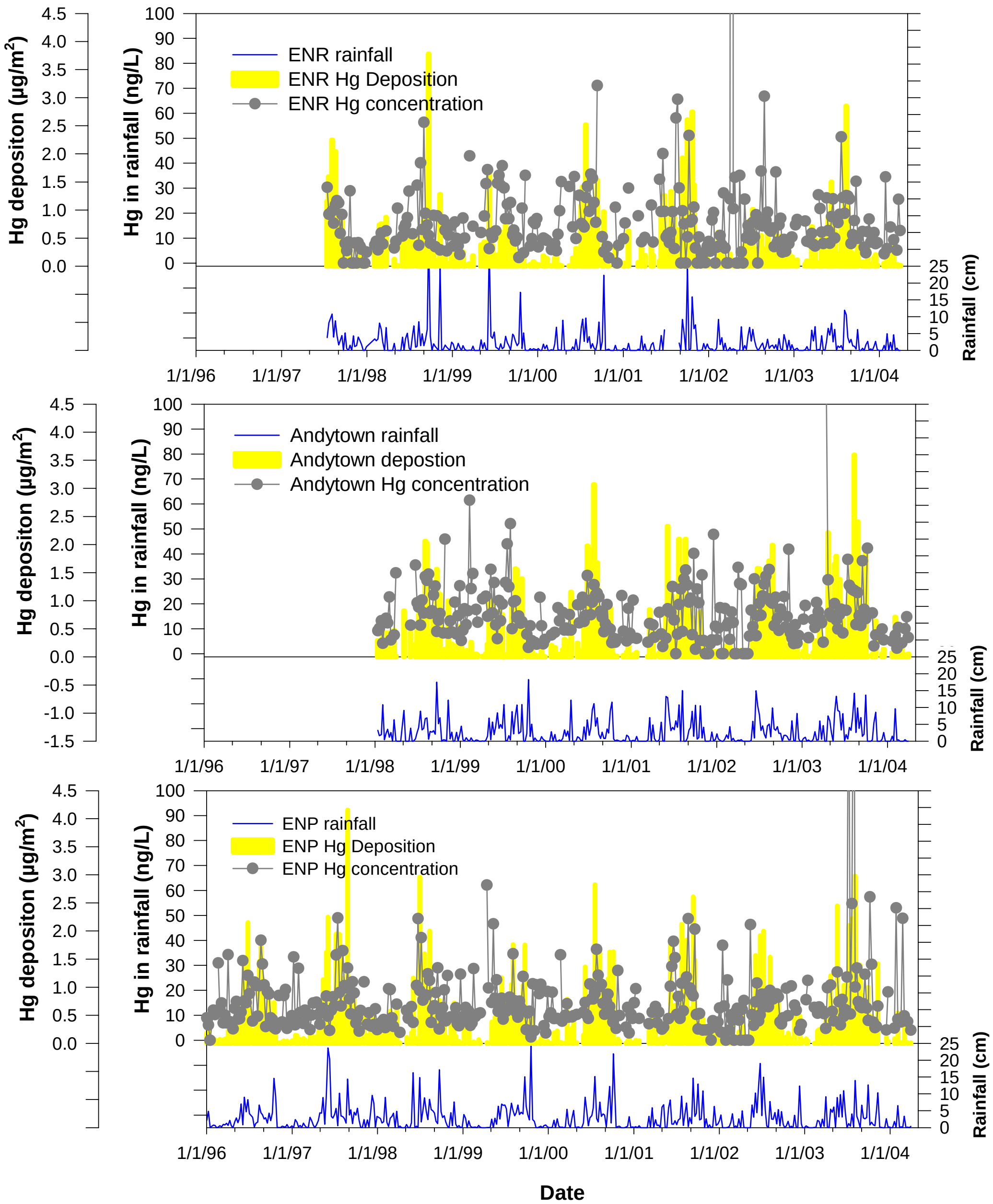

Figure 8. Time series of rainfall, rainfall Hg concentrations, and Hg rainfall deposition at the ENR Project, Andytown, and ENP Baird Research Center as reported by the MDN.

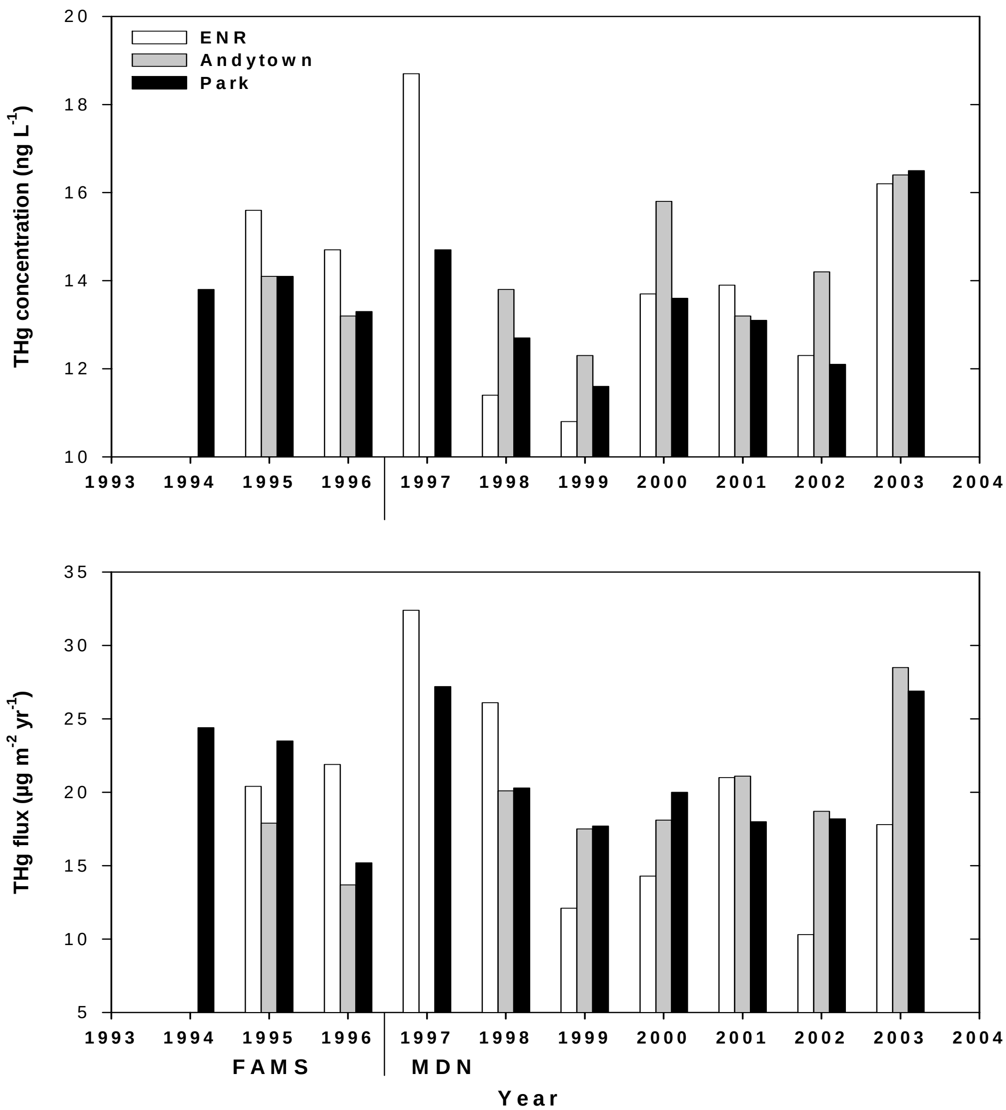

Figure 9. Time series of annual volume-weight concentration (top) and annual THg flux (bottom) at the three MDN stations (FAMS data from Guentzel et al. 2001).

It should be also recognized that the variability in seasonal rainfall (and for the reasons discussed above, THg in rain) in South Florida (e.g., wet winters, drier than usual summers) would tend to require even longer time series. The finding of no significant trends in the two recent assessments is also counter to an assessment by Atkeson et al. (in review). Using a novel approach involving the use of a seasonal dummy variable (month), they found a statistically significant decline ($p = 0.0413$) in volume-weighted concentrations at the ENP site. Their analysis indicated that volume-weighted concentrations had declined by approximately 3 ng/L between 1994–2002 due to factors other than seasonal dynamics and rainfall depth. However, it is important to note that the assessment by Atkeson et al. (in press) was completed prior to the availability of 2003 data, which, as already discussed, was markedly elevated compared to previous years.

Wet deposition (flux), which is a function of both concentration and rainfall, differed among sites in 2003 (**Table 3** and **Figure 9**). The lower deposition at the ENR site in 2003, relative to other two sites, was likely a result of less rainfall. The Belfort rain gauge recorded only 108 cm of rain at the ENR site, as compared to 173 cm at the Andytown site. Although there has been discrepancies between the ENR Belfort rain gauge and a nearby District tipping-bucket rain gauge in the past (for discussion, see Rumbold 2004), the 2003 MDN rain estimates were not inconsistent with District rain estimates (see DBKEY KN809). 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). Similar to concentration, a simple ANOVA found annual THg wet deposition differed among years ($F = 3.0$; $df = 9, 15$; $p = 0.03$), but not among sites ($F = 0.24$; $df = 2, 15$; $p = 0.79$); again, assumed no interaction between factors. Post-hoc, pairwise comparisons (Tukey Test) showed LSM deposition to differ between 1997 (29.9 µg m^{-2} yr^{-1}) and 1999 (15.8 µg m^{-2} yr^{-1}) and between 1997–2002 (15.7 µg m^{-2} yr^{-1}). Seasonal Kendall analysis again failed to show any long-term trends in the monthly deposition (final data set from 1997-2002) from ENR ($n = 59$ months, Tau = -0.034; $p = 0.83$), Andytown ($n = 60$ months, Tau = -0.067; $p = 0.62$) or ENP ($n = 84$ months, Tau = -0.111; $p = 0.24$; S. Hill, SFWMD, personal communication). Despite this lack of statistical difference in monthly deposition, annual deposition was elevated at Andytown and ENP relative to previous years (**Table 3** and **Figure 9**). Based on deposition rates measured in 2003 at all three sites, wet atmospheric loading of THg to the EPA ($9{,}054 \times 10^6$ m^2) was estimated to range from 161 to 258 kg per yr (or 0.4–0.7 kg per day). It is important to note that the upper range exceeds the USEPA (1998) estimates for atmospheric THg loading in 1994 (238 kg per yr) and in 1995 (206 kg per yr).

It should be noted that while the focus here is on wet deposition, dry deposition likely adds significantly (30–60 percent of wet deposited) to the overall atmospheric input (FDEP, 2003; Atkeson and Axelrad, 2004).

The results reported here for wet deposition of THg, along with results of monitoring of surface water at non-ECP structures (discussed in the next section), continue to show that the major source of mercury to the Everglades is from the air. 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).

In July 2001, FDEP began funding MeHg determination in four-week composite samples of bulk rainfall collected at these sites under the MDN program. Although the District is not required to summarize the results from this ancillary program, this data does provide useful information when interpreting ambient MeHg concentration in surface water. Median concentration (not volume-weight) in four-week composite samples of rainfall collected since

July 2001 was 0.12 ng MeHg/L at ENR (n = 35), 0.01 ng MeHg/L at Andytown (n = 33), and 0.06 ng MeHg/L at ENP (n = 44). Flux estimates were 5.3 ng m^{-2} for ENR, 1.1 ng m^{-2} for Andytown, and 2.8 ng m^{-2} for ENP; notice that both concentrations and flux rates are three orders of magnitude lower than for THg. These estimates were comparable to estimates reported for Minnesota (n = 284, 0.079 ng MeHg, 1.97 ng m^{-2}) and Wisconsin (n = 279, 0.078 ng MeHg, 2.6 ng m^{-2}), which have been monitoring MeHg in rain samples since 1995 [data provided by Bob Brunette, Hg Analytical Laboratory (HAL) Director, personal communication].

SURFACE WATER AT NON-ECP STRUCTURES

Table 4 and **Figures 10** and **11** 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 THg concentration observed during WY2004 was 8.7 ng/L and occurred at S-5A during the third quarter (**Figure 10**). Thus, there was no exceedance of the Florida Class III water quality standard for THg (12 ng/L) at the non-ECP structures monitored. The maximum MeHg concentration observed during WY2004 at a non-ECP structure was 0.63 ng/L, and occurred at L-28 during the third quarter of 2003 (**Table 4** and **Figure 11**). Currently, Florida has no Class III numerical water quality standard for MeHg.

In general, median concentrations of THg observed at individual structures during the past four quarters were similar or lower than medians observed for the period of record (**Table 4**), including S-5A, which has the highest median THg concentration among stations over the period of record. Median concentrations of MeHg observed during WY2004 were also similar to medians for the period of record. As observed in previous reports, THg concentrations were generally highest during the third quarter at the height of the wet season; MeHg exhibited less variability among quarters, but was highest during the second quarter (**Table 4**). When data were pooled across quarters, years and structures, median concentrations were 1 ng THg/L (n = 232) and 0.11 ng MeHg/L (n = 233).

Seasonal Kendall analyses found little indication of statistically significant trends in either THg or MeHg concentration at any of the sites. Calculated Tau values, which were based on four seasons, i.e., quarterly samples, ranged from -0.46 for THg at S-32 to +0.2 for MeHg at site L28 (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, which had P values of 0.06 (without correction for autocorrelation) and 0.02 (with autocorrelation correction). Nevertheless, caution must be exercised when interpreting these results given the period of record for this quarterly data set.

Table 4. Water column concentrations of THg and MeHg (ng/L) at non-ECP structures in 2003–2004.

Structure	Quarter	THg ng/L	remark **	WQS*	MeHg ng/L	remark **	% MeHg
L28	2nd Quarter	0.78		<WQS	0.063	I	8%
	3rd Quarter	3.00		<WQS	0.630		21%
	4th Quarter		V	<WQS	0.095		
	1st Quarter	0.80		<WQS	0.069	I	9%
	Median last 4 qt.	0.80			0.082		11%
	Median POR	1.50			0.110		11%
S10C	2nd Quarter	0.74		<WQS	0.150		20%
	3rd Quarter	0.95		<WQS	0.075	I	8%
	4th Quarter	0.51		<WQS	0.058	I	11%
	1st Quarter	0.57		<WQS	0.055	I	10%
	Median last 4 qt.	0.66			0.066		10%
	Median POR	0.95			0.081		8%
S12D	2nd Quarter	0.46		<WQS	0.091		20%
	3rd Quarter	0.96	A	<WQS	0.170		18%
	4th Quarter	0.97		<WQS	0.180		19%
	1st Quarter	0.63	A	<WQS	0.044	I	7%
	Median last 4 qt.	0.96			0.160		16%
	Median POR	0.98			0.150		15%
S140	2nd Quarter	1.30	A	<WQS	0.140		11%
	3rd Quarter	1.70		<WQS	0.120		7%
	4th Quarter		V	<WQS	0.200		41%
	1st Quarter	0.99		<WQS	0.075	I	8%
	Median last 4 qt.	1.30			0.130		10%
	Median POR	1.10			0.135		11%
S141	2nd Quarter	0.78		<WQS	0.170		22%
	3rd Quarter	1.60		<WQS	0.140		9%
	4th Quarter	0.93		<WQS	0.320		34%
	1st Quarter	0.51		<WQS	0.130		25%
	Median last 4 qt.	0.86			0.155		24%
	Median POR	1.08			0.140		14%
S151	2nd Quarter	0.91		<WQS	0.160		18%
	3rd Quarter	1.30		<WQS	0.093		7%
	4th Quarter	0.60		<WQS	0.100		17%
	1st Quarter	0.51	A	<WQS	0.086	I	17%
	Median last 4 qt.	0.76			0.096		17%
	Median POR	0.90			0.120		14%
S32	2nd Quarter	0.84		<WQS	0.085	I	10%
	3rd Quarter	1.00		<WQS	0.048	I	5%
	4th Quarter	0.60		<WQS	0.120		20%
	1st Quarter	0.57		<WQS	0.089		16%
	Median last 4 qt.	0.72			0.087		13%
	Median POR	0.85			0.120		14%

Table 4. Continued.

Structure	Quarter	THg ng/L	remark**	WQS*	MeHg ng/L	remark**	% MeHg
S334	2nd Quarter	0.74		<WQS	0.110		15%
	3rd Quarter	0.66		<WQS	0.130		20%
	4th Quarter	0.37	I	<WQS	0.078	I	21%
	1st Quarter	0.66		<WQS	0.110		17%
	Median last 4 qt.	0.66			0.102		18%
	Median POR	0.86			0.121		15%
S5A	2nd Quarter	1.40	A	<WQS	0.400		29%
	3rd Quarter	8.70		<WQS	0.200		2%
	4th Quarter	0.80	A	>WQS	0.170		21%
	1st Quarter	1.50		<WQS	0.110		7%
	Median last 4 qt.	1.45			0.185		14%
	Median POR	2.05			0.120		6%
S9	2nd Quarter	0.60		<WQS	0.060	I	10%
	3rd Quarter	1.40		<WQS	0.085	I	6%
	4th Quarter	0.13	I	<WQS	0.041	I	32%
	1st Quarter	0.14	I	<WQS	0.026	I	19%
	Median last 4 qt.	0.37			0.050		14%
	Median POR	0.74			0.058		8%
	Median 03-2	0.88	(10)¶		0.14	(10)	13%
	Median 03-3	1.35	(10)		0.12	(10)	8%
	Median 03-4	0.60	(8)		0.11	(10)	20%
	Median 04-1	0.60	(10)		0.08	(10)	13%
	Cum. Median 1st Q	0.90	(65)		0.07	(57)	10%
	Cum. Median 2nd Q	0.94	(51)		0.12	(52)	14%
	Cum. Median 3rd Q	1.60	(50)		0.18	(55)	17%
	Cum. Median 4th Q	1.01	(66)		0.09	(69)	10%

*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

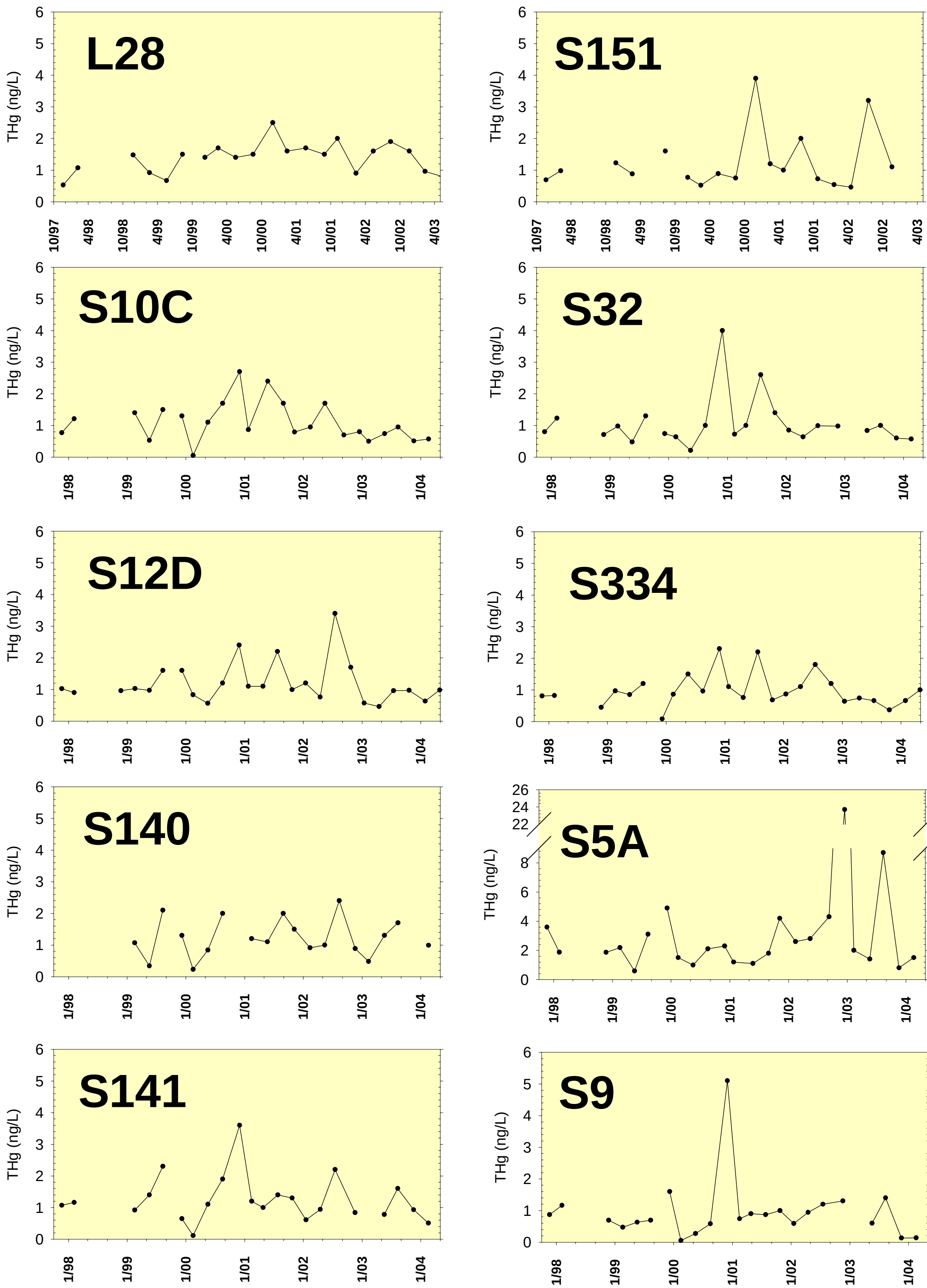

Figure 10. Concentrations of THg in unfiltered surface waters at 10 non-ECP structures for the period of record (i.e., 1997–2004). Note: Break in y-axis (THg concentration) in S-5A graph.

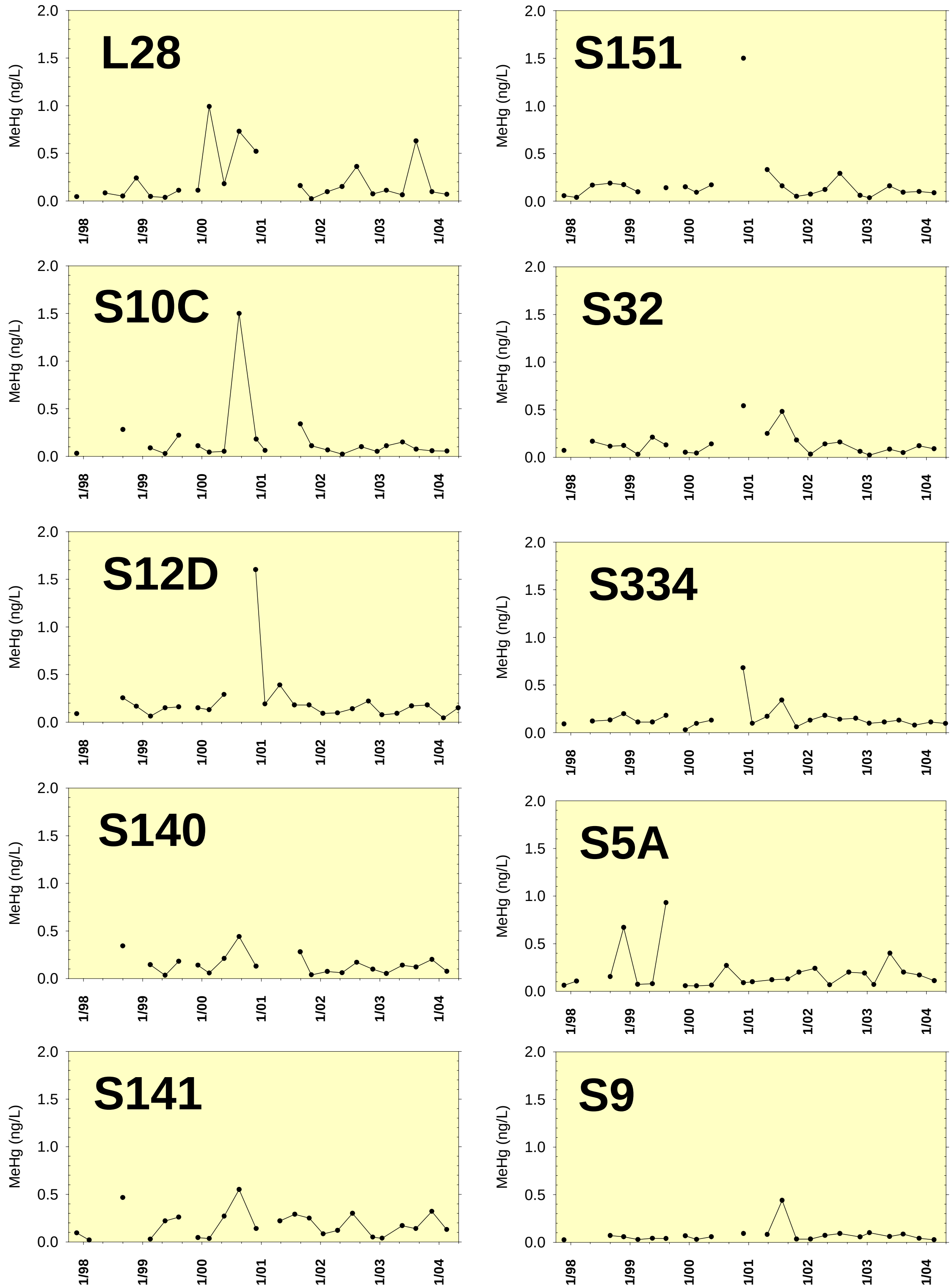

Figure 11. Concentrations of MeHg in unfiltered surface waters at 10 non-ECP structures for the period of record (i.e., 1997–2004).

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 5** through **7**, respectively. It should be noted that raw data for individual fish can be found at http://www.sfwmd.gov/org/ema/dbhydro/index.html. Fish collections were targeted at 12 downstream interior marsh sites (**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 the same water source was being sampled) 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 data sets 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 ensure that the chances of finding fish year-to-year are high). Although this may take a number of years at certain sites (e.g., 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 2003 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).

Mosquitofish

THg concentrations in mosquitofish (*Gambusia holbrooki*) collected from marsh sites in 2003 ranged from 6 ng/g at site L39F1 to 72 ng/g at site N2 (**Table 5**). The annual basin-wide average concentration was 38 ng/g (**Table 5)** (for locations, see **Figure 3**), which represents a 51-percent decrease from the 2002 basin-wide mean concentration. The 50th and 95th percentile tissue-Hg concentration in mosquitofish (i.e., aliquot means) for the period of record (1998–2003, n = 82) was 70 ng/g and 241 ng/g, respectively.

In 2003, THg levels in mosquitofish declined at most sites compared to the previous year (negative, between-year change) (**Table 5**); Holey Land and L5F1 were exceptions to this general trend. Levels have increased progressively (monotonically) in mosquitofish at L5F1 over the past four years. When sites sampled in three or more years were assessed, among-year differences in Hg levels in mosquitofish was statistically significant (ANOVA; df = 5,56; F = 28.6; $p < 0.001$). Pair-wise comparisons revealed levels in 2003 that differed from 1999 (Tukey HSD, $p < 0.05$; 2003 had the lowest basin-wide mean to date); 1999 levels differed from all other years as well ($p < 0.05$). As discussed in previous reports, mercury levels increased dramatically in mosquitofish in 1999 following a drydown and reflooding, decreasing substantially in 2000 and then rebounding (increasing) in 2001 (**Figure 12**). Between-year difference was also significant when 2003 levels were compared to 2001 ($p < 0.05$).

Table 5. Mean concentrations of THg in mosquitofish composites (*Gambusia* sp.) (ng/g wet weight) collected in 2003 from downstream sites. Value represents a mean of three analyses.

Location	THg (ng/g)	Between-yr. change (%)	Cum. Average (ng/g)
LOX4	NA	NA	83
CA2 F1 (L39F1)	6	NA	58
CA27 Alt (Z4)	58	-17%	70
CA27 Alt (N4)	72	-25%	118
Holey Land (North canal)	47	88%	48
Rotenberger Alt. (RotenF1)	18	-83%	109
Rotenberger Fish Camp (RotenFC)	24	-59%	41
Rotenberger rim canal (RotenC)	37	NA	37
CA2U3	51	-42%	113
CA33 Alt (L5F1)	63	21%	84
CA35alt2	35	-73%	113
Non-ECP North (CA3F1; end of L-28)	20	-53%	57
CA315	43	-43%	122
Non ECP South (CA3F2)	39	-8%	68
L67F1	46	-58%	129
annual mean	38	-51%	

NA = data not available.
Grandmean for POR (1998-03; aliquot means pooled across time and space) ±95%CI: n=82, 91±15; 50th and 95th percentile for POR is 70 ng/g and 241 ng/g, respectively.

Table 6. Mean concentrations (±1 SD; ng/g, wet weight) of THg in sunfish (*Lepomis* spp.) collected in 2003 from marshes downstream of the STAs within the EPA.

Target location	Sampling Location	Mean THg ng/g (±1SD, n)	Between-yr. change (%)	Grandmean of annual means
WCA1-LOX3	LOXF4	97 (±68, 20)	4%	138
WCA-2A F1	L39F1	90 (±88, 20)	49%	76
WCA-2A 2-7	Z4*	129 (±58,2)	-53%	148
	N4*	168 (±43,4)	NA	168
Holey Land	Holey Land	195 (±70, 20)	0%	109
Rotenberger	RotenC (canal)	179 (±208,20)	NA	179
WCA-2A U3	CA2U3	189 (±120, 20)	122%	148
WCA-3A 3	L5F1	126 (±48, 20)	-21%	96
WCA-3A 5	Alt. 2 site	167 (±99, 20)	-24%	205
Non-ECP North	CA3F1	125 (±43, 20)	-2%	122
WCA-3A 15	CA315	267 (±120, 20)	-25%	301
Non-ECP South	CA3F2	72 (±46, 20)	-31%	143
ENP P33 Marsh	L67F1	384 (±342, 20)	-9%	475
Average		196	-14%	

* 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=70, 190±33; 50th and 95th percentile site mean concentration was 145 and 436 ng/g, respectively.

Table 7. Standardized (EHg3) and arithmetic mean concentrations of THg in largemouth bass fillets (*Micropterus salmoides*) (ng/g, wet weight) collected in 2003 from ECP and non-ECP interior marsh sites.

Target Location	Sampling Location	EHg3 ± 95th CI (mean ±1SD, n) ng/g wet	Between-yr. Change (%)	Consumption advisory exceeded*	Cum. average EHg3
CA1-LOX3	LOX4	NC (1) (160±85, 20)	NA	No	501
CA2-F1	L39F1	300±57 (284±147, 20)	15%	No	285
CA2-7	Z4	NC (2) (540± NA, 1)	NA	Unknown	448
Holeyland	HOLYBC	582±109 (624±259, 20)	62%	Yes	374
Rotenberger	ROTENC	847±135 (454±397, 20)	NA	Yes	847
CA2-U3	CA2U3	752±81 (465±487, 19)	-16%	Yes	691
CA3-3	L5F1	NC (1) (843±315, 11)	NA	Likely	415
Non-ECP North	CA3F1	676±65 (575±557, 20)	19%	Yes	495
CA3-15	CA3-15	639±139 (461±240, 20)	-38%	Yes	894
Non-ECP South	CA3F2	442±152 (277±134, 9)	3%	No	436
ENP-P33	L67F1	1,556±266 (1,365±607, 20)	20%	Yes	1,332

* Florida limited fish consumption advisory threshold is 500 ng/g in 3-yr-old bass.
NC - not calculated for: (1) insignificant slope or (2) if poor age distribution. NA - not available.
Annual average EHg3 = 724 ng/g
Grandmean of site EHg3 for POR +95%CI: n = 40, 618 ±109 ng/g;
50th percentile EHg3 = 505 ng/g; 95th percentile = 1,327 ng/g.

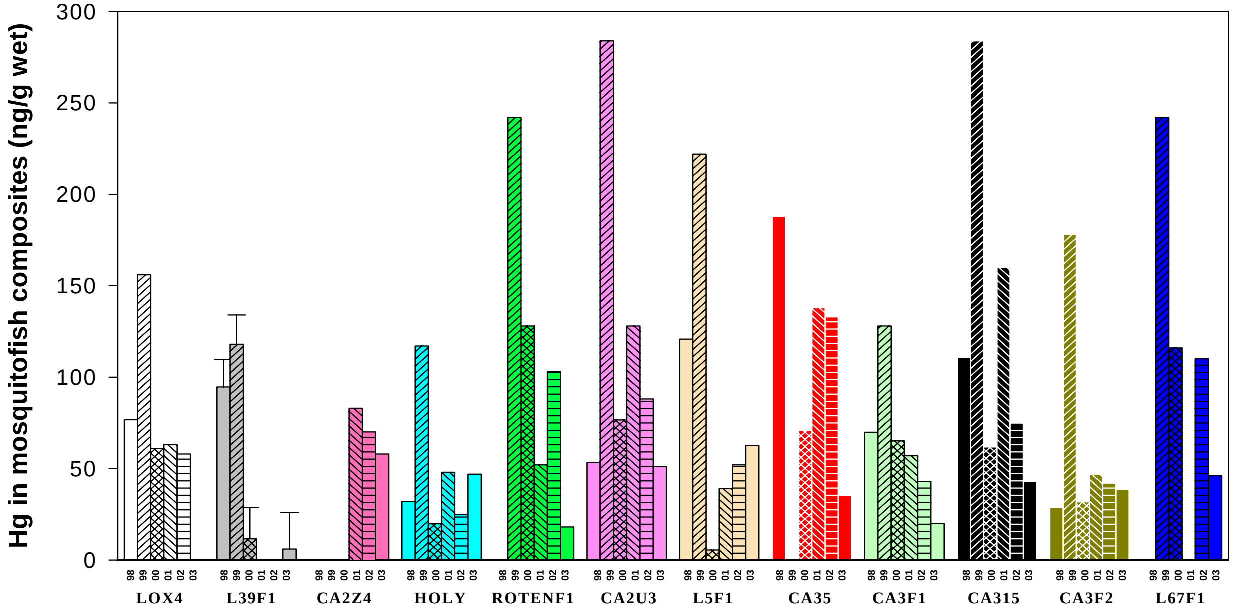

Figure 12. Hg concentrations in mosquitofish (*Gambusia* sp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2004). Not all sites were sampled in all years (for details, see **Table 5**).

Mosquitofish were selected as a representative, early-warning indicator (rapid responder) of MeHg availability because of their small size, short lifespan, relatively quick population turnover rate and low trophic position. Given the increase in 2003 in atmospheric THg loading and what is known regarding the availability of this new Hg for methylation (Krabbenhoft, USGS, personal communication), the decrease in Hg levels in the mosquitofish in 2003 seems counterintuitive. This apparent inconsistency cannot be explained at present, but may stem from the fact that the single annual collection of mosquitofish is completed over a limited time frame in September and October. Alternatively, one of the other influential factors (e.g., sulfate availability; see discussion regarding Hg levels in bass at CA315) may be controlling net methylation. The assessment of the impact of atmospheric loading in 2003 may not be fully complete until 2004 collections of large-bodied fish, which integrate exposure over the entire year, especially first-year bass.

Sunfish

THg concentration in sunfish (*Lepomis* spp.) collected from downstream sites in 2003 ($n = 226$) ranged from a low of 14 ng/g in a redear sunfish (*L. microlophus*) from site L39F1 to as high as 1,300 ng/g in a warmouth (*L. gulosus*) from L67F1 (**Table 6**). The grand mean of site means was 168 ng/g in 2003, which represents a 14-percent decrease from the previous year. However, as discussed below, caution should be exercised when interpreting basin-wide 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 there are one of two possible confounding factors (i.e., weight, species or both). As discussed in previous reports, attempts to use ANCOVA to evaluate patterns of mercury concentrations in sunfish using weight as a covariate were often inappropriate 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 in the past, when data were pooled across sites, fish species was a significant factor in tissue Hg concentration in 2003 (Kruskal-Wallis ANOVA on Ranks, $df = 3$, $H=34.6$, $p < 0.001$); THg was less concentrated in *L. microlophus* (redear, median 82 ng/g) than each of the other three species (Dunn's Method, $p < 0.05$), e.g., *L. macrochirus* (bluegill, median = 140 ng/g), *L. punctatus* (spotted sunfish, median = 185 ng/g), *L. gulosus* (warmouth, median = 250 ng/g); bluegill also differed from warmouth ($p < 0.05$). Other paired comparisons were not significant ($p > 0.05$).

As shown in **Figure 13**, sunfish continued to show significant spatial variability in THg levels in 2003 ($df = 12$, $H=90.6$, $p < 0.001$). As observed in previous years, sunfish caught at site L67F1 in 2003 had significantly greater mercury content (Dunn's Method, $p < 0.05$) than fishes from a number of other sites (e.g., CA3F2, L39F1, LOX4, ROTEN, CA3F1, L5F1). Likewise, sunfish from CA315 also differed in Hg levels compared to fish from a number of other sites (e.g., CA3F2, L39F1, LOX4, ROTEN). The Holey Land WMA differed from the three sites, each containing fish with lower levels (e.g., CA3F2, L39F1, LOX4). However, these fish caught in 2003 from the Holey Land WMA were also larger (median weight was 160 g) than fish from many other sites (e.g., CA3F2, L39F1, LOX4, N4, CA35Alt, ROTEN; Dunn's Method ($p < 0.05$) and this could account for the higher Hg levels (**Figure 13**).

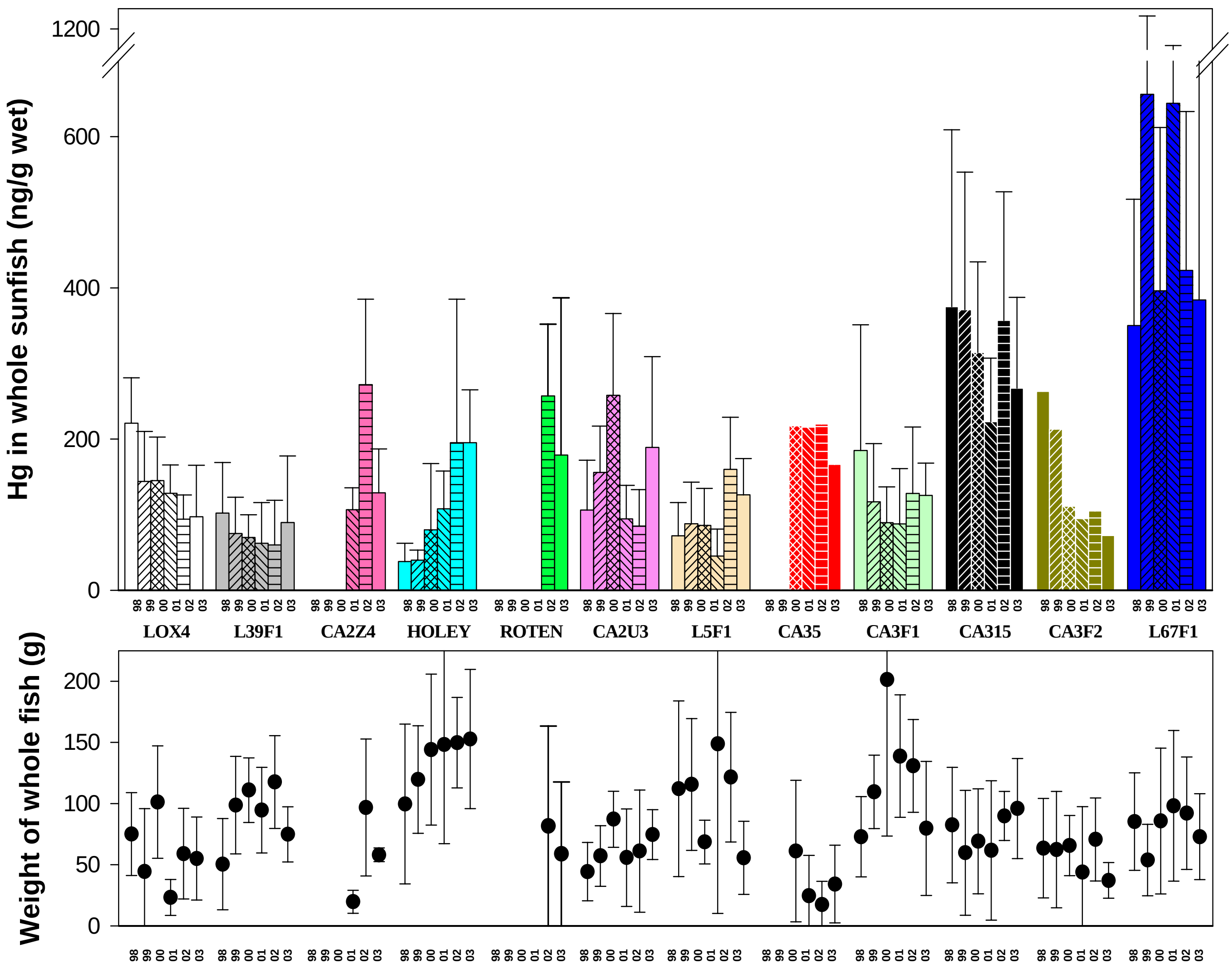

Figure 13. THg concentration (top) and weights (bottom) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2004).

With the exception of fish at CA2U3 and L39F1, average Hg levels in 2003 sunfish were similar or lower than levels observed in 2002 (**Table 6** and **Figure 13**).The between-year percent change in Hg levels (i.e., from 2002 to 2003) ranged from a 53-percent decrease at site Z4 to a 122-percent increase at site CA2U3. Although statistically significant (df = 5, H = 27.6, $p < 0.001$), the decline at LOX4 may not have been as steep as it appears in **Figure 13**. Only three fish were caught in 1998, and they were all warmouth. By comparisons, the 20 sunfish caught at LOX4 in 2003 were comprised of 35-percent warmouth, 50-percent bluegill and 15-percent redear. More informative is the finding that the 2003 levels at this site also differed from 1999–2000 (**Figure 13**), which were more appropriate comparisons due to similar species distributions. Sunfish collected from the Rotenberger WMA also exhibited between-year differences in mercury levels (df = 1, H = 5.5, $p < 0.02$), with significantly lower levels occurring in 2003 as compared to 2002. However, two different sites were sampled in 2002 and 2003 (i.e., ROTENF1, a marsh site, and ROTENC, a canal site), and spatial differences may have confounded any temporal differences. Further, only warmouth were collected in 2002, whereas four different lepomid species were collected from the canal in 2003. The warmouth were also slightly larger in size than the fish in the 2003 sample (difference was not significant; df = 1,28; F = 8.7, p = 0.06).

As reported last year, sunfish at site L5F1 contained greater concentrations of mercury in 2002, compared to each of the four previous years (df = 4, 93; F = 13.3; $p < 0.001$; post-hoc Tukey HSD); 2003 levels remained elevated and did not differ from 2002 levels ($p > 0.05$). Neither fish size nor species collected appeared to account for the increase in mercury in the last two years of the monitoring period. The likely reason for the higher MeHg in the fish was that this area supported a more favorable environment for methylation. The among-year differences in Hg burden in sunfish at CA3F2 (**Figure 13**) was also statistically significant (df = 5, H = 39.9, $p < 0.001$); with 2003 levels lower than levels in both 1998 and 1999 (Dunn's Method, $p < 0.05$). Hg levels have also varied in over time in sunfish at CA2U3 (df = 5, H = 43.7, $p < 0.001$). In a pairwise comparison, 2003 levels in sunfish at CA2U3 differed (were higher than) from 2002 levels ($p < 0.05$); this difference did not appear to be weight or species related. Among-year variability in Hg levels was also observed in sunfish collected at the Holey Land WMA (df = 5, H = 79.4, $p < 0.001$), with statistically significant pair-wise comparisons between 2003 (median = 190 ng/g) and 2000 (median = 59 ng/g), 1999 (median = 38 ng/g), and 1998 (median = 30 ng/g); 2002 Hg levels also differ from levels observed in the earlier years. As discussed in the previous report (Rumbold, 2004) the apparent continuous progressive increase in Hg levels from one year to the next (**Figure 13**) may in part be explained by differences in species of sunfish collected over time at Holey Land: redear sunfish were caught in higher proportions in 1998 (78 percent for redear) and 1999 (85 percent for redear) compared to later years (about 50 percent in 2000 and 2003) and, for the reasons stated above, this may explain the lower average mercury levels observed in those earlier years. As evident from **Figure 13**, sunfish caught at L67F1 in 2003 had tissue-Hg levels similar to fish collected in 2002 but much reduced compared to peak concentrations that occurred in 1999. 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). Nonetheless, Hg levels may have been even higher in the past in sunfish populations in the northern ENP. Levels reported for small bluegill (average length was 86 mm) caught in this general area in 1995–1998 (Loftus et al. 1998; LSM for 120 mm bluegill = 991 ±422 ng/g) were 70 percent higher than current levels (i.e., 2001–2003; LSM for 120 mm bluegill = 296 ±205 ng/g). Note that this comparison must be viewed with caution, however, due to the difference in analytical methods and instruments; no interlaboratory comparisons were available from that period.

Largemouth Bass

A total of 180 largemouth bass (*Micropterus salmoides*) were collected at 11 sites in September–October 2003. Tissue Hg concentration in individual bass ranged from a low of 56 ng/g in a fish from site L39F1 to has high as 2,500 ng/g in two different fish from sites CA3F1 and L67F1. It should be noted that the CA3F1 fish was almost 10 years old. Site-specific, age-standardized concentrations (expected for a three-year-old bass, EHg3) ranged from 300 ng/g at site L39F1, to 1,556 ng/g at site L67F1 (**Table 7** and **Figure 14**). It is noteworthy that for the first time FWC were able to collect bass from the Rotenberger WMA rim canal in 2003, and that these fish had the second highest (847 ng/g) EHg3 (**Table 7**). Calculation of EHg3 was not appropriate at sites LOX4, L5F1 or Z4 either because the tissue Hg-age relationship was not significant (first two sites) or because of small sample size (latter site). The average site-specific EHg3 value was 724 ng/g in 2003 (based on the 8 sites where it was appropriate to calculate an EHg3), which represents an 11-percent increase over the value estimated for 2002.

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 7** and **Figure 14**). Because of a statistically significant interaction between location and age ($F = 5.83$, $df = 7, 132$; $p < 0.001$), ANCOVA could not be used to assess differences in LSM Hg levels among all sites. However, when the sites were limited to CA315, ROTEN, WCA2U and L67F1 this interaction was no longer significant ($p = 0.06$), and tissue-Hg levels in bass at the latter site (L67F1) were found to differ from each of the other three sites ($F = 31.7$; $df = 3,71$; $p < 0.001$; Tukey-Kramer post-hoc test, $p < 0.05$); other pairwise comparisons were not significant.

Visual inspection of **Table 7** and **Figure 14** shows that of the sites where an EHg3 could be estimated, two showed a decrease (e.g., CA2U3, CA3-15), and three showed a marked increase (e.g., CA3F1, HOLYBC, L67F1) compared to the previous year; however, not all of these differences were statistically significant when the entire period of record was examined.

ANCOVA was not available to assess temporal differences in Hg levels in CA2U3 bass because of an interaction between year and bass age ($F = 6.97$, $df = 5, 107$; $p < 0.001$), i.e., between-year variability in slopes of regressions of Hg on age, which may hint at pulses of MeHg affecting specific age classes. Alternatively, the Hg data for CA315 bass satisfied the requirements for ANCOVA and was found to differ among years (e.g., 1999, 2002, 2003) ($F = 9.06$; $df = 2, 59$; $p < 0.001$), with levels lower occurring in 2003 as compared to other years (Tukey HSD, $p > 0.05$). This decrease has added significance because CA315 has been the recognized as the MeHg "hotspot" in the Everglades. Long-term downward trends in bioaccumulated Hg at this site and other Everglades sites have been generally ascribed to a reduction in atmospheric deposition (Atkeson and Axelrad, 2004). However, there is new evidence to suggest that sulfate concentrations have also declined at this site, which prompted some to argue that reduced sulfide reduction and methylation may in part account for the declines (D. Krabbenhoft, USGS, personal communication).

Mercury levels differed significantly among years in largemouth bass from the Holey Land ($F = 13.98$; $df = 5, 112$; $p < 0.001$), with higher levels occurring in 2003 than all other years except 2001 (Tukey HSD, $p > 0.05$). This apparent trend of increasing Hg levels in bass (**Figure 14**) as well as sunfish (**Figure 13**) suggests that conditions are becoming more favorable for methylation in the Holey Land. Accordingly, status and trends in Hg levels in these bass, along with bass from nearby Rotenberger WMA (because first sample had an elevated EHg3), warrant scrutiny in the future.

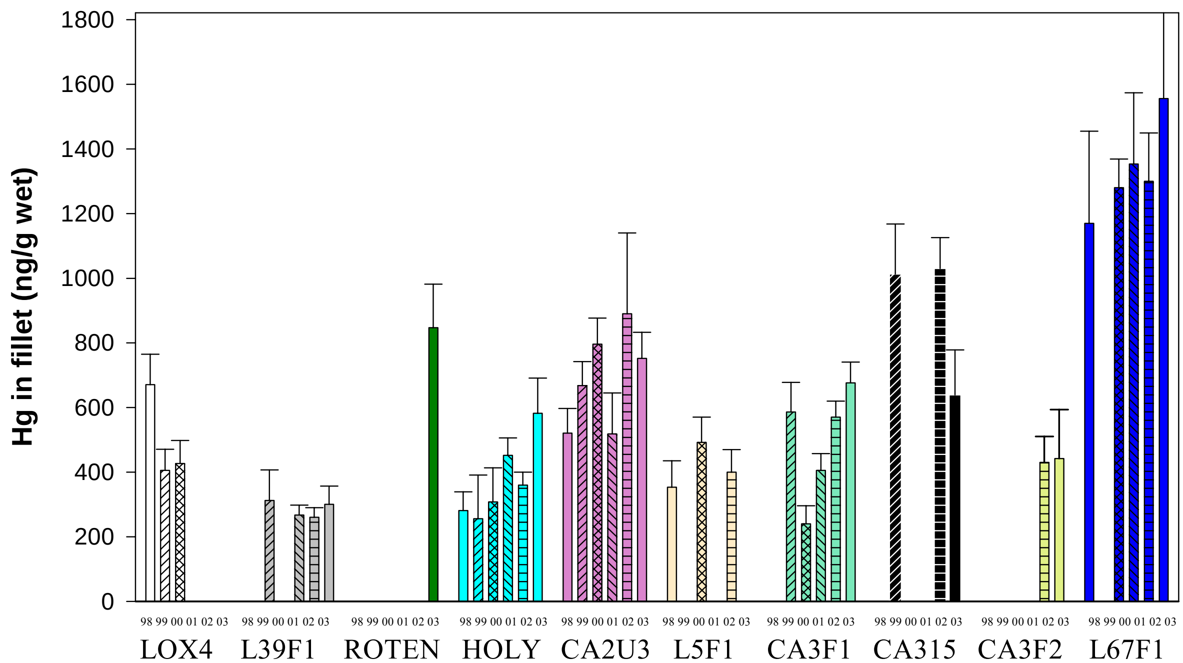

Figure 14. Standardized age (three-year-old) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at ECP and non-ECP sites for period of record (i.e., 1998–2004). EHg3 was not calculated if regressions were not significant or if age distributions were narrow (see **Table 7**).

Due to an interaction between year and age, ANCOVA was not available to assess temporal differences in bass at site CA3F1 ($F = 6.97$, $df = 5, 107$; $p < 0.001$). Finally, tissue-Hg levels did not differ significantly among years (e.g., 1998, 2000, 2001, 2002, 2003) in bass from site L67F1 ($F = 2.15$; $df = 4, 92$; $p = 0.8$). However, as was the case for sunfish, bass from the northern ENP may have had higher Hg levels in the past. Fish caught in 2003 (i.e., LSM for 280 mm bass, estimated whole-body concentration, = 704 ±101 ng/g) contained 40 percent less Hg than bass captured in this general area in 1995–1998 (Loftus et al., 1998; LSM for 280 mm bass, whole-body concentration = 1,164 ±161 ng/g). However, for the reasons discussed above, this comparison must be viewed cautiously.

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 2003, all mosquitofish (considered to be at TL 2–3, depending on age; Loftus et al., 1998) were below both the USFWS and USEPA criterion. Alternatively, sunfish, which are at TL 3 (*L. gulosus* at TL 4; Loftus et al., 1998) at 10 out of 13 sites (77 percent), contained mean THg concentrations approaching or exceeding the predator protection criteria (**Table 6**). 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 = 0.69 x fillet THg; Lange et al., 1998) approached or exceeded the guidance value for TL 4 fish at 7 out of 10 sites. 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 a probabilistic risk assessment done recently that focused on STA-2 but included other reference areas (Rumbold, in press). That assessment found current exposures continue to exceed effects thresholds, and have increased slightly over the past five years at sites within WCA-2 and WCA-3. Simulations indicated MeHg exposures were greatest at site L67F1 in the northern ENP. Although the extent of any likely adverse effects from this exposure cannot be quantified at this time, the probability of exceeding the lowest observed adverse effect level (LOAEL) suggested a high likelihood that dietary MeHg exposure to fish-eating wildlife in the northern ENP could reach a level at which some adverse effects may be expected.

WADING BIRD FEATHERS FROM ECP INTERIOR MARSHES

In 2004, feather samples were collected from 10 nests in each of the two colonies. Feather THg concentrations ranged from 0.27 µg/g to 4.8 µg/g, with an overall mean concentration (two colonies pooled) of 1.4 ± 1.1 µg/g. As evident from **Table 8**, feather Hg levels in 2004 were the lowest levels observed during the six years that the District's monitoring program has been in place, and were much lower than values reported by Frederick et al. (1997). However, caution must again be used when interpreting these results because the THg concentrations in nestling feathers are often dependent on the duration of exposure and thus the age of the bird. In recent years, regression of feather Hg concentrations on bill length (i.e., as an age surrogate) have not been statistically significant. This lack of statistical significance (i.e., concentration did not increase with age) has been interpreted as an indication that exposure had been reduced to a level such that growth dilution overwhelmed daily intake. Of interest is that in 2004 regressions were statistically significant at both the Cypress City (df = 1, 8 F = 15.5 p = 0.004) and L67 colonies (df = 1, 8; F = 5.9; p = 0.04). Based on these regressions, standardized feather-Hg concentration was found to be much reduced compared to standardized concentrations observed in the mid 1990s, and compared to levels observed in 1999 and 2000 (**Table 8**), thus confirming the initial conclusion based on non-standardized concentrations.

Establishing a benchmark for critical feather THg concentration has also been difficult because of observed or suspected interspecies differences in mercury sensitivity, particularly between piscivores and nonpiscivores, and between freshwater birds and seabirds. However, Bouton et al. (1999) and Spalding et al. (2000) reported results of a controlled dosing study that combined feather analysis with toxicological observations of great egrets. They dosed great egret juveniles with MeHg-containing gelatin capsules at 0.5 mg Hg/kg food (n = 5) and found subtle behavioral changes and statistically significant differences in blood chemistry, liver biochemistry, and weight index (Bouton et al., 1999; Frederick et al., 1979; Spalding et al., 2000). At five weeks, chicks in this dose group had 19 µg/g THg in feathers and showed a significant decline in packed cell volume (i.e., lowest observed effects level) (Spalding et al., 2000). Based on those findings, egret nestlings at the two Everglades colonies, with estimated standard concentrations ranging up to 4.7 ±2 µg/g (95th upper confidence level) in a 4–5 week old chicks (> 7.1 cm bill), do not appear to be at risk of toxicological effects from MeHg in 2004.

Table 8. THg (µg/g) concentrations growing scapular feathers collected annually from great egret nestlings at the two wading birds colonies in WCA-3A, standardized LSM for bird with 7.1 cm bill (arithmetic mean concentration ± 1SD, n).

Colony	1994 *1	1995 *	1999	2000	2001	2002	2003	2004
JW1	21 ± 6 (25 ±8, 9)	14 ±3 (NA, 8)	7 ±1 (4 ±2, 13)	7 ±1 (3 ±2, 10)	Failed to initiate nesting	Colony abandoned	Failed to initiate nesting	Failed to initiate nesting
L67	16 ± 4 (NA, 27)	16 ±6 (16 ±6, 14)	NC (4 ±2, 20)	NC (3 ±1, 10)	NC (7 ±3, 13)	NC (2 ±0.5, 6)	NC (5 ±2, 3)	3.7 ±2 (1.3 ±1, 10
Cypress City							NC (6 ±2, 15)	4.7 ±2 (1.5 ±1,10

* Data from Frederick et al. (1997).
[1] Concentrations standardized to a bill length of 5.6 cm.
NC – not calculated where slope of regression was not significant (p > 0.05).
Estimated mean age of sampled nestling, based on bill length, was 16 days in 1994, 24 days in 1995, 15 days in 1999, 16 days in 2000, 15 days in 2001 and 13 days in 2002 and 2003.

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 ECRs (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 WY2004 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. Studies and monitoring programs identified during this search are discussed below.

From February–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. Although summarized in previous years, results of the 2004 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 2004 breeding season (for details, see Chapter 6 of the 2005 SFER – Volume I; also see Cook, in prep). In 2004, the estimated number of wading bird nests in South Florida was 45,885, which represents a 36-percent increase over 2003 (see Chapter 6 of the 2005 SFER – Volume I). As was the case in the peak year of 2002 (best nesting since 1940s), the increase in 2004 over 2003 was attributed to large numbers of white ibis nesting. As observed in the past, WCA-3 supported the largest number of nests (66 percent). This was followed by WCA-1 (28 percent) and ENP (6 percent), with very few nests being found in WCA-2. This spatial pattern has been relatively consistent since 1998. As discussed previously, nesting effort is controlled by a combination of multiple factors including, but not limited to, the numbers of birds in the Everglades basin (i.e., varies depending on conditions in SE US), rainfalls during the wet season, as well as timing and rapidity of the drydown (Gawlik, 2000). At the outset conditions were favorable, as heavy rains caused reversals in the drydown and as a result, breeding success varied among species (see Chapter 6 of the 2005 SFER – Volume I).

In summary, during this reporting year the District is unaware of any evidence that would support any conclusion that wading bird foraging (or nesting) patterns have been significantly altered or impacted by construction or operation of the STAs, or that such changes in foraging patterns would have led to an increased exposure to MeHg via consumption of MeHg-contaminated fish; however, this conclusion remains tentative, pending the results from 2004 SRFs.

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