

Appendix 3B-1: Annual Permit Compliance Monitoring Report for Mercury in Downstream Receiving Waters of the Everglades Protection Area

Darren Rumbold¹, Nicole Howard, 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 calendar year 2005.

The key findings presented in this appendix are as follows:

1. Difficulties were encountered in rainfall collection for mercury analysis in 2005 due to passage of tropical storms and hurricanes (e.g., Katrina, Rita, and Wilma). Consequently, estimates for both volume-weighted (wet) concentration and wet deposition are highly uncertain for 2005, especially for values reported at the southern most site, Everglades National Park Baird Research Center.
2. The maximum total mercury (THg) concentration observed at non-Everglades Construction Project (non-ECP) water control structures was 3.3 nanograms per liter (ng/L) at S-140 during the second quarter of 2005. This value was below the Florida Class III water quality standard of 12 ng THg/L. The maximum water-column methylmercury (MeHg) concentration at a non-ECP structure was 1 ng/L, which also occurred at S-140 during the second quarter of 2005. Currently, Florida has no Class III numerical water quality standard for MeHg. After more than eight 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 mercury levels ranging from 3 nanograms per gram (ng/g) to 62 ng/g and an average basinwide concentration of 28 ng/g. This average concentration level represents a 39 percent decrease

¹ Florida Gulf Coast University, Fort Myers, FL

from the basinwide mean concentration in 2004; the basinwide concentration was also much reduced compared to the peak of 177 ng/g that occurred in 1999.

4. Sunfish (*Lepomis* spp.) collected from downstream sites had mercury levels ranging from 13 ng/g to 750 ng/g. The basinwide average concentration in sunfish was 185 ng/g, representing a 15 percent increase from the previous year. Yet, the 2005 basinwide concentration was slightly reduced as compared to the peak of 228 ng/g observed in 1998. However, as discussed in previous consolidated reports, trend analysis was confounded by differences in the size of fish collected, species of lepomid collected, or both. When the dataset was censored to only look at bluegill (*L. macrochirus*) and normalized mercury levels based on fish length, only site CA2U3 had statistically significant among-year differences when compared to 2005. Data from 2005 for site CA2U3 contained levels higher than all previous years, except 2000 and 2004, which were not included in the analysis due to small sample size.
5. Fillets from individual largemouth bass (*Micropterus salmoides*) collected from downstream sites had tissue mercury concentrations ranging from 10 ng/g to 1,700 ng/g. Site-specific, age-standardized concentrations (estimated for a three-year-old bass symbolized as EHg3) ranged from 250 ng/g to 1,130 ng/g. The basinwide EHg3 was 666 ng/g in 2005 as compared to the peak of 724 ng/g observed in 2003. Standardized mercury levels fluctuated at a number of sites in 2005 as compared to previous years, in some cases, reversing previous trends. However, mercury levels at two sites, CA2U3 and CA315, increased in both bass and sunfish.
6. Great egret (*Ardea alba*) feathers were collected from a total of 14 nestlings at two colonies in Water Conservation Area 2 in early 2006. Feather THg concentrations ranged from 1.3 micrograms per gram ($\mu\text{g/g}$) to 8.8 $\mu\text{g/g}$, with an overall mean concentration (two colonies pooled) of $4 \pm 2.4 \mu\text{g/g}$. Levels in 2005 were much reduced compared to the range of 14 $\mu\text{g/g}$ to 21 $\mu\text{g/g}$ observed in chicks in 1994 and 1995. Based on published benchmarks, egret nestlings sampled in 2006 do not appear to be at risk of toxicological effects from MeHg.
7. Although most of the trends indicate that South Florida's mercury problem has improved, a number of concerns remain. First, several areas continue to be MeHg hotspots or have shown reversing (i.e., increasing) trends in recent years, e.g., site HOLYBC in Holey Land Wildlife Management Area, site CA3F1 in Water Conservation Area 3, and site L67F1 in the Everglades National Park. Second, based on guidance from the U.S. Fish and Wildlife Service and the U.S. Environmental Protection Agency on mercury 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. Lastly, most of South Florida remains under fish consumption advisories for the protection of human health.

INTRODUCTION

This appendix is the annual permit compliance report for calendar year 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, 1 East, 2, 3/4, 5, and 6 (STA-1W, STA-1E, STA-2, STA-3/4, STA-5, and STA-6). This report includes the monitoring results in 2005. The results of monitoring mercury within the STAs are presented separately in Appendix 5-5 of the *2007 South Florida Environmental Report – Volume I*.

Following this introduction, this report consists of five main sections: (1) background, (2) summary of the Mercury Monitoring and Reporting Program, (3) quality assessment, (4) monitoring results, and (5) recommendations for optimizing the monitoring program.

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 (49,540 acres). 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 WCA-2B); Water Conservation Areas 3A and 3B (WCA-3A and WCA-3B); and Everglades National Park (Park or ENP).

Despite these legislations and goals, concerns were raised that the restoration effort might inadvertently worsen the Everglades mercury problem while reducing downstream eutrophication (Mercury Technical Committee, 1991). Mercury is a persistent, bioaccumulative, toxic pollutant that can build up in the food chain to levels harmful to human and ecosystem health. Widespread elevated concentrations of mercury were first discovered in freshwater fish from the Everglades in 1989 (Ware et al., 1990). Based on the 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 or elemental mercury problem. MeHg is more toxic and bioaccumulative than the inorganic or elemental form. Elsewhere in the world, industrial discharge or mine runoff (e.g., chlor-alkali plant in Lavaca Bay in Texas, New Idria Mine in California, and Idrija Mercury Mine in Slovenia) can contain total mercury (THg) concentrations much greater (in some areas three-hundredfold higher) than that 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 (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. This methylation process is extraordinarily effective in the Everglades, possibly due to the availability of sulfate (Gilmour and Krabbenhoft, 2001; Renner, 2001; Bates et al., 2002).

To provide assurance that the ECP was not exacerbating the mercury problem, construction and operation permits for the STAs, issued by the FDEP, required that the District monitor the levels of THg and MeHg in various abiotic (e.g., water and sediment) and biotic (e.g., fish and bird tissues) media, within both the downstream receiving waters and the STAs (Appendix 5-5 of this volume).

SUMMARY OF THE MERCURY MONITORING AND REPORTING PROGRAM

PRE-OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

Levels of THg and MeHg in various compartments (i.e., abiotic and biotic 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, the report 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.

Rainfall

From 1992 through 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 more than 95 percent of the annual mercury 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 are 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 to measure wet deposition

(i.e., dry deposition is not measured; for locations, see **Figure 1**). These samples were analyzed for THg.

MERCURY DEPOSITION NETWORK

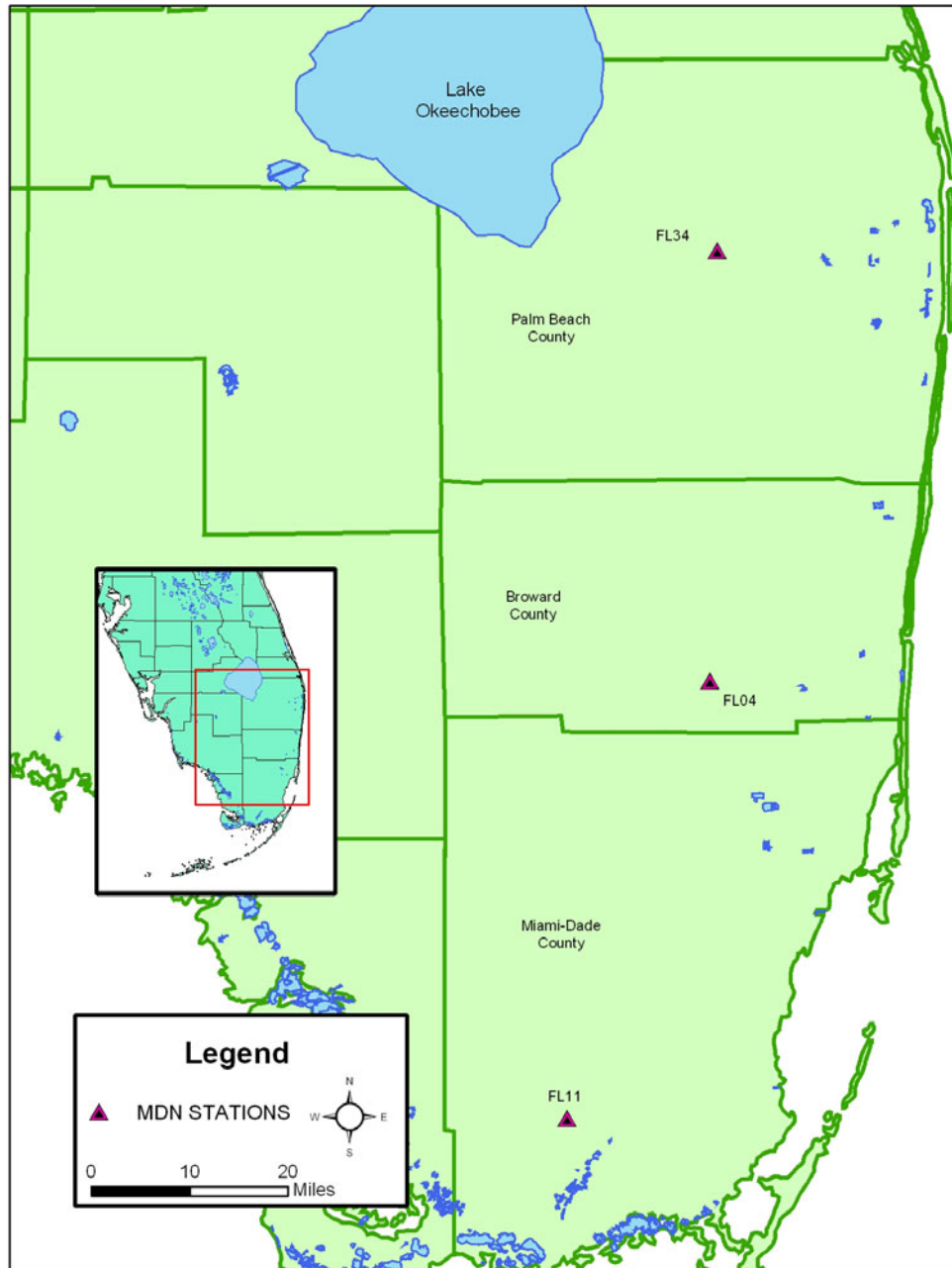


Figure 1. Map showing mercury deposition monitoring sites.

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, and S-190/L-28 interceptor (**Figure 2**). These samples were analyzed for THg and MeHg.

HGLE SAMPLING LOCATIONS

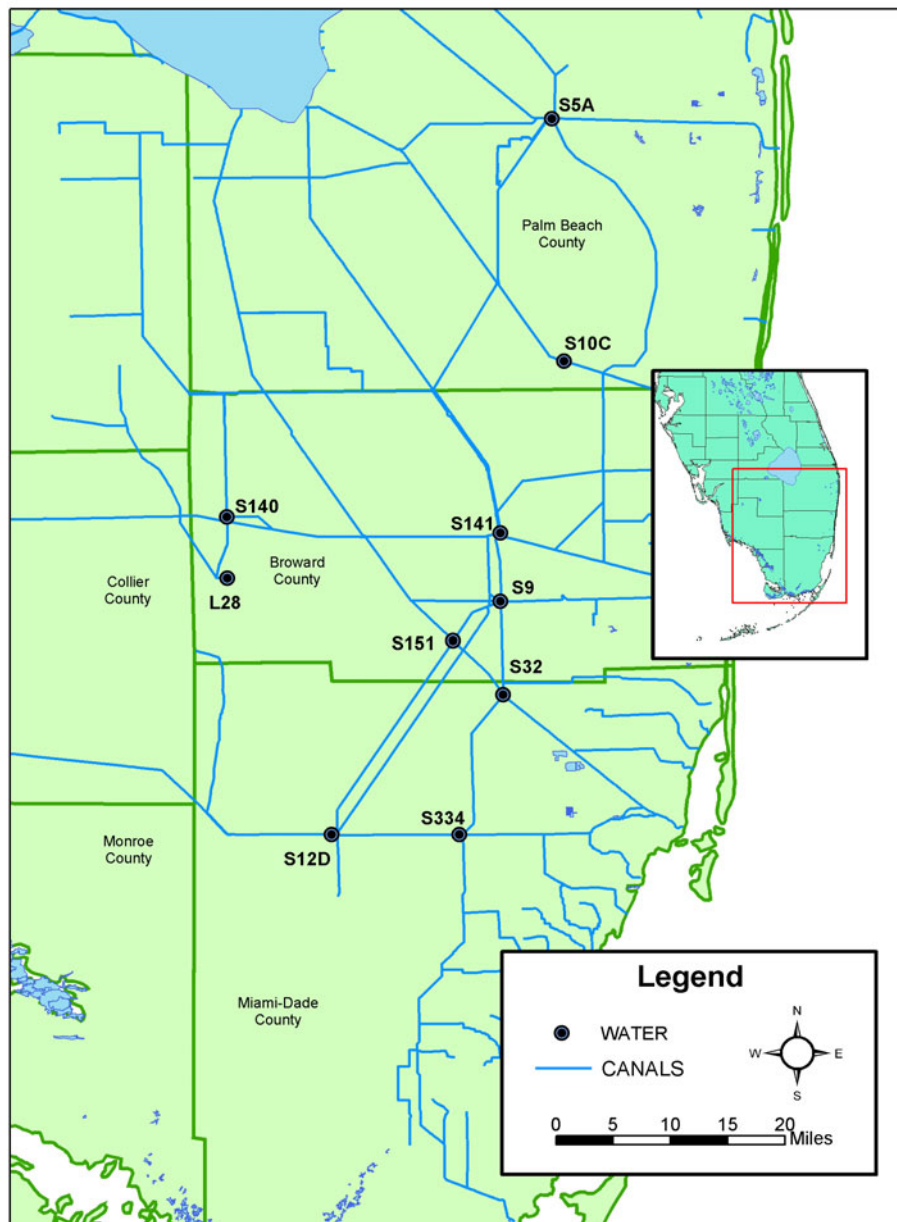


Figure 2. Map showing non-ECP structures where unfiltered surface water is collected quarterly to monitor concentrations of total mercury (THg) and methylmercury (MeHg). Not required under the permit, sampling at sites S-32 and S-334 was discontinued in October 2005.

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 (**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 three weeks and have an average lifespan of only four to five 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

Up to 20 sunfish (*Lepomis* sp.) were also collected at the same 12 downstream interior marsh sites using electroshocking techniques (**Figure 3**). Sunfish are thought to have an average lifespan of four to seven years in the wild. Each whole fish was analyzed for THg. Sunfish occur widespread and are the preferred prey for a number of fish-eating species in the Everglades; therefore, this species was selected as an indicator of mercury exposure to wading birds and other fish-eating wildlife.

Top-Predator Fish

Using electroshocking techniques, up to 20 largemouth bass (*Micropterus salmoides*) were also collected at these 12 downstream interior marsh sites (**Figure 3**); the fillets were analyzed for THg. Largemouth bass are long lived (oldest bass collected as part of this effort was nine years old) and have been monitored at several Everglades sites since 1989. Therefore, bass were selected as an indicator of potential human exposure to mercury.

HGFS SAMPLING LOCATIONS DOWNSTREAM WATERS FISH

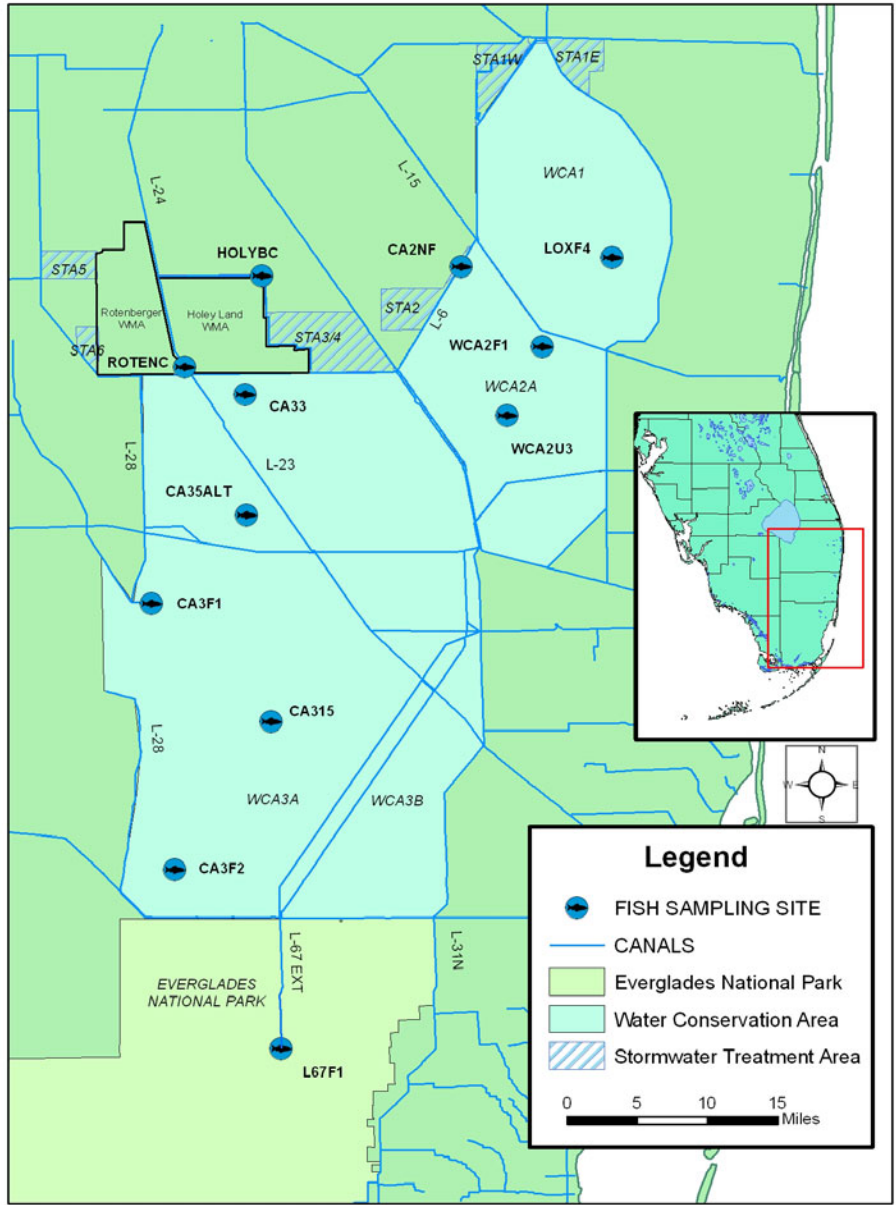


Figure 3. Map showing collection sites for monitoring Hg levels in mosquitofish, sunfish, and largemouth bass.

Tissue concentrations in each of these three monitored fish species will reflect ambient MeHg levels, i.e., their exposure is 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. Conversely, 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, sunfish and bass represent exposure at higher trophic levels (TLs) with a requisite time lag for trophic exchange. While focusing on a three-year-old bass is appropriate to evaluate exposure to fishermen, it complicates the data results by only interpreting tissue concentration integrated over a three-year period. The key is to use these species-related differences to better assess MeHg availability within the system.

More than 85 percent of the mercury found in the muscle tissue of fish is in the methylated form (Grieb et al., 1990; Bloom, 1992). Therefore, the analysis of fish tissue for THg, which is a more straightforward and less costly procedure than the analysis for MeHg, can be interpreted as being equivalent to the analysis of MeHg.

Feathers

To monitor temporal trends in mercury bioaccumulation of fish-eating wildlife, the District collects feathers from great egret (*Ardea alba*) nestlings and compares the results to similar collections made in 1994 and 1995 by Frederick et al. (1997; later published by Sepulveda et al., 1999). In accordance with the U.S. Army Corps of Engineers (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**). Under appropriate state and federal permits, feathers are collected (for THg analysis) from the oldest nestling in 10 nests in each of the two different nesting colonies. This is a modification from the sampling scheme initially proposed, which would have involved collecting molted feathers from post-breeding adults, either 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 not 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 are in the District's Everglades Mercury Monitoring Plan revised in March 1999 (Appendix 1 of the Quality Assurance Protection Plan, June 7, 1999).

HGBM MERCURY SAMPLING LOCATIONS

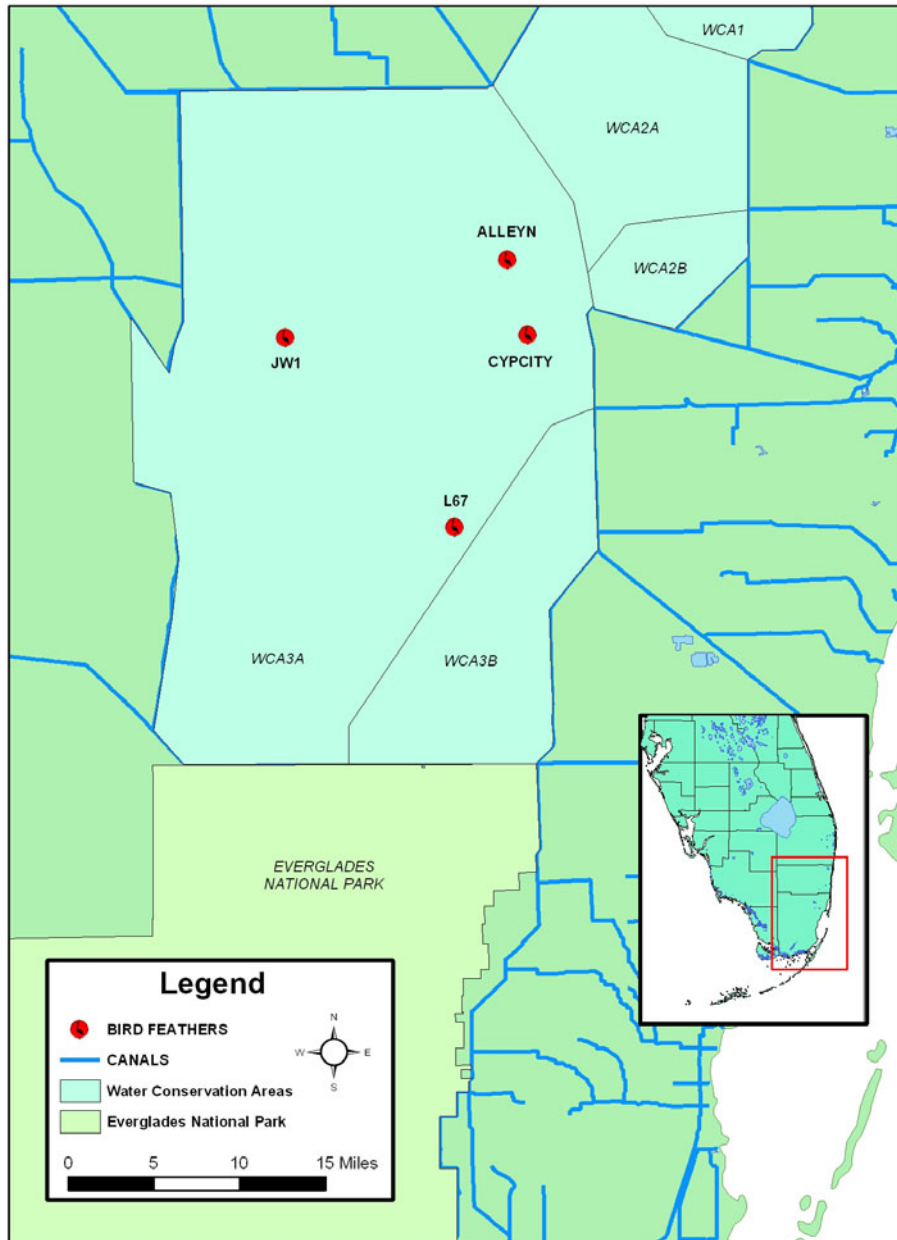


Figure 4. Map showing colonies where great egret nestling feathers have been collected. Note: Although efforts are made to repeatedly collect from the same colony, colonies are sometimes inactive or abandoned, thus, requiring collection at an alternate colony.

QUALITY ASSESSMENT FOR THE MERCURY MONITORING PROGRAM

This section is a quality assessment of the District's Mercury Monitoring Program during Water Year 2006 (WY2006) (May 1, 2005–April 30, 2006) and, where appropriate, an evaluation of the accuracy, precision, and completeness of the data quality. 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 and quality control (QA/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 implementing the project to ensure that the appropriate types 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.

QC measures are incorporated during the sample collection and laboratory analysis to evaluate the quality of the data. These 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 the use of standard reference materials. Typical external QC checks include split and blind studies, independent performance audits, and periodic proficiency examinations. Data comparability is a primary concern because mercury-related degradation of water quality is defined here as relative to baseline data generated by one or more laboratories. It is important to establish and maintain comparability of the performance and results among participating laboratories assessing the reporting units and calculations, database management processes, and interpretative procedures. Comparability of laboratory performance must be ensured if the overall goals of the Mercury Monitoring Program are to be realized.

Laboratory Quality Control

Data for this program were generated by the FDEP and Battelle Marine Sciences (BMSL) Laboratory (FDEP being the primary lab and BMSL the secondary), both of which are certified by the Florida Department of Health under the National Environmental Laboratory Accreditation Program. The following methods were utilized when analyzing samples for THg and MeHg during WY2006: 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 Accreditation Conference (NELAC), the specific reference

method, and the Mercury Monitoring Program. Methods used by both FDEP and BMSL 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 343 field QC samples, including field kit prep blanks (FKPB), equipment blanks [both laboratory-cleaned equipment blanks (EB) and field-cleaned equipment blanks (FCEB)], and replicate samples (RS) were collected for both THg and MeHg surface water samples at STA-1W, STA-1E, STA-2, STA-3/4, STA-5, STA-6, and non-ECP structures during WY2006. These field QC check samples represented approximately 36 percent of the 949 water samples collected during this reporting period. The results of the field QC blanks are summarized in **Table 1**. 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 mercury 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) add little value to the assessment of data quality and are no longer a requirement, FDEP FBs were eliminated in WY2003.

Table 1. Frequency of field QC blanks from STA-1W, STA-1E, 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					
Field QC 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	31	6.4	1	0.210	0	0	31	6.6	1	0.026	0	0
EB	47	9.8	4	0.535	2	4.3	47	10.0	11	0.030	1	2.1
FCEB	45	9.4	4	0.370	1	2.2	45	9.6	11	0.028	1	2.2
FB	2	0.4	0	N/A	0	0	1	0.2	1	0.036	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 WY2006 was 481 THg and 468 MeHg

³ Mean concentration of contaminated QC samples

⁴ Analyte was detected in the blank

N/A No Answer

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, 47 replicate, unfiltered surface water samples (23 THg and 24 MeHg) collected at STA-1W, STA-1E, STA-2, STA-3/4, STA-5, STA-6, and non-ECP structures were processed during the course of WY2006. **Table 2** reflects the results of the sample analyses.

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

Analyte	n/Sets	Precision (% RSD)			
		Minimum	Maximum	Mean	Median
Surface Water THg	23	0	38.4	13.4	11.4
Surface Water MeHg	24	0	31.0*	10.1	8.1
Mosquitofish THg	81	0	53.0	6.7	5.6

* Sample result less than PQL-associated data not flagged

MOSQUITOFISH COMPOSITE SAMPLES

To monitor spatial and temporal patterns in mercury residues in small-bodied fishes, individual mosquitofish (100 to 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 intersample 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 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 WY2006, the mean RSD in THg concentrations among the 81 composite triplicate aliquots was 6.7 percent (**Table 2**).

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. Consequently, uncertainty is introduced in the representative sample describing the population and can hinder statistical comparisons. To

assess the representative composite samples, five replicate sets (RS) of mosquitofish composites were collected during WY2006; one routine set consisting of 100 to 250 individual fish were composited along with two replica sets each consisting of 100 to 250 individual fish. Unlike abiotic media that may change little over the time period for collecting replicate samples, dip-netting mosquitofish likely disperses the local population. Consequently, the resampled population may not represent a true replicate of the first sample. The mean percent RSD in THg concentrations among the five RS mosquitofish composite aliquots was 14.5 percent (minimum = 6.7 percent, maximum = 24.2 percent, median = 14.1 percent).

Interlaboratory Comparability Studies

To ensure further 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 interlaboratory splits were performed for surface water in WY2006. In a recent “round robin” (i.e., interlaboratory comparison study) of 11 laboratories that analyzed ambient surface water samples from Florida, the FDEP was ranked as performing satisfactorily (Niu and Tintle, 2005). On a scale of 0 to 5, with 5 as the best, the FDEP ranked 3.67 for both THg and MeHg determination; FGS ranked 3.33 for THg and 4.33 for MeHg. The BMSL, which was recently contracted by the District as the secondary mercury laboratory, ranked 4.33 for THg and 4 for MeHg.

FISH

Six mosquitofish composites collected during WY2006 were sent for independent analysis. THg concentration (average of triplicate aliquots) ranged from less than the method detection limit (MDL) to 0.044 milligrams per kilogram (mg/kg). The relative percent difference (RPD) range between aliquot means was 5.3 percent and 91.4 percent.

One hundred and fifty-three large-bodied fish (whole sunfish homogenates and fillets of largemouth bass) collected during WY2006 were sent to BMSL, a secondary laboratory, for independent analysis (**Figure 5**). The analytical concentration for THg ranged from 0.0059 to 0.982 mg/kg compared to the FDEP analytical range of 0.0064 mg/kg to 0.9 mg/kg. The interlaboratory RSD ranged from 0 to 193.6 percent, with a mean of 19.6 percent. Aliquots of five fish, which had unusually high RPD between splits or that appeared as outliers, were sent in for rework; the results will be reported at a later date. A signed rank test found significant differences between the two labs for mercury levels reported in paired fish ($W = 2,633$, $p = 0.015$) with BMSL reporting a slightly higher median (**Figure 5**).

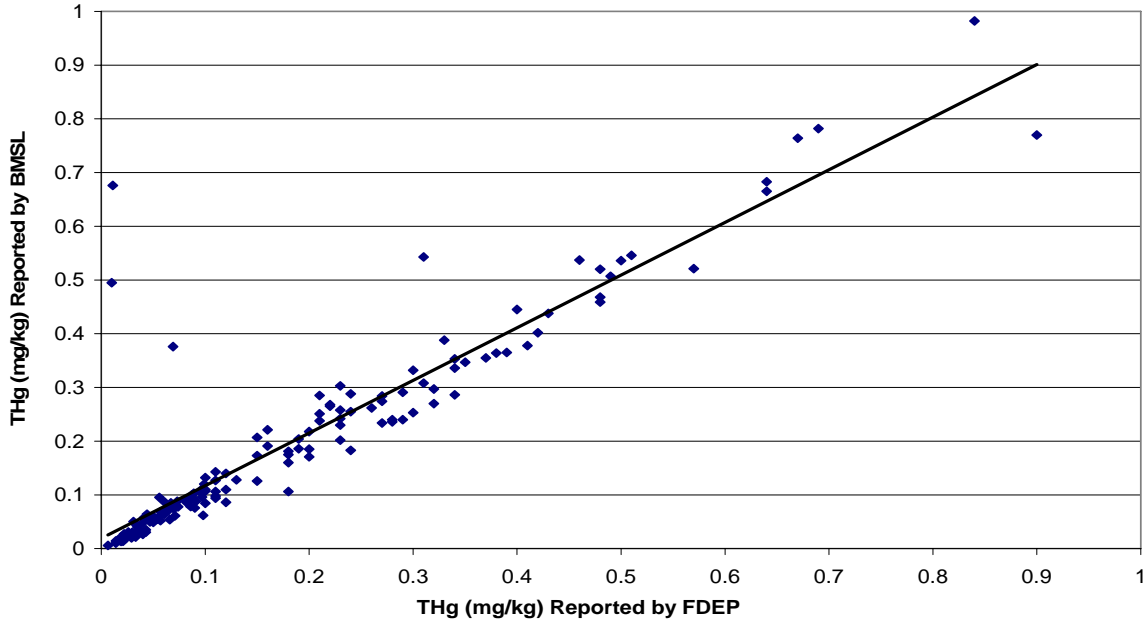


Figure 5. Interlaboratory comparison of THg determination in tissues of large-bodied fish.

SEDIMENT

Sediment split samples were collected and submitted in triplicate to three laboratories: BMSL, Frontier Geosciences (FGS), and FDEP. Analytical range for MeHg reported by BMSL was from 0.0002 mg/kg to 0.00064 mg/kg, by the FDEP was from 0.00021 mg/kg to 0.00099 mg/kg, and by FGS was from 0.00018 mg/kg to 0.00023 mg/kg. Analytical range for THg reported by BMSL was from 0.0857 mg/kg to 0.217 mg/kg, and by FGS was from 0.09 mg/kg to 0.123 mg/kg. The calculated RSD range for MeHg was 12 to 63 percent. The RSD range for THg was 4 to 38 percent.

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 multi-year, multi-season datasets. 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 obtained data, some of which were unusable due to fatal qualifiers, should be approached cautiously, and results should be viewed as approximations only.

Monitoring mercury concentrations in aquatic animals provides several advantages. However, interpretability of residue levels in animals can be problematic due to the confounding influences of the age or species. 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). 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 centimeter (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 (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 was used; possible covariates were considered separately. The assumptions of normality and equal variance were tested by the Kolmogorov-Smirnov and Levene Median tests, respectively. Datasets that either lacked homogeneity of variance or departed from normal distribution were natural-log transformed and reanalyzed. If transformed data met the assumptions, then it was 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 multi-group null hypothesis was rejected, then the groups were compared using either Tukey HSD (Honestly Significant Difference) 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/> (as of June 6, 2006). Last year's report noted that difficulties were encountered due to the landfall of four hurricanes in 2004 (Rumbold et al., 2006). In 2005, the pattern and difficulties continued with the passage or near misses of hurricanes Katrina (fourth week of August), Rita (third week of September) and Wilma (fourth week of October). In 2004, the northern most station, ENR, was most affected. In 2005, the southern station, ENP, was most significantly affected by the first two storms. During these events, the collectors recorded significant precipitation with little THg. All three collectors were non-functioning during Hurricane Wilma. Therefore, among-year differences in both volume-weighted concentration and deposition must be viewed with caution.

Notwithstanding the uncertainties caused by the storms, 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 and tall, convective thunderclouds that can scavenge particulate mercury and water-soluble reactive gaseous mercury from the middle and upper troposphere. This observation is consistent with those 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**).

Table 3. THg concentration data (ng/L; wet-only) from the compliance sites of the MDN in calendar year 2005. Annual point estimates are also based on calendar year.

Week Ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
1/4/2005	N/A	6.17	N/A
1/11/2005	N/A	N/A	10.93
1/18/2005	N/A	5.41	8.77
1/25/2005	N/A	N/A	31.47
2/1/2005	N/A	N/A	8.84
2/8/2005	N/A	21.09	24.37
2/15/2005	N/A	N/A	N/A
2/22/2005	N/A	N/A	N/A
3/1/2005	N/A	18.71	14.73
3/8/2005	5.49	6.39	8.04
3/15/2005	4.55	4.82	6.03
3/22/2005	3.08	3.66	3.88
3/29/2005	20.41	N/A	17.73
4/5/2005	13.4	N/A	N/A
4/12/2005	17.16	7.74	9.27
4/19/2005	10.99	N/A	N/A
4/26/2005	31.17	N/A	43.07
5/3/2005	27.98	69.23	N/A
5/10/2005	9.17	9.54	9.91
5/17/2005	25.21	N/A	N/A
5/24/2005	8.19	14.82	11.1
5/31/2005	18.87	17.12	32.82
6/7/2005	10.83	15.77	15.76
6/14/2005	15.31	7.73	2.77
6/21/2005	10.51	14.6	11.88
6/28/2005	12.8	10.56	17.07
7/5/2005	11.7	19	8.1
7/12/2005	17.8	N/A	7
7/19/2005	27.8	23.5	19.7
7/26/2005	31.3	38.6	15.3
8/2/2005	23.3	29.5	20.9
8/9/2005	40.8	28.9	22.3
8/16/2005	28.1	N/A	16.8
8/23/2005	14.4	31.6	15.2
8/30/2005	11.1	3.5	2.5
9/6/2005	5.5	30.4	21.7
9/13/2005	21.4	16.7	21.4
9/21/2005	N/A	N/A	5.7
9/27/2005	10.3	N/A	13.9
10/4/2005	15.27	N/A	13.01
10/11/2005	5.5	6.93	8.21
10/18/2005	7.58	19.6	14.11
11/1/2005	N/A	N/A	N/A
11/8/2005	8.32	N/A	N/A

Table 3. Continued.

Week Ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
11/15/2005	N/A	N/A	N/A
11/22/2005	9.19	N/A	9.87
11/29/2005	N/A	N/A	N/A
12/6/2005	13.18	12.8	11.69
12/13/2005	6.42	4.8	9.52
12/20/2005	5.73	22.19	5.66
12/27/2005	N/A	N/A	N/A
Volume-Weight Concentration (ng/L)			
1996*			14.1
1997*	18.7	N/A	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	13.7 ^a	14.7	14.7
2005 ^b	11.7	13.7	10.6
Deposition Annual ($\mu\text{g}/\text{m}^2$)			
1996*			17.2
1997*	32.4	N/A	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	a	18.3	18.7
2005 ^b	11.5	14.5	17.5

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

a Rain gauge malfunction; in 2004, several trips missed due to four 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

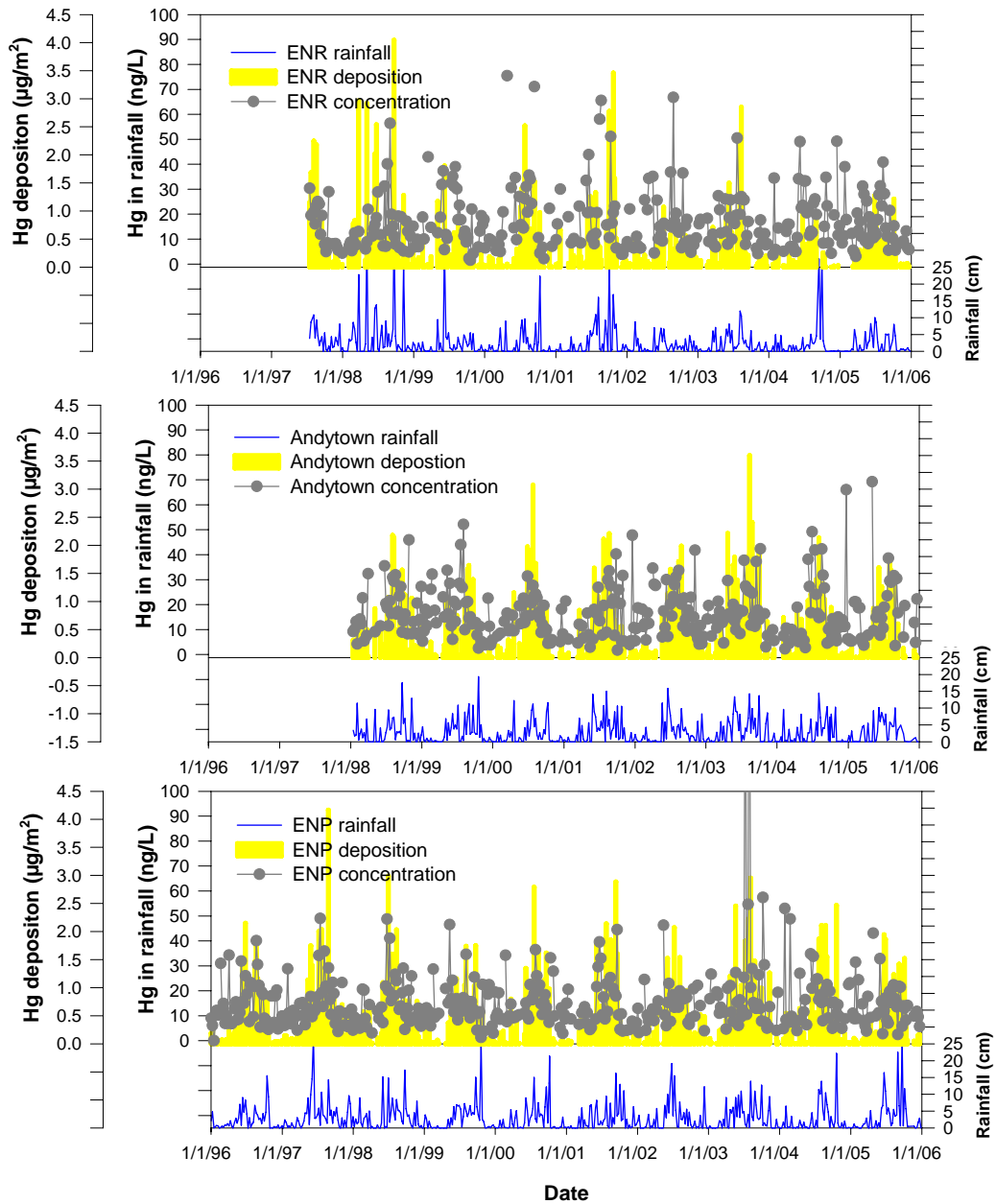


Figure 6. Time series of rainfall, rainfall Hg concentrations, and wet Hg deposition at the ENR Project, Andytown, and ENP Baird Research Center, as reported by the MDN.

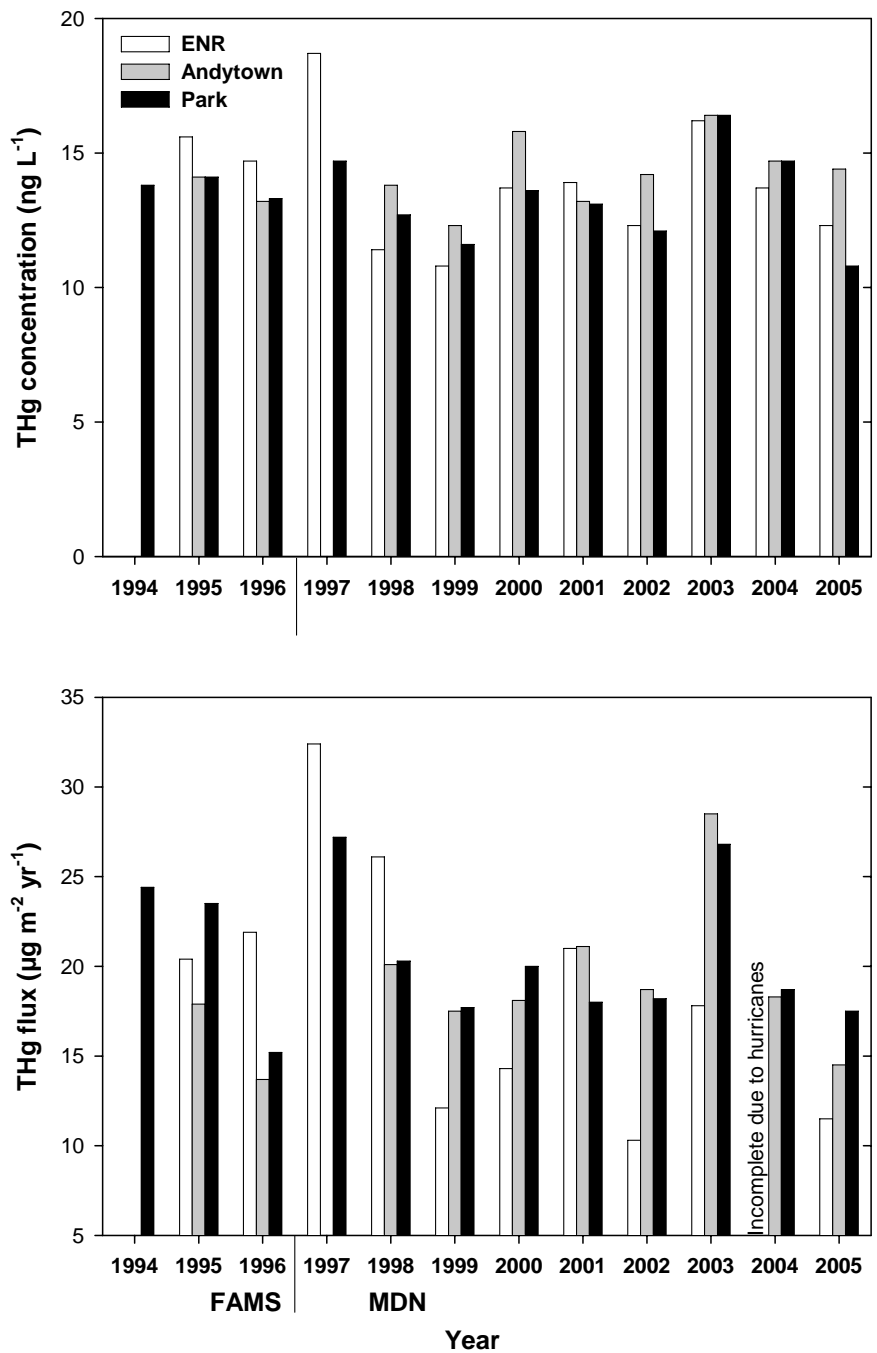


Figure 7. Time series of annual volume-weighted concentration (top) and annual THg flux (bottom) at the three MDN stations (FAMS data from Guentzel et al., 2001).

Annual volume-weighted THg concentrations differed among the three sites in 2005 (**Table 3**) with an apparent marked decline at ENP. However, if the data from the storm events are removed from the calculations for ENP, volume-weighted THg concentrations increase to over 14 nanograms per liter (ng/L; note, value reported in table).

A seasonal Kendall analyses (of ranks) revealed no significant trends in monthly median THg concentrations at ENR (1997–2005; n = 97 months; Tau = -0.037; p = 0.67), Andytown (1998–2005; n = 92 months; Tau = 0.016; p = 0.88) or ENP sites (1996–2005; n = 117 months; Tau = 0.07; p = 0.35; S. Hill, SFWMD, personal communication, June 16, 2006). 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”). Although deposition was highly variable, a seasonal Kendall analysis still did not show any long-term trends in the monthly deposition at either ENR (n = 97 months; Tau = -0.106; p = 0.21), Andytown (n = 92; Tau = -0.132; p = 0.13), or ENP (n = 117; Tau = 0) (S. Hill, SFWMD, personal communication, June 16, 2006).

Based on an average deposition rate measured at the three sites, wet-only atmospheric loading of THg to the EPA ($9.01 \times 10^9 \text{ m}^2$) was estimated at 131 kilograms per year (**Table 4**); however, this value is highly uncertain given the impacts from the storms in 2005. While the focus here is only on wet deposition, dry deposition likely adds significantly (30 to 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 ^d	3.2 ^c
2005	131 ^e	9.8 ^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 Everglades Agriculture Area (EAA) into the EPA.
- b Rumbold (2005)
- c Sum of loads at S-5A, S-6, S-7, and S-8 over Calendar Year 2005
- d Rumbold et al. (2006)
- e Value highly uncertain due to passage or near misses of hurricanes Katrina (fourth week of August), Rita (third week of September), and Wilma (fourth week of October) in 2005

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 (**Figure 2**). The maximum water-column THg concentration observed during 2005 was 3.3 ng/L that occurred at S-140 during the second quarter (**Figure 8**). This value did not exceed the Florida Class III water quality standard of 12 ng THg/L. As discussed in previous reports, when the entire period of record is examined, site S-5A had the greatest THg concentration level (median was 1.9 ng THg/L) compared to all other sites except L-28 (median = 1.48 ng THg/L) (Kruskal-Wallis ANOVA on ranks; $H = 57.3$; $df = 7$; $p < 0.0001$ using Dunn's method of pairwise multiple comparisons). The only other significant pairwise comparison was between L-28 and S-9, the latter having a lower median. Owing to pump operation, S-5A often has highly elevated total suspended solids and, consequently, elevated water-column THg concentrations.

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

Structure	Quarter	THg		MeHg		% MeHg
		ng/L	remark **	WQS*	ng/L	
L-28	1 st May–Jul	1.10		<WQS	0.11	10%
	2 nd Aug–Oct	3.30	A	<WQS	0.40	12%
	3 rd Nov–Jan	0.94		<WQS	0.11	12%
	4 th Feb–Apr	0.65	A	<WQS	0.11	17%
	Median	1.02			0.11	12%
	Median POR	1.48			0.11	11%
S-10C	1 st May–Jul	0.80		<WQS	0.16	20%
	2 nd Aug–Oct	2.00		<WQS	0.23	12%
	3 rd Nov–Jan	0.62		<WQS	0.14	23%
	4 th Feb–Apr	0.30	I	<WQS	0.11	37%
	Median	0.71			0.15	21%
	Median POR	0.87			0.105	11%
S-12D	1 st May–Jul	1.60		<WQS	0.27	17%
	2 nd Aug–Oct		J3	<WQS	0.24	
	3 rd Nov–Jan	0.55	A	<WQS	0.22	40%
	4 th Feb–Apr	0.62		<WQS	0.11	18%
	Median	0.62			0.23	18%
	Median POR	0.98			0.150	15%
S-140	1 st May–Jul	0.91		<WQS	0.07	I 8%
	2 nd Aug–Oct	2.50		<WQS	0.38	15%
	3 rd Nov–Jan	0.77		<WQS	0.08	I 10%
	4 th Feb–Apr	0.50		<WQS	0.13	26%
	Median	0.84			0.10	13%
	Median POR	1.07			0.13	11%
S-141	1 st May–Jul		J3	<WQS	0.63	
	2 nd Aug–Oct	2.60		<WQS	0.28	11%
	3 rd Nov–Jan	0.76		<WQS	0.19	25%
	4 th Feb–Apr	0.46	A	<WQS	0.099	22%
	Median	0.76			0.24	23%
	Median POR	1.07			0.17	15%
S-151	1 st May–Jul		J3	<WQS	0.14	
	2 nd Aug–Oct	5.00		<WQS	0.21	4%
	3 rd Nov–Jan	1.10	A	<WQS	0.24	22%
	4 th Feb–Apr	0.56		<WQS	0.15	27%
	Median	1.10			0.18	22%
	Median POR	0.91			0.14	14%
S-32	1 st May–Jul		J3	<WQS	0.095	
	2 nd Aug–Oct	1.90	A	<WQS	0.16	5%
	3 rd Nov–Jan	0.94		<WQS	0.16	20%
	4 th Feb–Apr	0.40		<WQS	0.07	I 16%
	Median	0.94			0.13	17%
	Median POR	0.89			0.12	14%

Table 5. Continued.

Structure	Quarter	THg			MeHg		% MeHg	
		ng/L	remark **	WQS*	ng/L	remark **		
S-334	1 st May–Jul	1.10		<WQS	0.087	I	8%	
	2 nd Aug–Oct		J3	<WQS	0.120			
	3 rd Nov–Jan	0.44		<WQS	0.089		20%	
	4 th Feb–Apr	0.58		<WQS	0.150		26%	
	Median	0.58			0.104		20%	
	Median POR	0.865			0.111		15%	
S-5A	1 st May–Jul	2.00		<WQS	0.063	I	3%	
	2 nd Aug–Oct	28.00		>WQS	0.086	I	0%	
	3 rd Nov–Jan	1.90		>WQS	0.062	I	3%	
	4 th Feb–Apr	2.10		<WQS	0.240		11%	
	Median	2.05			0.074		3%	
	Median POR	2.05			0.110		6%	
S-9	1 st May–Jul		J3	<WQS	0.060	I		
	2 nd Aug–Oct	2.50		<WQS	0.085	I	6%	
	3 rd Nov–Jan	0.26	I	<WQS	0.041	I	32%	
	4 th Feb–Apr	0.26	I	<WQS	0.026	I	19%	
	Median	0.26			0.056		12%	
		Median POR	0.72			0.058		8%
		Median 1 st Q	1.10	(6) [¶]		0.102	(10)	13%
		Median 2 nd Q	2.55	(8)		0.220	(10)	11%
		Median 3 rd Q	0.76	(10)		0.125	(10)	19%
		Median 4 th Q	0.53	(10)		0.110	(10)	20%
		Cum. Median 1 st Q	1.10	(55) [¶]		0.140	(60)	12%
		Cum. Median 2 nd Q	1.60	(54)		0.180	(61)	11%
		Cum. Median 3 rd Q	0.91	(70)		0.092	(87)	10%
		Cum. Median 4 th Q	0.90	(77)		0.095	(65)	14%

* Class III Water Quality Standard (WQS) 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, (n), is number of unqualified values used to calculate median.

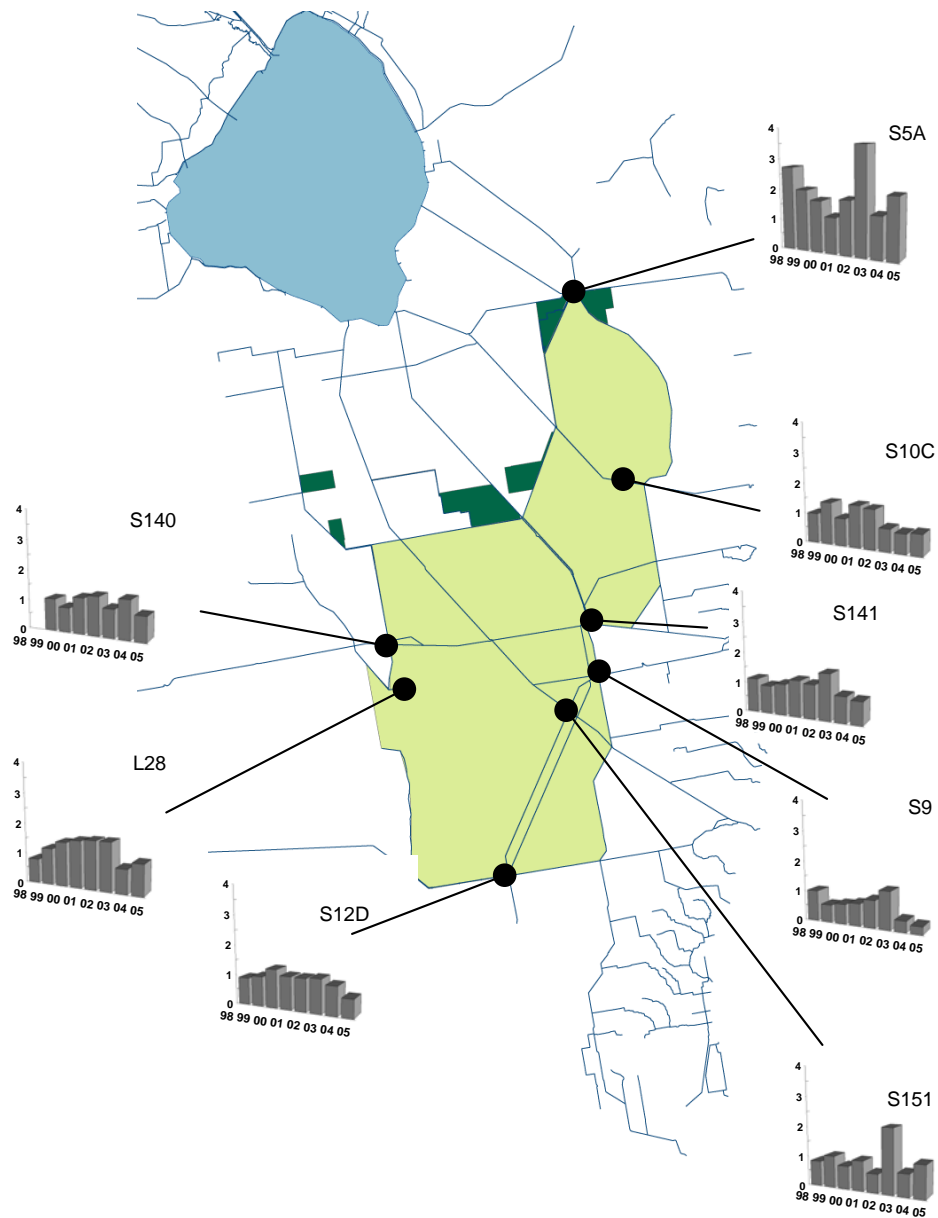


Figure 8. Annual median THg concentrations for period of record (POR) at stations sampled under project code HGLE.

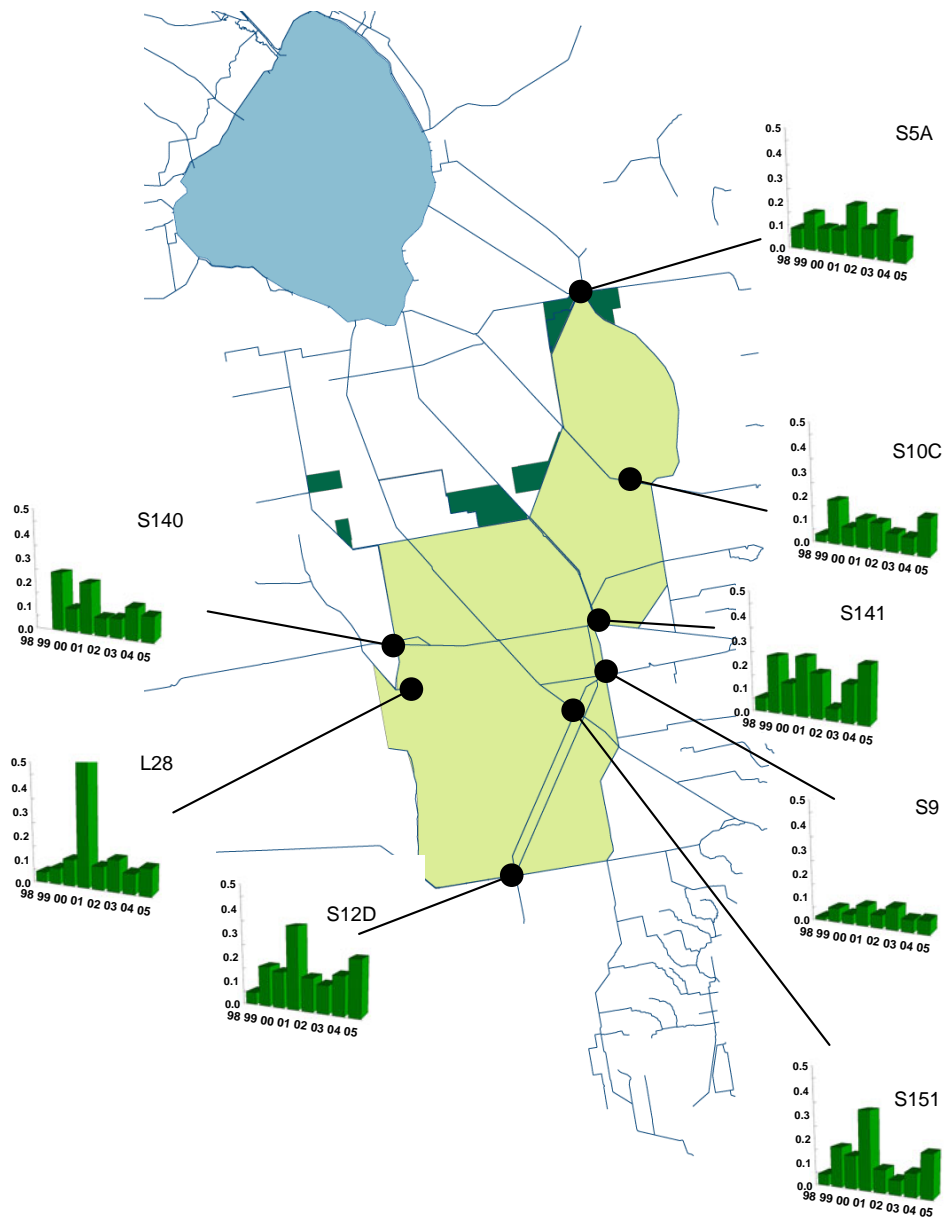


Figure 9. Annual median MeHg concentrations for POR at stations sampled under project code HGLE.

The maximum water-column MeHg concentration observed during 2005 at a non-ECP structure was 1 ng/L that occurred at S-140 at the time of the peak THg (**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 pattern showed that site S-9 typically had the lowest concentration; this difference was statistically significant in pairwise comparisons with all other structures except S-10C ($H = 28.8$; $df = 7$; $p < 0.0001$). No other pairwise comparisons were significant.

After more than eight 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 ($n \leq 33$), ranged from -0.30 to +0.28 for THg and from -0.11 to +0.35 for MeHg (a negative Tau indicates a decreasing trend, whereas a positive Tau indicates an increasing trend). None of the "p" values (both with and without autocorrelation correction) were significant ($p < 0.05$) with autocorrelation correction (assessment by S. Hill, SFWMD, personal communication, June 16, 2006).

As observed in previous consolidated reports (Rumbold et al., 2006), concentrations of both THg and MeHg were generally highest during the late summer months of July–September (i.e., third quarter) of the calendar year.

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. Raw data for individual fish can be found at www.sfwmd.gov/org/ema/dbhydro/index.html or through the District's web site at www.sfwmd.gov under the *What We Do, Environmental Monitoring, DBHYDRO Browser* section. 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; and CA315 or WCA-3A-15) have been monitored by the FWC since 1993. If fish could not be collected from a targeted marsh site due to inaccessibility, poor habitat, or both, collections defaulted to nearby marshes or, in some cases, canals where fish were more plentiful if source water was similar (approval for these alternate sites was received from the FDEP on March 5, 2002; correspondence from F. Nearhoof, FDEP).

Table 6. Mean concentrations (ng/g wet weight) of THg in mosquitofish composites (*Gambusia* sp.) collected in calendar year 2005 from downstream sites. Value represents a mean of three analyses.

Location	THg (ng/g)	Between-Year Change (%)	Cumulative Average (ng/g)
LOX4	40	-17%	72
CA2 F1 (L39F1)	9	-31%	42
CA27 Alt (Z4)	14	-75%	56
CA27 Alt (N4)	15	-82%	91
Holey Land (North canal)	21	-22%	42
Rotenberger Alt. (RotenF1)	19	73%	82
Rotenberger rim canal (RotenC)	53	36%	43
CA2U3	39	-53%	100
CA33	22	-58%	37
CA35alt2	62	-21%	77
Non-ECP North (CA3F1; end of L-28)	25	79%	48
CA315	56	12%	105
Non ECP South (CA3F2)	24	-31%	58
L67F1	23	-54%	98
Annual mean	28	-39%	

N/A Data not available

Note: Fish were successfully captured at CA2F1 in 2006 and contained 3 ng Hg/g.

Grandmean for POR (1998-2005; aliquot means pooled across time and space): n=112;

76 ±12 ng/g; 90th percentile for POR is 180 ng/g.

Table 7. Mean concentrations (± 1 SD; ng/g wet weight) of THg in sunfish (*Lepomis* spp.) collected in calendar year 2005 from marshes within the EPA downstream of the STAs.

Target Location	Sampling Location	Mean THg ng/g (± 1 SD, n)	Between-Year Change (%)	Grand Mean of Annual Means
WCA1-LOX3	LOXF4	117 ($\pm 31, 20$)	19%	131
WCA-2A F1	L39F1	35 ($\pm 27, 20$)	-26%	68
WCA-2A 2-7	Z4*	153 (1)	N/A	210
	CA2N4	125 ($\pm 65, 20$)	N/A	146
Holey Land	Holey Land	146 ($\pm 41, 16$)	-32%	127
Rotenberger	RotenC (canal)	134 ($\pm 41, 20$)	14%	172
WCA-2A U3	CA2U3	239 ($\pm 137, 20$)	46%	161
WCA-3A 3	3A-3	127 ($\pm 67, 20$)	-17%	140
WCA-3A 5	Alt. 2 site	192 ($\pm 103, 20$)	66%	188
Non-ECP North	CA3F1	139 ($\pm 81, 20$)	-21%	131
WCA-3A 15	CA315	204 ($\pm 73, 20$)	35%	283
Non-ECP South	CA3F2	123 ($\pm 63, 20$)	43%	134
ENP P33 Marsh	L67F1	243 ($\pm 200, 20$)	-44%	441
Average		185	15%	

* Unable to collect 20 fish from each site

N/A Data not available due to the absence of fish at the site

Note: Fish captured at CA2F1 in 2006 contained 63 ± 49 ng Hg/g, n = 5

Grand mean of site means (pooled across space and time) for POR (1998–2005) $\pm 95\%$

CI: n = 94, 186 ± 134 ; 50th and 95th percentile site mean concentration was 145 and 441 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 2005 from ECP and non-ECP interior marsh sites.

Target Location	Sampling Location	EHg3 ± 95 th CI (mean ± 1SD, n) ng/g wet	Between-Year Change (%)	Cumulative Average EHg3
CA1-LOX3	LOX4	480 ± 52 (270 ± 110, 20)	N/A	491
CA2-F1	L39F1	250 ± 40 (220 ± 140, 20)	9%	270
CA2-7	N4	720 ± 143 (690 ± 430, 20)	N/A	720
Holey Land	HOLYBC	740 ± 100 (800 ± 290, 20)	14%	454
Rotenberger	ROTENC	NC (2) (470, 1)	N/A	847
CA2-U3	CA2U3	750 ± 139 (490 ± 165, 20)	27%	686
CA3-3	CA33	NC (2) (570 ± 120, 11)	N/A	N/A
Non-ECP North	CA3F1	579 ± 57 (500 ± 156, 17*)	-22%	537
CA3-15	CA315	680 ± 120 (450 ± 170, 20)	5%	802
Non-ECP South	CA3F2	NC (2) (250 ± 70, 9)	N/A	436
ENP-P33	L67F1	1,130 ± 190 (1,020 ± 310, 20)	-5%	1,283

NC Not calculated for (1) insignificant slope or (2) poor age distribution

N/A Not available

* Excludes three values (sample numbers P24467-12, -13, -14) that exhibited excessive RPD in inter-lab splits

Annual average EHg3 = 666 ng/g

Grand mean of site EHg3 for POR +95% CI: n = 53, 632 ± 90 ng/g; 90th percentile = 1.17 ng/g

To preserve long-term datasets that are crucial for temporal trend assessment, reverting to the original target site will involve sampling at both the alternate and the original site for some period to assess spatial differences. Accordingly, sampling will revert to the original targeted site only after it has been established that long-term hydrology and habitat restoration has occurred to insure chances of finding fish year-to-year are high. Although reverting may take a number of years at certain sites (e.g., sites WCA-2-F1, WCA-3-3, and WCA-3-5), it will prevent alternating collections between the two sites and disruption of data continuity.

Fishes collected in 2005 showed both spatial and temporal patterns in tissue mercury concentrations. In keeping with the primary objective of the Mercury 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 mercury concentrations are important, particularly if there has been a variation from pre-ECP conditions established by the FWC. Therefore, spatial patterns will be reviewed in detail only where there have been changes over time to determine the interaction between treatment effects.

Mosquitofish

Mercury levels in mosquitofish collected from marsh sites in 2005 ranged from 3 nanograms per gram (ng/g) at site WCA2F1 to 62 ng/g at site CA35alt2 (**Table 6** and **Figure 10**). The annual basinwide average concentration in mosquitofish collected in 2005 was 28 ng/g (**Table 6**) (for locations, see **Figure 3**), which represents a 39 percent decrease from the 2004 basinwide mean concentration. The aliquot means (90th percentile) of tissue-mercury concentrations in mosquitofish for the period of record (1998–2005; n = 112) was 180 ng/g. In 2005, THg levels in mosquitofish declined at 11 of 15 sites (**Table 6**).

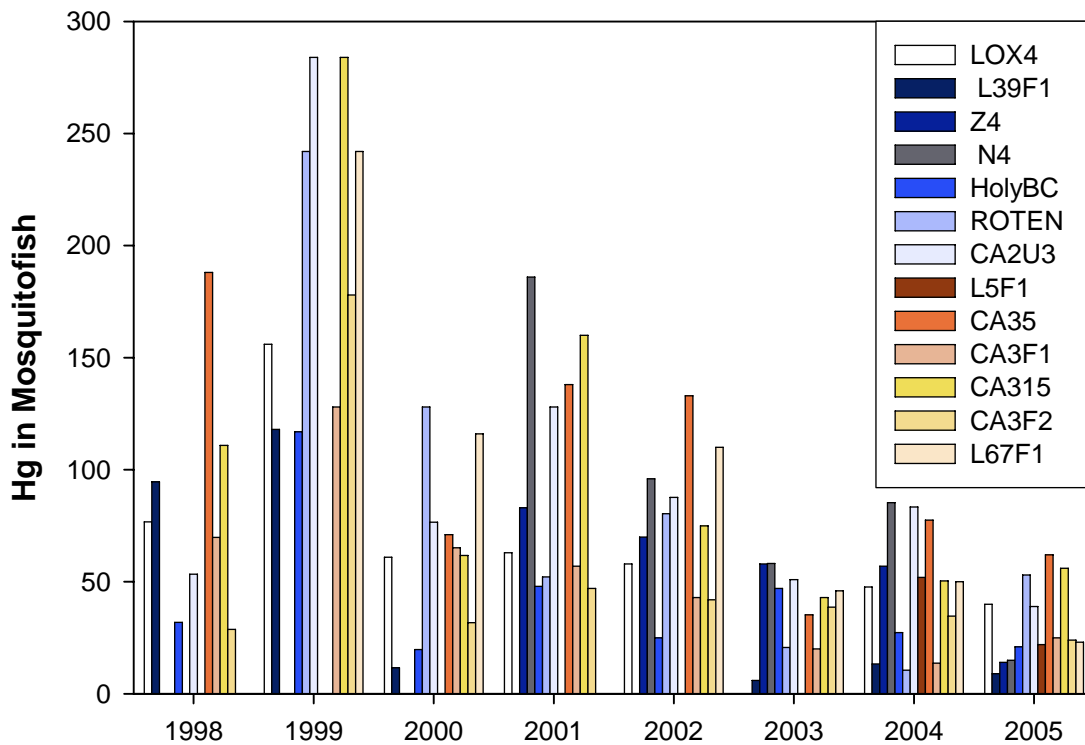


Figure 10. Hg concentrations in mosquitofish (*Gambusia* sp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2005). Not all sites were sampled in all years (for details, see **Table 6**).

Sunfish

Mercury levels in sunfish (*Lepomis* spp.) collected from downstream sites in 2005 (n = 218) ranged from a low of 13 ng/g in a redear sunfish (*L. microlophus*) from site L39F1 to a high of 750 ng/g in a bluegill (*L. macrochirus*) from site L67F1. This pattern of minimum and maximum at these two sites has been consistent for several years. The grand mean of both site means was 185 ng/g in 2005, which represents a 15 percent increase from the previous year.

Because of differences in sizes and species of *Lepomis* collected, results must be interpreted with caution. 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 consolidated reports (Rumbold et al., 2006), attempts to use ANCOVA to evaluate patterns of mercury concentrations in sunfish using weight as a covariate were often unavailable because concentration-weight relationship slopes were either not significant or not parallel for each year. The lack of a strong concentration-size relationship likely resulted from interspecies differences among the *Lepomis* spp. in growth and bioaccumulation factors, which are likely a function of diet.

As observed over the past seven years when data was pooled across sites, fish species was a significant factor in tissue mercury concentration in 2004 (Kruskal-Wallis ANOVA on Ranks; df = 3; H = 39.19; p < 0.001). Mercury levels were lower in *L. microlophus* (redeer, median = 96 ng/g) than each of the other three species (Dunn's method, p < 0.05): *L. macrochirus* (bluegill, median = 140 ng/g), *L. punctatus* (spotted sunfish, median = 140 ng/g), and *L. gulosus* (warmouth, median = 160 ng/g). No other paired comparison between species was significant (p > 0.05; Dunn's Method, p > 0.05). These species-specific medians were nearly identical to 2004 values.

In 2005, sunfish continued to show significant spatial patterns in mercury levels (**Table 7; Figure 11**; df = 12; H = 8736; p < 0.001). Fish from site L39F1 contained the lowest median concentration (25 ng/g) and differed from most other sites (p < 0.05). Interestingly, this median concentration did not differ significantly from levels observed in fish collected for the first time from the nearby marsh site (CA2F1), which contained the second lowest median concentration. However, fish at both of these sites differed significantly in mercury levels from fish further downstream at site WCA2U3. Except for the paired comparison between WCA2U3 and CA2F1, all other statistically significant paired comparisons (Dunn's Method) involved site L39F1.

From visual inspection of **Figure 11**, sunfish appeared to exhibit clear temporal variability in mercury burdens; however, these apparent trends were confounded by temporal differences in size or species of lepidid collected. For example, the marked decline in mercury levels in fish from Holey Land may be an artifact from a sample with greater numbers of redear (11 of 16 fish) as compared to previous samples. Similarly, the decline in mercury levels in fish apparent at site L67F1 may be due to increased numbers of redear (9 of 20). To exclude this variability due to species and size, the dataset was censored to assess only bluegill ranging in size from 102 to 178 millimeters (mm) for temporal trends (to reduce size-related effects further, mercury levels were normalized by dividing measured concentration by total length of the fish).

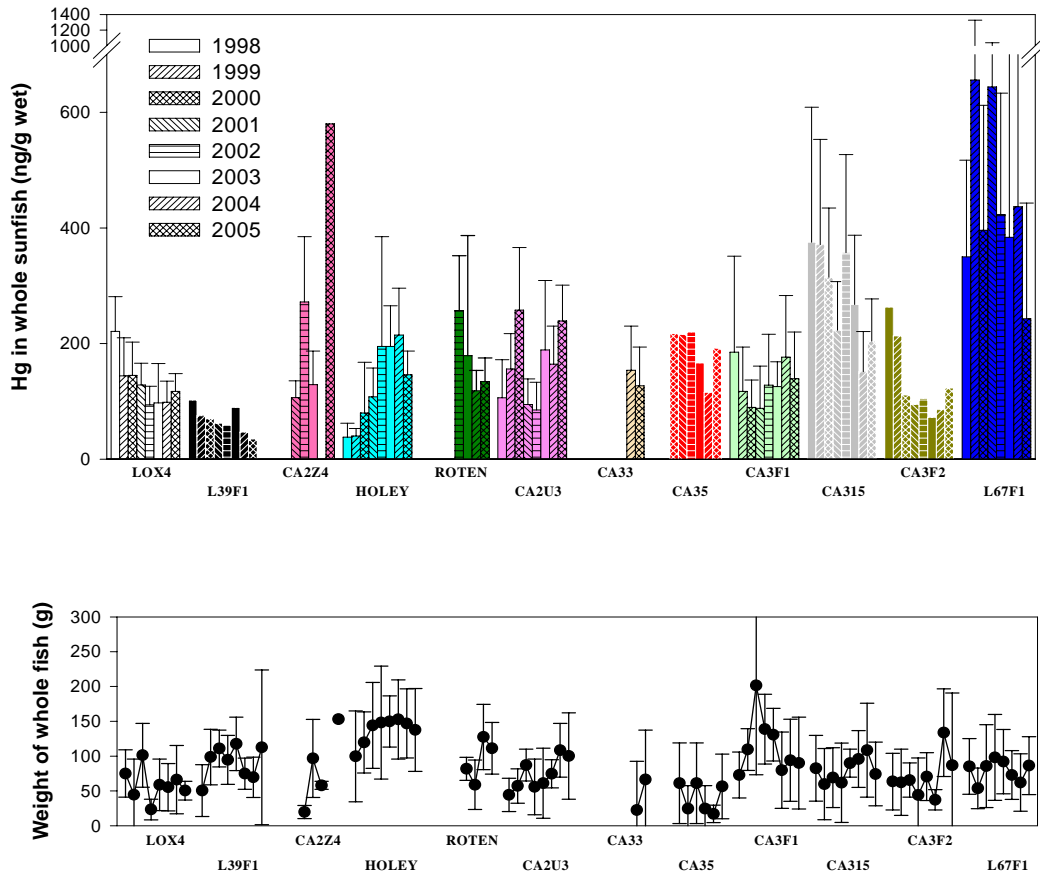


Figure 11. THg concentration (top) and weights (bottom) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the POR.

This analysis showed significant among-year differences at a number of sites. However, there was only one case that involved a comparison to 2005. In this instance, normalized mercury levels in bluegill collected in 2005 differed from previous years at site CA2U3; data from 2005 contained higher levels as compared to all previous years, except 2000 and 2004, which were not included in the analysis due to small sample size. Hence, the apparent declines in 2005 discussed above for HOLYBC and L67F1 sites were not demonstrated in bluegill.

Largemouth Bass

A total of 183 largemouth bass were collected at 13 downstream sites from October through November 2005. Despite the best efforts of the FWC (who were contracted to electrofish at these sites), bass could not be collected from site WCA2F1. The bass that were collected had tissue mercury concentrations ranging from a low of 10 ng/g in a 2.8-year-old fish from site CA3F1 to 1,700 ng/g in a 5.8-year-old fish from site N4. Site specific, age-standardized concentrations (expected in EHg3) ranged from 250 ng/g at site L39F1 to 1,130 ng/g at site L67F1 (**Table 8** and **Figure 12**); however, the latter was reported for information only and is almost identical to the 2004 estimate. As discussed further below, the mercury-age relationship regression for L67F1 fish was not statistically significant ($f = 2.95$; $df = 1.18$; $p = 0.10$). Calculation of EHg3 was not appropriate at sites ROTENC, CA3F1, CA33, and CA3F2 either because the tissue mercury-age relationship was not significant or because of small sample size. Based on the sites where it was appropriate to calculate site-specific EHg3, the grand mean values was 679 ng/g in 2005, which represents a 0.4 percent increase over the grand mean estimated for 2004.

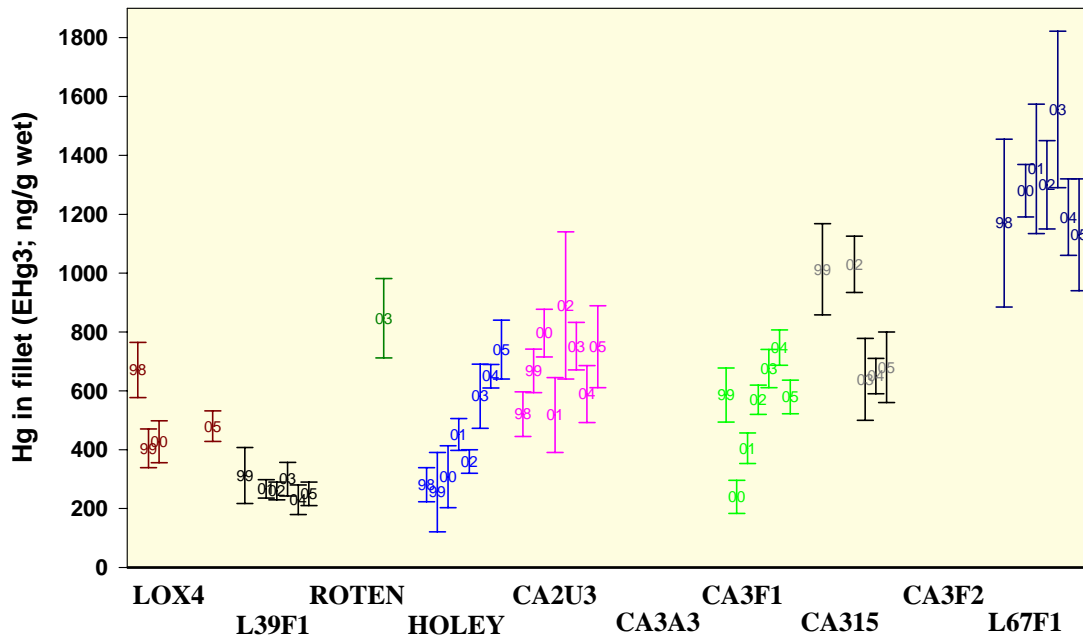


Figure 12. Age standardized (class 3 year) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at downstream sites for the POR of 1998–2005. EHg3 was not calculated if regressions were not significant or if age distributions were narrow (see **Table 8**).

Largemouth bass exhibited spatial patterns in tissue mercury concentrations similar to those observed in sunfish, with higher levels generally being found at the southern sites (**Table 8** and **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 ($f = 2.4$; $df = 7, 140$; $p = 0.02$), ANCOVA could not be used to assess differences in LSM mercury levels among all sites.

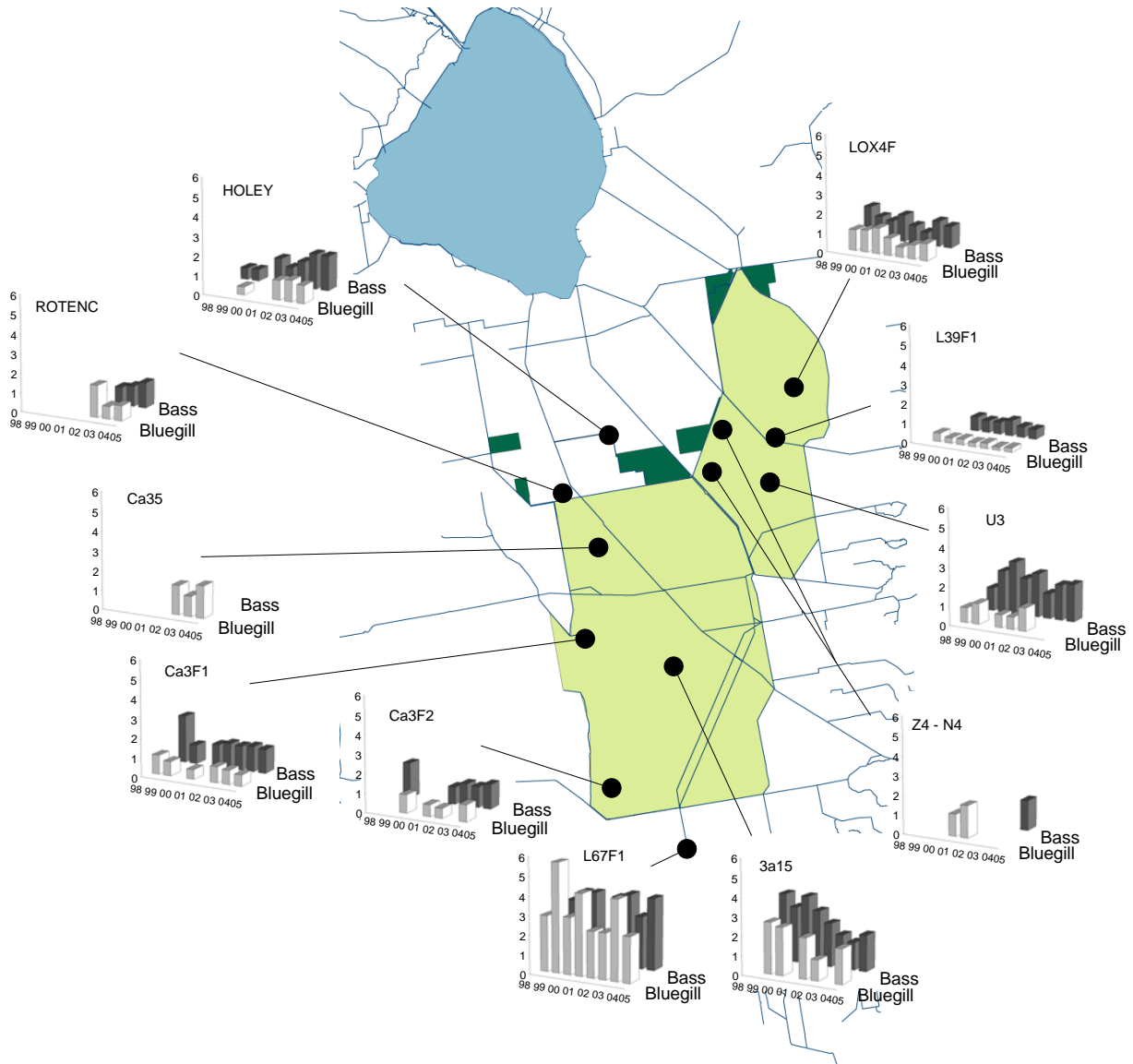


Figure 13. Spatial and temporal patterns in Hg levels in young largemouth bass (i.e., less than 1.8 years old) and bluegill sunfish (4–7 inches in length). Hg levels in fish were further normalized by dividing concentration in a given fish by its total length. Hence, the units of the y-axis is mg/kg/m (*cf.* Brumbaugh et al., 2001). Few large fish were collected from site CA2N4 in NW WCA-2 (reported along with Z4) and from site CA33 in NE WCA-3.

As advocated in previous consolidated reports, the focus in evaluating temporal patterns should be on long-term trends and not year-to-year differences. To control some of the aleatory uncertainty driving variability, tissue concentration in largemouth bass were standardized to an expected mean concentration in three-year-old fish. As evident in **Figure 12**, even standardized tissue concentrations in bass vary year-to-year with a median percent change of 19 percent. Yet, subtle but important changes can be obscured by focusing on EHg3 rather than on a specific year-class. For example, as reported earlier, the calculated EHg3 for L67F1 must be interpreted with caution because the mercury-age regression was not statistically significant. Nonetheless, the regression was informative in that the slope appeared to have been reduced by a marked increase in very young bass (less than 0.8 year old) as compared to older bass. The comparative increase in mercury levels in young bass in 2005 and 2004 is shown in **Figure 13**. Although this increase may suggest that young bass were exposed to higher ambient MeHg in 2005, a similar increase was not evident in bluegill (**Figure 13**) and, thus, must be interpreted cautiously. Two sites that showed similar year-to-year variation for both sunfish and bass were CA2U3 and CA315; these sites warrant further scrutiny next year (**Figures 11 through 13**).

Based on **Figure 12**, the most obvious progressive trends of increasing mercury in fish are occurring at CA3F1 and the Holey Land Wildlife Management Area (Rumbold, 2005; Rumbold et al., 2006). The apparent drop in mercury levels in 2005 fish from site CA3F1 should be viewed with some caution because of QC issues with at least three reported values. ANCOVA was not used to assess temporal differences in mercury levels in bass from CA3F1 because of significant between-year variability in slopes of regressions of mercury on bass age ($f = 14.01$; $df = 6, 122$; $p < 0.001$). ANCOVA was used to assess mercury trends at site HOLEYBC (interaction between age*year, $p = 0.49$; model without interaction, $f = 50.07$; $df = 8, 150$; $p < 0.001$). Although the 14 percent increase in the 2005 EHg3 was not statistically different from 2004 levels (Tukey post hoc comparison of LSM mean, $p = 0.45$), 2005 levels differed from all previous years (Tukey comparisons, $p < 0.01$). As discussed last year (Rumbold et al., 2006), mercury in fish at the Holey Land Wildlife Management Area have reached levels that may pose a threat to fish-eating wildlife; steps should be taken to understand and correct the factors to abate and possibly reverse this trend.

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 U.S. Fish and Wildlife Service (USFWS) has proposed a predator protection criterion of 100 ng/g of THg in prey species (Eisler, 1987). Likewise, the USEPA has proposed in a Mercury Study Report to the U.S. Congress a criteria of 77 ng/g and 346 ng/g for trophic level (TL) 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997).

In 2005, mosquitofish (considered to be at TL 2 and 3, depending on age; Loftus et al., 1998) did not exceed either the USEPA or the USFWS criterion (**Table 6**). However, 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 two sites (i.e., L39F1 and WCA2F1; **Table 7**). As discussed previously by Rumbold (2005), this finding is significant because sunfish represent the preferred prey item of many fish-eating species in the Everglades. Likewise, whole body concentrations of mercury in largemouth bass (where whole body THg concentration = $0.695 \times$ fillet THg; Lange et al., 1998) approached or exceeded the guidance value for TL 4 fish at roughly half the sites (e.g., N4, HOLYBC, CA2U3, CA33, CA315, and L67F1). Based on these findings, 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.

WADING BIRD FEATHERS FROM ECP INTERIOR MARSHES

As reported last year, feather collection was coordinated through the District's Everglades Research Division in 2005. 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. Regrettably, unusually poor nest initiation by the egrets at the Alley North colony resulted in the locating of only three active nests containing nestlings of an appropriate age. Feather samples collected from those nestlings were shipped to the FDEP Chemistry Lab in July 2005 and, consequently, results were not reported last year but instead are reported this year (**Table 9**). THg concentrations averaged 4 ± 1 micrograms per gram ($\mu\text{g/g}$) in the three feather samples, which is consistent with levels observed in birds of similar age collected from other colonies in past years.

Table 9. Standardized least square mean of THg ($\mu\text{g/g}$) for a chick with a 7.1 cm bill (arithmetic mean concentration \pm 1 SD, n) in growing scapular feathers collected annually from great egret nestlings (2 to 3 weeks old) at colonies within WCA-3A.

	JW1	L67	Cypress City	Alley North
1994 ^{*1}	21 ± 6 (25 ± 8 , 9)	16 ± 4 (N/A, 27)		
1995 [*]	14 ± 3 (N/A, 8)	16 ± 6 (16 ± 6 , 14)		
1999	7 ± 1 (4 ± 2 , 13)	NC (4 ± 2 , 20)		
2000	7 ± 1 (3 ± 2 , 10)	NC (3 ± 1 , 10)		
2001	Failed to initiate nesting	NC (7 ± 3 , 13)		
2002	Colony abandoned	NC (2 ± 0.5 , 6)		
2003	Failed to initiate nesting	NC (5 ± 2 , 3)	NC (6 ± 2 , 15)	
2004	Failed to initiate nesting	4 ± 2 (1 ± 1 , 10)	5 ± 2 (2 ± 1 , 10)	
2005	NS	Failed to initiate nesting	NS	NC (4 ± 2 , 3)
2006	NS	NC (5 ± 2 , 6)	NS	NC (3 ± 2 , 8)

* Data from Frederick et al. (1997)

¹ Concentrations standardized to a bill length of 5.6 cm

N/A Not available

NC Not calculated where slope of regression was not significant ($p > 0.05$)

NS Not sampled

Estimated mean age of sampled nestlings 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, 13 days in 2002 and 2003, 12–14 days in 2004, 12 days in 2005, and 28–29 days in 2006.

In early 2006, feather samples were collected by the District's Water Quality Monitoring Division from a total of 14 nests at the two colonies. Feather THg concentrations ranged from 1.3 µg/g in a chick from Alley North estimated to be 26 days old to 8.8 µg/g in a chick from the L67 colony thought to be 32 days old, with an overall mean concentration (both colonies pooled) of 4 ± 2.4 µg/g. Regression of feather mercury concentrations on bill length (i.e., as an age surrogate) were not statistically significant if the colonies were run separately; however, when pooled across colonies the regression was significant ($df = 1, 12; f = 6.8; p = 0.02$) with a LSM for a chick with a 7.1 cm bill equal to 3.7 ± 1.1 µg/g. As shown in **Table 9**, this LSM and the arithmetic mean concentrations are much lower than similar metrics for samples collected in the mid 1990s.

Establishing a benchmark for critical feather THg concentration has 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. Great egret juveniles were dosed with MeHg-containing gelatin capsules at 0.5 mg Hg/kg food ($n = 5$) and were found to have subtle behavioral changes and statistically significant differences in blood chemistry, liver biochemistry, and weight index (Bouton et al., 1999; 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 sampled in 2006 do not appear to be at risk of toxicological effects from MeHg.

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 consolidated reports (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 2005 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 at <http://dlis.dos.state.fl.us/cgi-bin/services/index.cfm>). No new reports in 2005 were found; however, various individuals or agencies made systematic aerial and ground surveys of foraging and nesting wading birds in South Florida during the early 2006 breeding season. These reports were not final at the date of this report (for details, see Chapter 7 of the 2006 SFER – Volume I).

OPPORTUNITIES FOR OPTIMIZING THE MONITORING NETWORK

Following discussions between the District and FDEP on January 23–24, 2006, it was agreed that the mercury monitoring requirements contained under Section 4 of *Downstream Receiving Water Monitoring* for each of the EFA STA permits were to be omitted during the renewals process and instead codified in the non-ECP structures permit upon renewal. The existing non-ECP plan contains similar language (see Condition 11) to that of the STA monitoring plans; the exception being the exact number of sites for large-bodied fish collection. To resolve this issue, the District has submitted an updated non-ECP mercury monitoring plan for approval by the FDEP.

In the updated non-ECP mercury monitoring plan, the District also attempted to resolve the long standing issue of primary versus alternate fish collection sites. In accordance with sampling requirements contained in both the older non-ECP structures permit and the EFA STA permits, large-bodied fish collections were originally targeted at a total of 12 downstream marsh sites in the interior of the WCAs and ENP (District's Everglades Mercury Monitoring Plan revised in March 1999; Appendix 1 of the Quality Assurance Protection Plan, June 7, 1999). Despite these efforts, fish have not been collected from a number of the targeted marsh sites over the monitoring period due to inaccessibility, poor habitat, or both. Consequently, collections defaulted to nearby marshes or, in some cases, canals (if source water were similar) where fish were more plentiful. Collection at these alternate sites was formally approved by the FDEP in March 2002, through a minor modification of Condition 4(i), which was modified to read "allow for alternates sites for fish sample collection when the primary site is inaccessible" (correspondence from F. Nearhoof, FDEP). For certain alternative sites, long-term data sets have now been established. To preserve these long-term sites, the District has submitted for approval a monitoring plan that eliminates sampling sites that have not successfully produced fish since 1998 and thereby makes the alternate sites the primary sites.

Finally, the District also recommends that the requirement for quarterly collection of water samples at structures S-5A, S-9, S-10C, S-12D, S-140, S-141, S-151, and S-190 be omitted from the updated non-ECP mercury monitoring plan. This request is based on Condition 11(d) of the existing permit which states "after an initial period of three years, the permittee may request a reduction in the frequency of mercury monitoring or the number of monitoring locations based on consistent compliance with state water quality standards, including applicable narrative or numeric criteria, and the absence of any adverse impacts attributable to mercury." The District contends that this criterion has been satisfied through the following:

1. The finding that of the 2,199 unfiltered water samples collected and analyzed for THg between 1997 and 2005 (and which met all quality controls), 75 percent had a concentration less than 2 ng THg/L, whereas less than 1 percent exceeded the State's Water Quality Standard (WQS) of 12 ng/L (Rumbold and Lange, 2006).
2. The consensus that atmospheric loading is the dominant source of THg to the Everglades (Stober et al., 2001; Atkeson and Parks, 2002; Rumbold et al., 2006; **Table 4**).
3. The growing body of evidence that *in situ* methylation of fresh inorganic mercury in direct rainfall is the primary driver for MeHg biomagnification in fish at Everglades marsh sites, as opposed to loading of THg or MeHg from upstream (Harris et al., 2003; Gilmour et al., 2004).
4. The THg and MeHg concentrations and loads in upstream canals are a poor predictor of biomagnification in downstream fish (Rumbold and Lange, 2006).

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