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

Mark Gabriel, Nicole Howard, Fran Matson, Shane Atkins and Darren Rumbold[1]

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). All results displayed in this report for fish and surface water are based on calendar year 2006. Results for quality assurance/quality control are for Water Year 2007 (WY2007).

The key findings presented in this appendix are as follows:

1. The total annual deposition for the Everglades Protection Area in 2006 was 134 kg-Hg/yr, which is a 2.2 percent increase from 2005. The above is the average of stations FL11 (Everglades National Park) and FL34 (Everglades Nutrient Removal). Due to closure of station FL04 (Andytown) on October 17, 2006, and its re-establishment as station FL97 (Western Broward County) on November 21, 2006, complete annual depositions could not be calculated for these stations. As a result of difficulties associated with sampling handling, low precipitation, and mechanical failures and passage of Tropical Storm Ernesto several periods were missing for 2006 for all stations. Consequently, estimates for both the volume-weighted (wet) concentration and annual wet deposition are to be viewed with caution.
2. The maximum total mercury (THg) concentration observed at non-Everglades Construction Project (non-ECP) water control structures was 5.1 nanograms per liter (ng/L) at S-5A during the second quarter of 2006. 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 0.4 ng/L, which also occurred at S-5A during the forth quarter of 2006. Currently, Florida has no Class III numerical water quality standard for MeHg. Little indication of statistically significant temporal trends have been found in either THg or MeHg concentrations (or percent MeHg) at any of the individual structures after eight years of monitoring.
3. Mosquitofish (*Gambusia holbrooki*) collected from downstream marsh sites had mercury levels ranging from 15 nanograms per gram (ng/g) at site CA2NF to 69 ng/g at site L67F1. The average basin-wide concentration for 2006 was 46 ng/g. This average concentration level

[1] Florida Gulf Coast University, Fort Myers, FL

represents a 55 percent increase from the basin-wide mean concentration in 2005. The average 2006 basin-wide concentration was much reduced compared to the 1999 peak of 177 ng/g.

4. Sunfish (*Lepomis spp*.) collected from downstream sites had mercury levels ranging from 13 ng/g at site L39F1 to 650 ng/g at site CA33. The basin-wide average concentration in sunfish was 152.5 ng/g, representing a 0.16 percent increase from 2005. The 2006 basin-wide concentration was slightly reduced 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, sites CA35ALT and L67F1 had statistically higher THg levels than all other sites.
5. Fillets from individual largemouth bass (*Micropterus salmoides*) collected from downstream sites had tissue mercury concentrations ranging from 64 ng/g at site ROTENC to 3,400 ng/g at site L67F1. Site-specific, age-standardized concentrations (estimated/expected for a three-year-old bass symbolized as EHg3) ranged from 247 ng/g at site L39F1 to 1,130 ng/g at site L67F1. The basin-wide EHg3 was 660 ng/g in 2006 as compared to the peak of 724 ng/g observed in 2003. Standardized mercury levels increased at three of the six sites' analyses in 2006 using age-standardization. The Holey Land Tract continues to show a steady linear increase in THg levels.
6. Great egret (*Ardea alba*) feathers were collected from a total of 20 nestlings at two colonies in Water Conservation Area 3A (WCA-3A) in early 2007. Feather THg concentrations ranged from 0.9 micrograms per gram (μg/g) in a chick from Cypress City to 9.2 μg/g in a chick from the L67 colony. The overall mean concentration (two colonies pooled) was 4 ± 2.4 μg/g. Levels in 2007 were much reduced compared to the range of 14 μg/g to 21 μg/g observed in chicks in 1994 and 1995. Based on published benchmarks, egret nestlings sampled in 2007 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 the Holey Land Wildlife Management Area, site CA3F1 in WCA-3, and site L67F1 in Everglades National Park. From 2005 to 2006 there was an increase in all fish types at nearly all non-ECP stations. 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 2006, summarizing results of monitoring mercury in the downstream receiving waters of the Everglades Protection Area. This report satisfies the mercury-related reporting requirements of the Florida Department of Environmental Protection Everglades Forever Act permits [Section 373.4592, Florida Statutes (F.S.)], including permits for Stormwater Treatment Areas 1 West, 1 East, 2, 3/4, 5, and 6. This report includes the monitoring results of 2006. The results of monitoring mercury within the Stormwater Treatment Areas are presented separately in Appendix 5-7 of this volume. 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 Everglades Forever Act (Section 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 Stormwater Treatment Areas (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 Everglades Protection Are (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 (the ENP or Park).

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 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 Florida Department of Environmental Protection (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 5-7 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 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**). In mid-2006 measurements at the Andytown station ended. The tower supporting measurements was moved to a new location in Western Broward County [hereafter referred to as Broward County station (FL97)]. Measurements at the Broward County station began at the end of November of 2006.

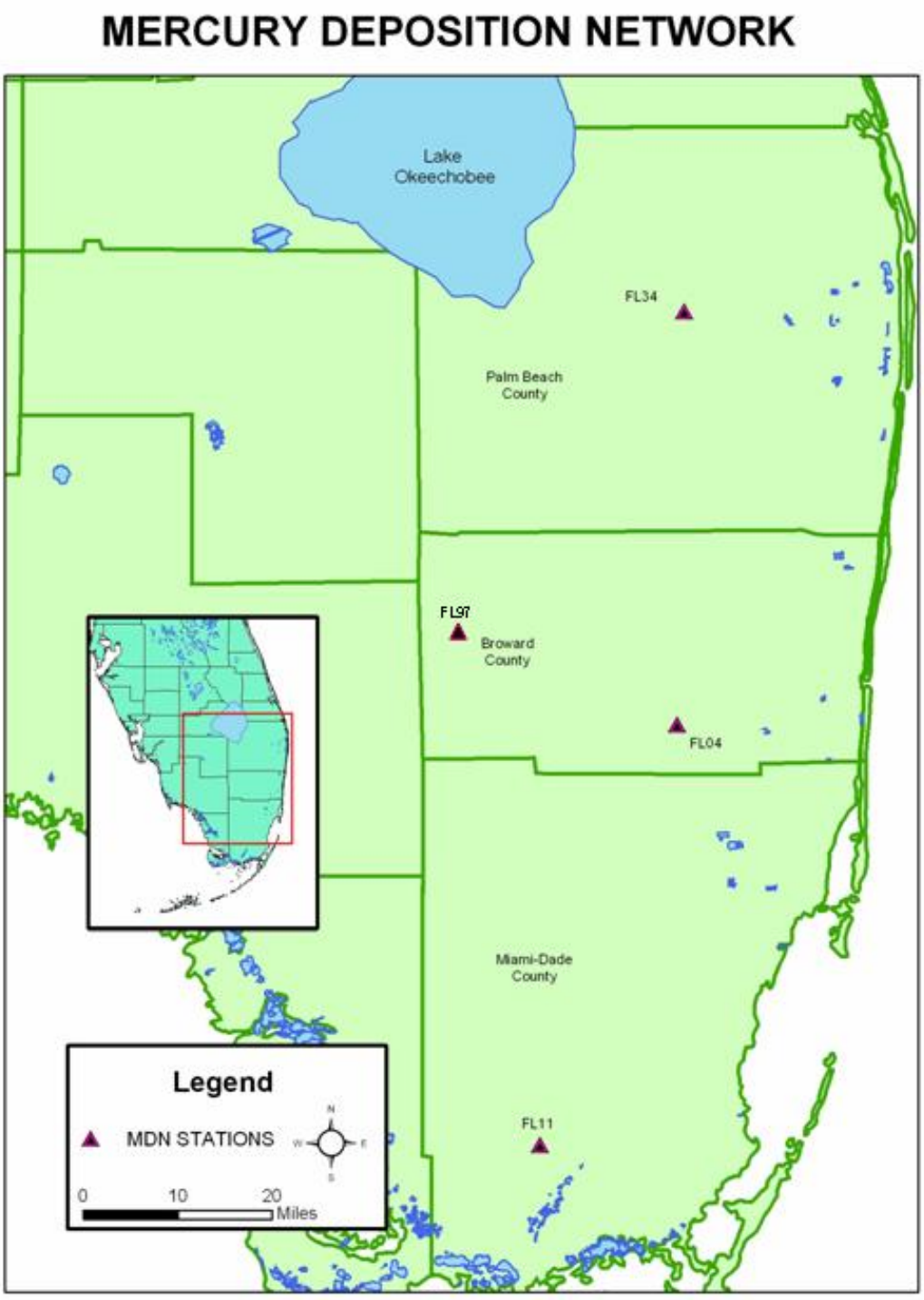

Figure 1: Map showing mercury deposition monitoring sites

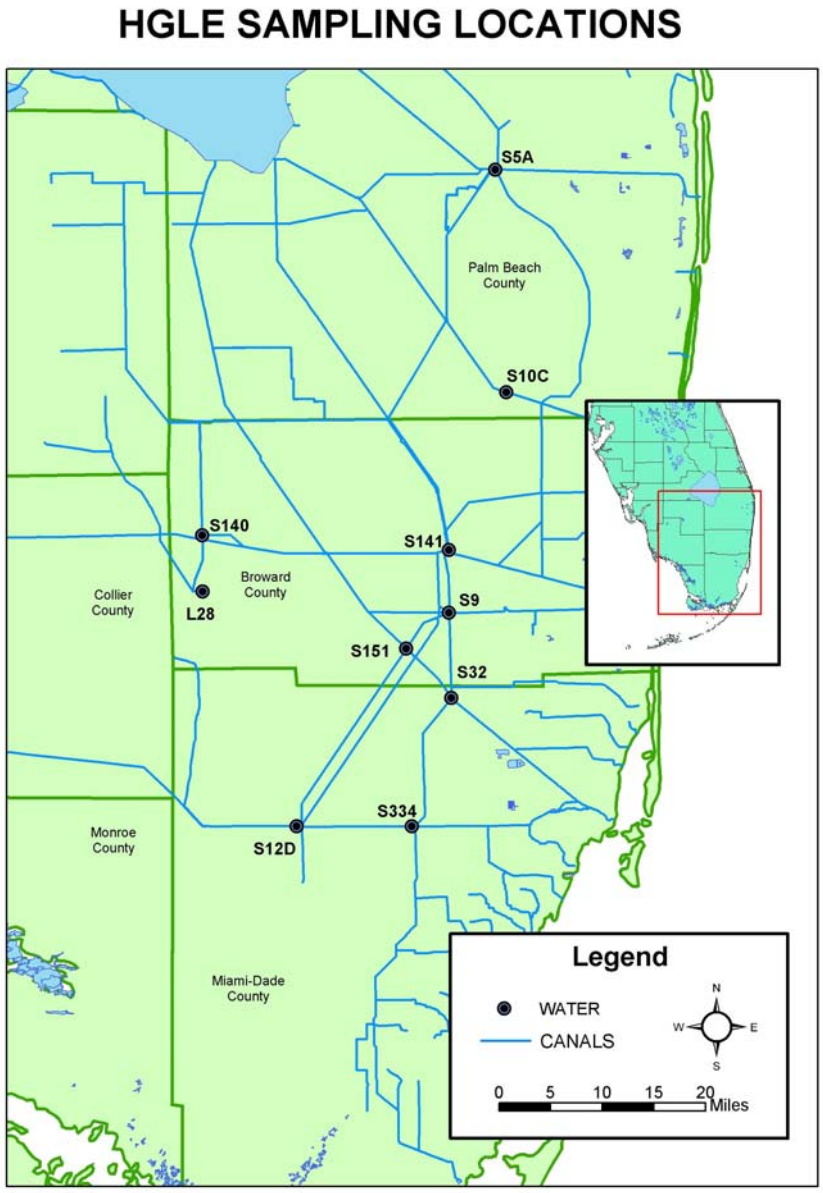

Figure 2. Map showing non-ECP structures where unfiltered surface water is collected quarterly to monitor concentrations of THg and methylmercury (MeHg). Sites S-32 and S-334 that were not required under the permit were dropped in October 2005.

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.

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 are 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.

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.

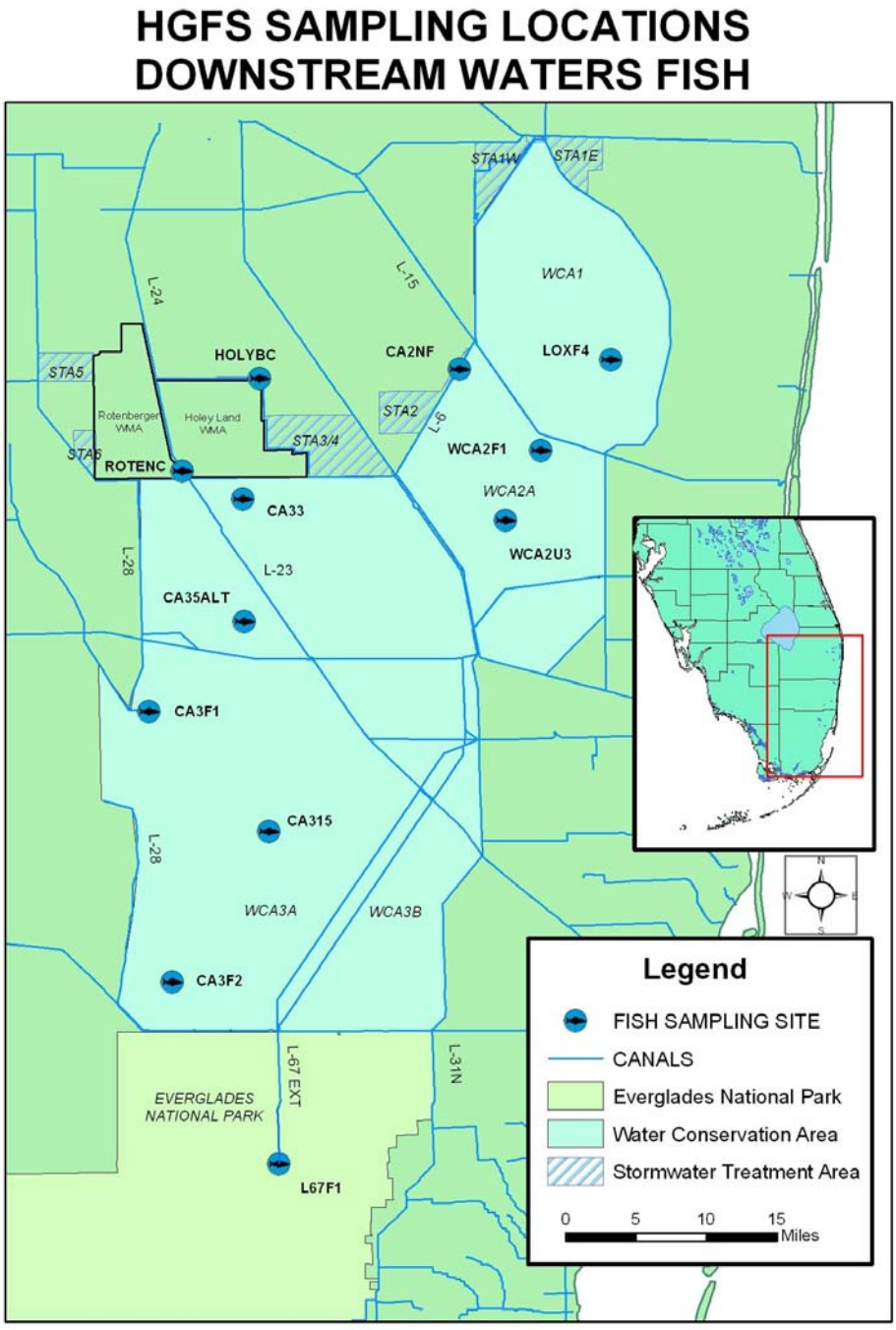

Figure 3: Map showing collection sites for monitoring Hg levels in mosquitofish, sunfish, and largemouth bass

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 other priorities were more urgent, egg collection was 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).

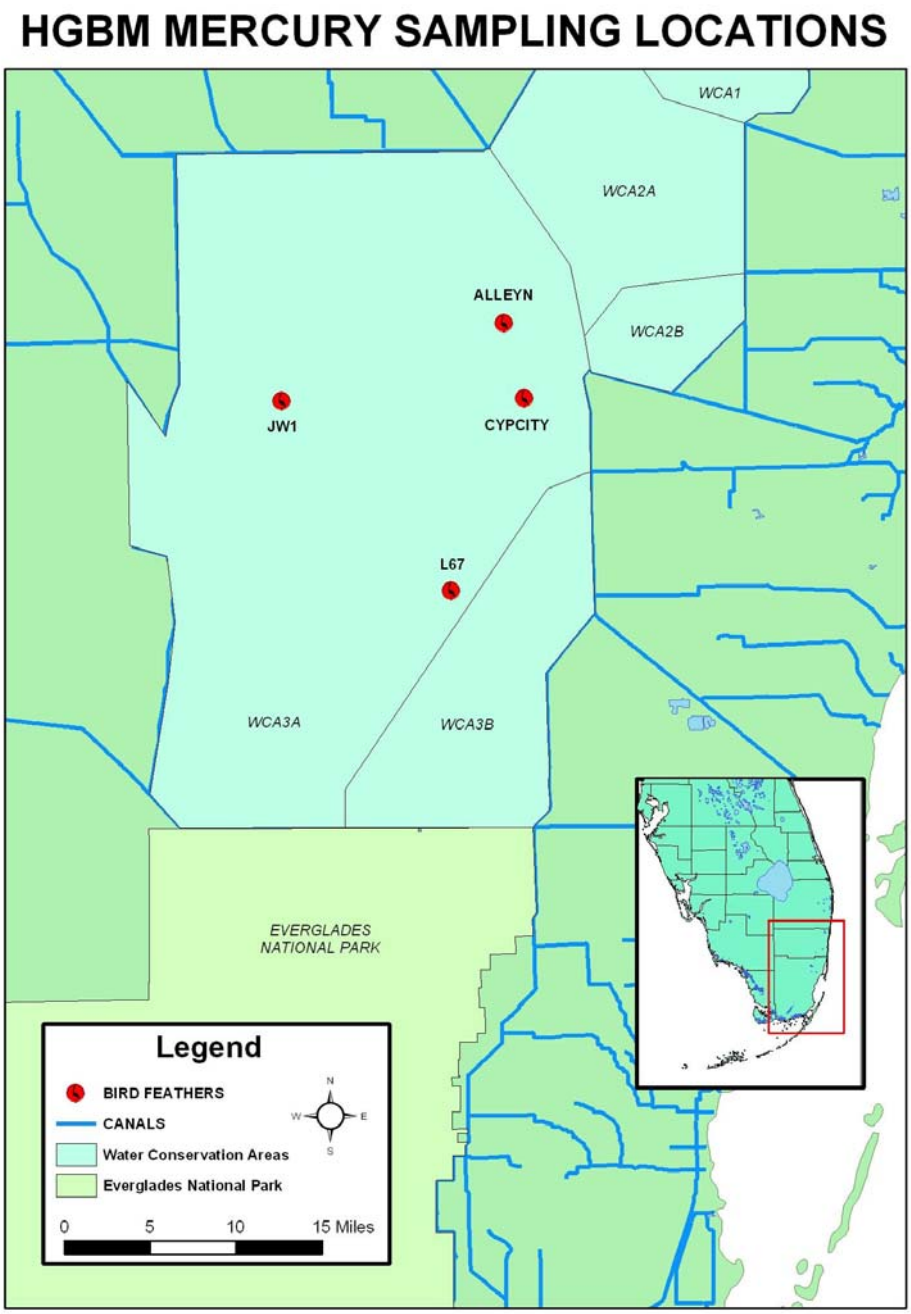

Figure 4. Map showing colonies where great egret nestling feathers have been collected. Explanatory note: Although efforts are made to repeatedly collect from the same colony, colonies are sometimes inactive or abandoned thus requiring collection at an alternate colony.

QUALITY ASSESSMENT FOR THE MERCURY MONITORING PROGRAM

This section is a quality assessment of the District's Mercury Monitoring Program during Water Year 2007 (WY2007) (May 1, 2006–April 30, 2007) 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.

Quality control 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. Quality-control 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 or Battelle) Laboratory (the FDEP being the primary lab and the BMSL the secondary), both of which are certified by the Florida Department of Health under the National Environmental Laboratory Accreditation Program (NELAC). The following methods were utilized when analyzing samples for THg and MeHg during WY2007: 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 the FDEP and the 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 the FDEP and NELAC-accrediting authorities.

FIELD QUALITY-CONTROL SAMPLES

A total of 156 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 (project code HGLE) during WY2007. These field QC check samples represented approximately 32 percent of the 476 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 add little value to the assessment of data quality and are no longer a requirement, the FDEP field blanks were eliminated in WY2003.

Table 1. Frequency of QC field blanks (FB) 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 J[5] Flagged	% Flagged
FKPB	11	4.6	1	0.10	0	0	11	4.6	1	0.026	0	0
EB	19	7.9	2	0.40	2	10	19	7.9	2	0.029	1	5.2
FCEB	16	6.7	1	0.25	0	0	16	6.7	2	0.023	1	6.2
FB	0	NA	NA	NA	NA	NA	0	NA	NA	NA	NA	NA

1 FKPB-Field kit preparation blank, EB-Lab-cleaned equipment blank, FCEB-Field-cleaned equipment blank collected at the end of the sampling event.

2 Total number (n) of surface water samples collected from these structures/sites during WY2007 was 238 THg and 238 MeHg.

3 Mean concentration of contaminated QC samples.

4 Analyte was detected in both the sample and the blank.

5 Estimated value; not accurate.

NA 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, 59 replicate, unfiltered surface water samples (29 THg and 30 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 WY2007. **Table 2** reflects the results of the sample analyses. For surface water two replicate samples (RS) were matched with one surface water sample. For mosquitofish one replicate sample was matched with one routine sample.

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.

		*PRECISION (% Difference)			
Analyte	**n**	**Minimum**	**Maximum**	**Mean**	**Median**
Surface Water THg	29	0	109	27	15
Surface Water MeHg	30	0	103	19	15
Mosquitofish THg	24	0	63	20	20

$$* \frac{|RS - S|}{\left(\frac{RS + S}{2}\right)} x100$$

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 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 WY2007, the mean percent difference between replicate and routine samples for the 24 aliquots was 20 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. 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 re-sampled population may not represent a true replicate of the first sample. For WY2007, out of six replicate sets (each set containing three aliquots) RSDs ranged between 2 and 40 percent with a mean of 14 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 and Fish

No inter-laboratory splits were performed for surface water or fish in WY2007.

Sediment

For WY2007, a total of four sediment split samples were submitted to the BMSL for THg and MeHg analysis. These samples were split from a total of six sediment samples that were later submitted to the FDEP for THg and MeHg analysis. Sediment THg values for the FDEP ranged from 0.085 to 0.093 mg/Kg (n = 3), and MeHg ranged from 0.0011 to 0.0013 mg/Kg (n = 3). THg in sediment for Battelle were 0.0954 and 0.107 (mg/Kg) and 0.0013 and 0.0014 (mg/Kg) for MeHg. The inter-variability of MeHg and THg for each laboratory could not be compared due to the varying number of samples between each lab; therefore dataset variances were not calculated. However, comparing percent differences provides some measure of laboratory precision. After averaging all datasets the percent difference in MeHg between labs was 11 percent; for THg, the difference was 8 percent.

In 2006, the District conducted a performance evaluation (PE study) to assess the ability of the District's contract laboratories to generate analytical data for THg and MeHg of acceptable quality. The following analytical laboratories were used in the PE study: Battelle Marine Sciences Laboratory (Battelle), Florida Department of Environmental Protection laboratory (FDEP), and Frontier Geosciences (Frontier). Further details on this study are presented in the *Performance Evaluation Study of the Analysis of Total Mercury and Methylmercury in Sediment* (see attachment).

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). 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. If multi-group null hypotheses were rejected under ANOVA then the group were compared using either Tukey HSD (Honestly Significant Difference; for equal sized data sets) test or the Tukey-Kramer test (for unequal sized data sets). The assumptions of normality and equal variance were tested by the Kolmorogov-Smirnov and Levene Median tests, respectively. Datasets that either lacked homogeneity of variance or departed from normal distribution were natural-log transformed and 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 or Kruskal-Wallis Rank sum tests. If the multi-group null hypothesis was rejected, then groups were compared using either Nemenyi test (for equal sized data sets) or Dunn's Method (for unequal sized data sets). Pearson Product moment (or the non-parametric equivalent Spearman Rank Order) was used to evaluate the relationship between two parameters. Linear regression was used to develop a line of best fit (linear model) between two parameters.

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 Everglades Nutrient Removal (ENR) Project (i.e., STA-1W), Florida Power and Light's Andytown substation, the Baird Research Center in the ENP, and the Everglades-Western Broward County (FL97) (**Figure 1**). Operation of FL97 began on November 14, 2006, following shut-down of the Andytown substation (October 16, 2006). For more information on MDN and to retrieve raw data, refer to the NADP's web site at http://nadp.sws.uiuc.edu/mdn/. In 2004, difficulties were encountered due to the landfall of four hurricanes (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 northernmost 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. Missing samples at each station in 2006 were due to a combination of no precipitation, sample-handling issues, and mechanical failure. The missing data for station FL04 from October 24 through November 14, 2006, was due to its closure. Missing data for FFL9797 from September 2 through 5, 2006, was due to the passage of Tropical Storm Ernesto.

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, Figure 6,** and **Figure 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 2006. Annual point estimates are also based on calendar year.

Week Ending	ENR (FL34)	Andytown (FL04), Broward (FL97)	ENP (FL11)
1/3/06	NA	NA	NA
1/10/06	NA	15.8	NA
1/17/06	17.68	7.29	NA
1/24/06	12.05	11.3	8.27
1/31/06	9.09	NA	9.1
2/7/06	9.31	1.49	10.0
2/14/06	8.85	0.03	NA
2/21/06	NA	NA	6.82
2/28/06	23.07	1.47	NA
3/7/06	NA	NA	NA
3/14/06	NA	NA	NA
3/21/06	NA	NA	9.83
3/28/06	10.19	15.7	NA
4/4/06	NA	NA	13.67
4/11/06	16.97	10.6	NA
4/18/06	2.63	4.90	21.2
4/25/06	11.65	NA	NA
5/2/06	17.85	17.0	NA
5/9/06	NA	NA	10.8
5/16/06	6.21	8.89	4.46
5/23/06	7.08	4.80	9.34
5/30/06	12.95	21.6	20.1
6/6/06	30.75	15.8	13.1
6/13/06	NA	16.7	17.5
6/20/06	11.48	16.1	9.40
6/27/06	11.6	7.80	9.39
7/4/06	NA	18.9	26.7
7/11/06	12.8	14.7	12.1
7/18/06	8.60	18.8	11.1
7/25/06	29.4	22.3	12.5
8/1/06	21.8	12.1	13.8
8/8/06	NA	15.4	19.6
8/15/06	NA	12.6	13.4
8/22/06	27.7	29.9	22.7
8/29/06	8.90	NA	NA
9/5/06	12.4	12.6	NA
9/12/06	NA	17.1	NA
9/19/06	NA	10.7	NA
9/26/06	NA	14.6	NA
10/3/06	11.8	11.3	19.2
10/10/06	6.00	21.2	13.3
10/17/06	25.2	13.1	35.4
10/24/06	NA	NA	5.09
10/31/06	13.4	NA	12.4
11/7/06	10.7	NA	NA
11/14/06	NA	NA	11.3

Table 3. Continued.

Week Ending	ENR (FL34)	Andytown (FL04), Broward (FL97)	ENP (FL11)
11/21/06	9.50	***11.7***	19.9
11/28/06	10.5	***8.50***	9.10
12/5/06	11.4	***26.6***	5.80
12/12/06	27.5	***12.2***	NA
12/19/06	5.60	***11.0***	10.2
12/26/06	7.40	***7.40***	8.89
Volume-Weight Concentration (ng/L)			
1996*			14.1
1997*	18.7	NA	14.7
1998*	11.4	13.8	12.7
1999*	10.8	12.3	11.6
2000*	13.7	15.8	13.6
2001*	13.9	13.2	13.1
2002*	12.3	14.2	12.1
2003*	16.1	16.4	16.4
2004*	13.7[a]	14.7	14.7
2005*	11.7	13.7	10.6
2006	12.6	14.9	12.4
Deposition Annual ($\mu g/m^2$)			
1996*			17.2
1997*	32.4	NA	27.2
1998*	26.1	20.1	20.3
1999*	12.1	17.5	17.7
2000*	14.3	18.1	20.0
2001*	21.0	21.1	18.0
2002*	10.3[a]	18.7	18.2
2003*	17.8	28.5	26.8
2004*	[a]	18.3	18.7
2005*	11.5	14.5	17.5
2006	14.4	NA[a]	15.4

* Adapted from SFER, 2007

[a] Rain gauge malfunction; in 2004, several trips missed due to four hurricanes.

NA Not available due to mechanical problems with collector, failure to meet QC criteria, or no precipitation

NA[a] No calculation due to (1) discontinuation of station FL04 and (2) not enough data existed for station FL97 to calculate annual deposition

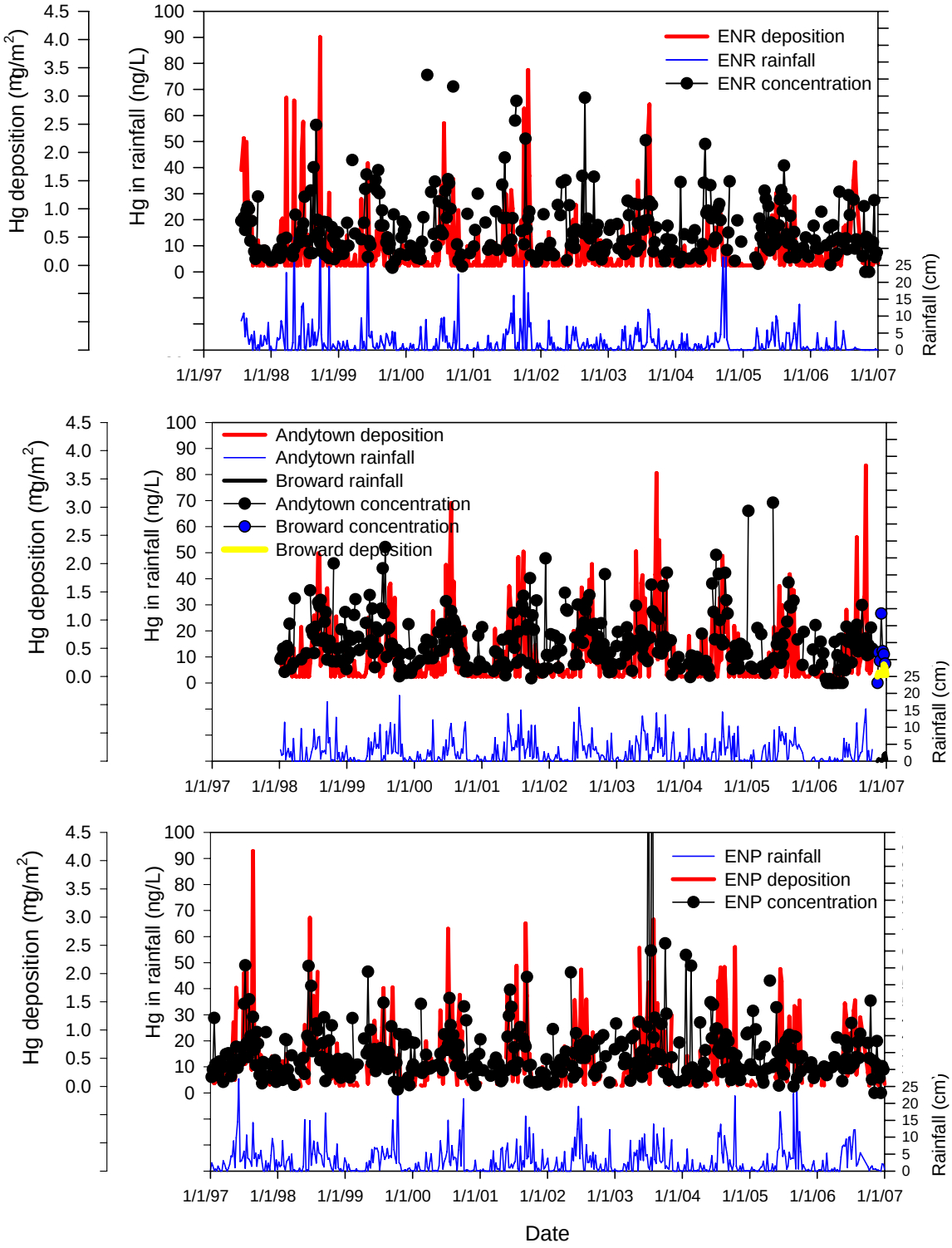

Figure 6. Time series of rainfall, rainfall Hg concentrations, and wet Hg deposition at the ENR Project, Andytown, ENP Bair Research Center, and Broward County, as reported by the MDN.

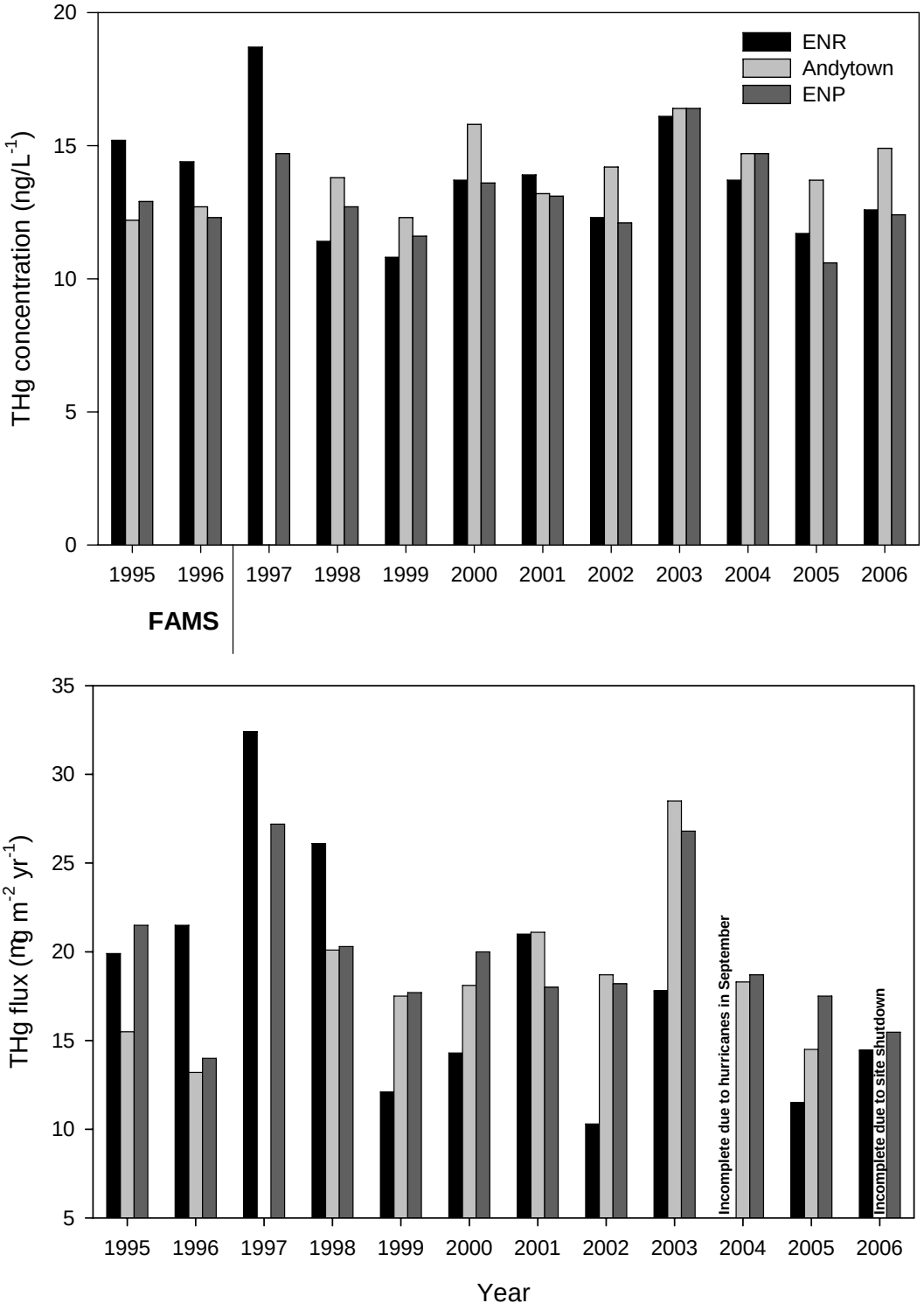

Figure 7. Time series of annual volume-weighted concentration (top) and annual THg flux (bottom) at three MDN stations (FAMS data from Guentzel et al., 2001).

Annual volume-weighted THg concentrations differed slightly among the ENR and ENP stations in 2006; however, both stations were significantly different from the Andytown and Broward County stations (**Table 3**). The above calculations were not performed for FL97 and FL04 because of incomplete year-long time series. No apparent trends are visible for each station aside from a slight drop for ENR from 1997 to 2004.

A seasonal Kendall analyses (of ranks) revealed no significant trends in monthly median THg concentrations at ENR (1997–2006; n = 109 months; Tau = 0.026; p = 0.74), Andytown (1998–2006; n = 102 months; Tau = -0.038; p = 0.65) or ENP sites [1996–2006; n = 127 months; Tau = 0.03; p = 0.06 (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"). Seasonal Kendall analysis still did not show any long-term trends in the monthly deposition at Andytown (n = 102; Tau = -0.038; p = 0.65), or ENP [n = 127; Tau = 0 (S. Hill, SFWMD, personal communication, June 16, 2006)]; however, did so for ENR (n= 109; Tau = -0.156; p = 0.043) which may have been linked to the decreasing trend in rainfall for the same period of record (POR) (n = 112; Tau = -0.212; p = <0.001).

Based on an average deposition rate measured at the three sites, wet-only atmospheric loading of THg to the EPA (9.01×10^{9} m^2) was estimated at 134 kilograms per year (**Table 4**). 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). This estimate should be viewed with caution as 12 percent of the quarterly samples were not available at site FL11 due to issues associated with the passage of Tropical Storm Ernesto and daily mechanical problems.

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]
2006	134[f]	2.7[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.

f Based on average annual loading from FL34 and FL11.

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 2006 was 5.1 ng/L at S-5A during the second quarter (**Figure 8**). This value did not exceed the Florida Class III water quality standard of 12 ng THg/L. As shown in previous reports, statistical differences exist between sites when the entire POR is examined (Kruskal-Wallis ANOVA on ranks; $H = 85.8$; $df = 7$; $p < 0.0001$). Site S-5A had the greatest THg concentration (median 2.0 ng/L) compared to all other sites. Using Dunn's method of pairwise multiple comparisons 10 out of 28 comparison displayed significant differences ($p < 0.05$) with S-5A different from all others. Other significant comparisons were between L-28 and S-9, S-141 and S-9, and S-140 and S-9 with the former sites in each comparison having the higher 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 2006.

Structure	Quarter	THg			MeHg		% MeHg
		ng/L	remark **	WQS	ng/L	remark **	
L28	Jan - March	1.8		<WQS	0.14		7.8
	April - June	1.9		<WQS	0.12		6.3
	July - Sept	1.2		<WQS	NA		
	Oct – Dec	0.93		<WQS	0.064	I	6.9
	Median	1.5			0.12		6.9
	Median POR	1.4			0.11		7.6
S-10C	Jan - March	1.6		<WQS	0.047	I	2.9
	April - June	2.1		<WQS	0.09	I	4.3
	July - Sept	1.6	A	<WQS	NA		
	Oct – Dec	0.59		<WQS	0.049	I	8.3
	Median	1.6			0.049		4.3
	Median POR	0.95			0.09		9.9
S-12D	Jan - March	0.92		<WQS	0.17		18.5
	April - June	0.84		<WQS	0.085	I	10.1
	July - Sept	1.6		<WQS	0.13		8.1
	Oct – Dec	1.1		<WQS	0.23		20.9
	Median	1.01			0.15		14.3
	Median POR	1			0.16		16.0
S-140	Jan - March	1.4		<WQS	0.09		6.4
	April - June	0.72		<WQS	0.08	I	11.1
	July - Sept	3.1		<WQS	NA		
	Oct – Dec	0.64	A	<WQS	0.08	I	12.5
	Median	1.06			0.08		11.1
	Median POR	1.1			0.13		11.8
S-141	Jan - March	1	A	<WQS	0.11		11.0
	April - June	1.4		<WQS	0.17		12.1
	July - Sept	2.4		<WQS	NA		
	Oct – Dec	1		<WQS	0.06	I	6.0
	Median	1.2			0.11		11.0
	Median POR	1			0.18		15.7
S-151	Jan - March	1.5		<WQS	0.16		10.7
	April - June	0.81	A	<WQS	0.19		23.5
	July - Sept	1.7		<WQS	NA		
	Oct – Dec	1		<WQS	0.07	I	7.0
	Median	1.25			0.16		10.7
	Median POR	1			0.15		15.0

Table 5. Continued.

Structure	Quarter	THg			MeHg		% MeHg
		ng/L	remark **	WQS	ng/L	remark **	
S-5A	Jan - March	3		<WQS	0.40		13.3
	April - June	2.8		<WQS	0.07	I	2.5
	July - Sept	5.1		<WQS	0.33		6.5
	Oct – Dec	1		<WQS	0.05	I	4.7
	Median	2.9			0.20		5.6
	Median POR	2			0.14		7.0
S-9	Jan - March	1.1		<WQS	0.04	I	3.6
	April - June	0.39	I	<WQS	NA		
	July - Sept	0.86	A	<WQS	NA		
	Oct – Dec	0.52		<WQS	0.02		3.8
	Median	0.69			0.03		3.7
	Median POR	0.69			0.05		3.7
	Median Jan - March	1.4			0.09		7.77
	Median April - June	1.4			0.125	(1)	9.61
	Median July - Sep	1.6			0.23	(6)	20.9
	Median Oct – Dec	0.92			0.064		7.0

* Class III Water Quality Standard (WQS) of 12 ng/L

** For qualifier definitions, see FDEP Rule 62-160: "A" – averaged value; "I" – below PQL; Flagged values and values that were labeled QC type RS (Replicate sample) or SS (Split sample) value were not used in calculating medians.

NA Not available due to analytical flagging

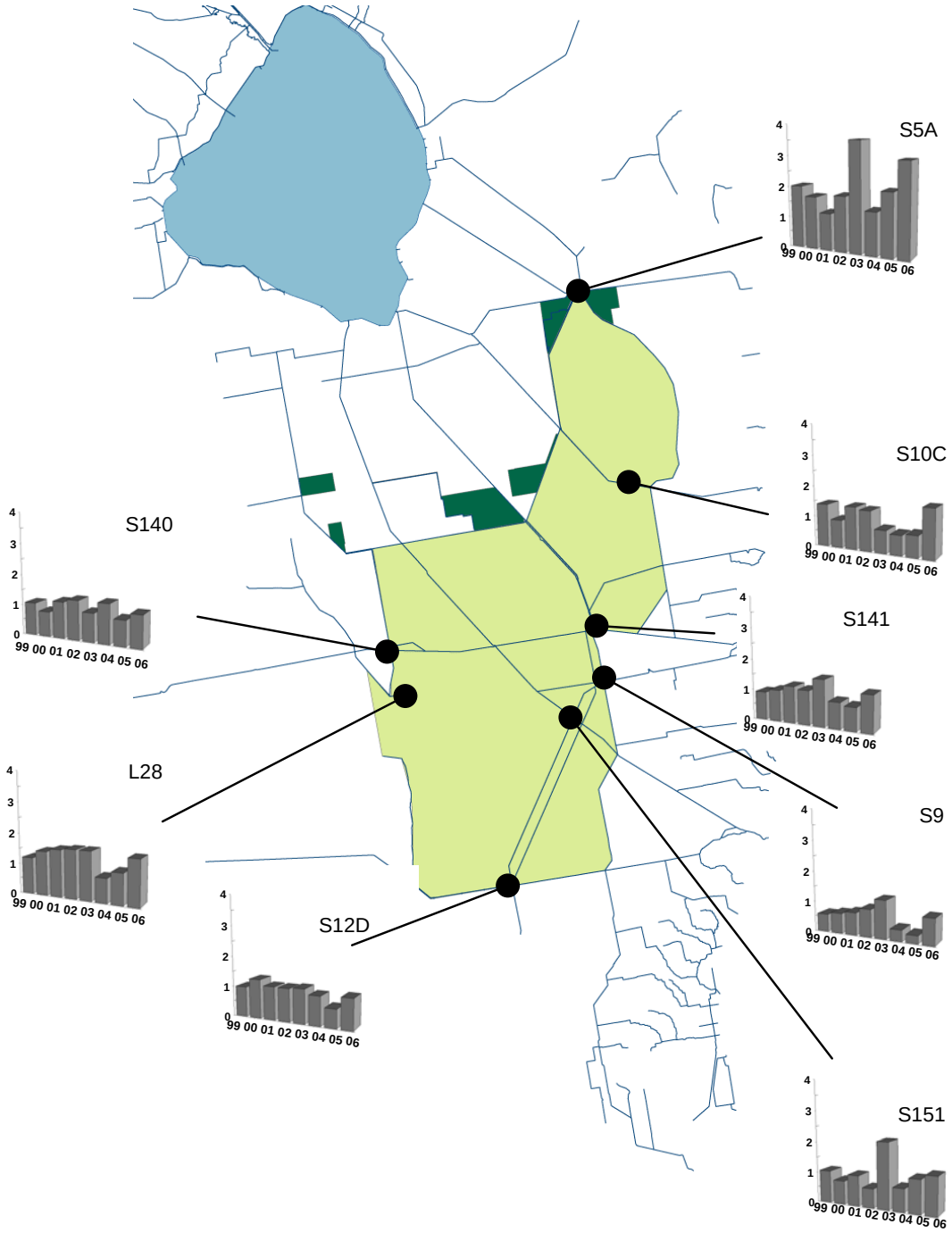

Figure 8. Annual median THg concentrations for period of record (POR) at stations sampled under project code HGLE.

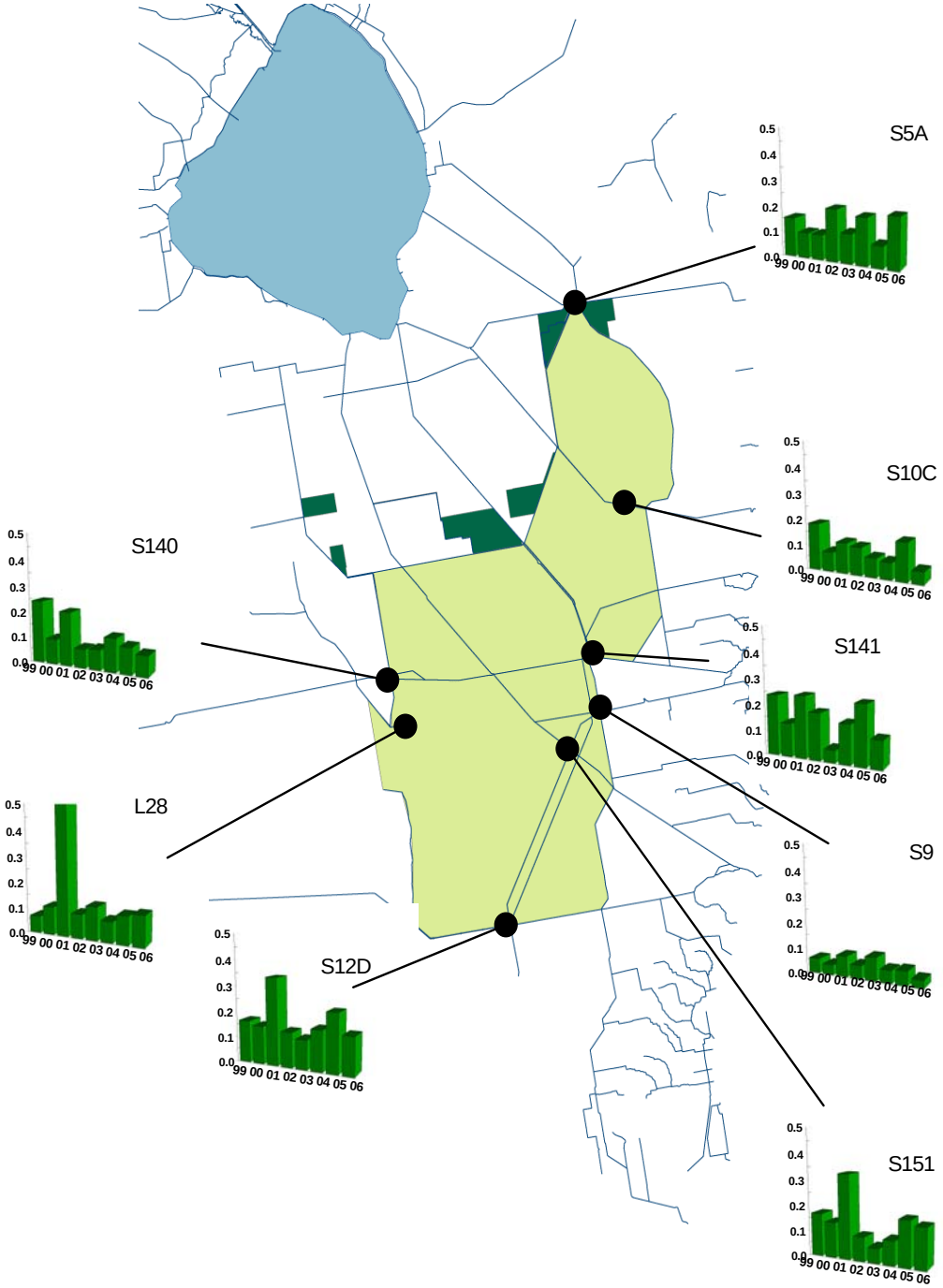

Figure 9. Annual median MeHg concentrations for POR at stations sampled under project code HGLE.

The maximum water-column MeHg concentration observed during calendar year 2006 at a non-ECP structure was 0.4 ng/L, which occurred at S-5A (**Table 5**). Currently, Florida has no Class III numerical water quality standard for MeHg. When the entire POR is examined for MeHg, the most obvious spatial pattern showed that site S-9 typically had the lowest concentration among all sites. Statistically significant differences in MeHg levels were also present (Kruskal-Wallis; $H = 43.7$; $df = 7$; $p < 0.0001$). Pairwise comparisons showed that S-9 was different from all structures (Dunn's test; $p < 0.05$) except S-10C and L-28. No other pairwise comparisons were significant.

After more than nine 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 -0.18 to +0.25 for THg and from -0.09 to +0.24 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, the 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 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 the 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 2006 from downstream sites. Value presents a mean of three analyses.

LOCATION	THg (ng/g)	Between-Year Change (%) (2005 to 2006)*	Cumulative Average (ng/g)
LOXF4	32.6	-18.5	69.0
CA2F1 (L39F1)	16.3	81.1	28.0
CA27 Alt (Z4)	38.6	175.7	93.0
CA2NF	16.6	10.7	116.0
Holey Land (north canal)	41.6	98.1	46.0
Rotenberger Alt. (RotenF1)	44.6	134.7	82.0
Rotenberger rim canal (RotenC)	47.6	-10.2	47.0
WCA2U3	50.6	29.7	112.0
CA33	53.6	143.6	65.0
CA35ALT	56.6	-8.7	93.0
Non-ECP North (CA3F1; end of L-28)	59.6	138.4	54.0
CA315	62.6	11.8	98.0
Non ECP South (CA3F2)	65.6	173.3	42.0
L67F1	68.6	198.3	123.0
Annual mean	**46.8**	**82.7**	

* ((2006-2005)/2005)x100
NA Data not available
Note: Grandmean for POR (1998 to 2006; aliqouts pooled across time and space) n = 531; 72.1 ± 63.1 ng/g; 90% conf. interval is 72.1 ± 4.4; 75th percentile for the POR is 91 ng/g; 90th percentile for the POR is 150 ng/g

Table 7. Mean concentrations (±1 SD; ng/g wet weight) of THg in sunfish (*Lepomis* spp.) collected in calendar year 2006 from marshes within the EPA downstream of the STAs.

TARGET LOCATION	SAMPLING LOCATION	Mean THg ng/g (± 1SD, n)	Between-Year Change (%) (2005 to 2006)$	Grand Mean (1998 to 2006) (ng/g)
WCA1-LOX3	LOXF4*	109 (±38,14*)	-6.8	123
WCA-2A-F1	L39F1	73 (±89,20)	108.6	69
WCA-2A-2-7	Z4	NA		180
	CA2NF	70 (±41,20)	-44.0	70
Holey Land	Holey Land	162 (±52,20)	11.0	149
Rotenberger	RotenC (canal)*	122 (±89,15*)	-9.0	153
WCA-2A-U3	WCA2U3	226 (±94,20)	-5.4	170
WCA-3A-3	CA33	166 (±129,20)	30.7	130
WCA-3A-5	CA35ALT	273 (±67,19*)	42.2	197
Non-ECP North	CA3F1	72 (±52,20)	-48.2	123
WCA-3A-15	CA315	268 (±112,20)	31.4	288
Non-ECP South	CA3F2	61 (±62,20)	-50.4	124
ENP P-33 Marsh	L67F1	225 (±159,20)	-7.4	421
Average		**152**	**4.37**	

* Unable to collect 20 fish
$ ((2006-2005)/2005)x100
NA Data not available
Note: Grand mean of sites (pooled across space and time) for POR (1998 to 2006) ± 95% C1 of mean: n = 1927, 177 ± 7.9 ng/g; 50th and 95th percentiles for POR were 130 and 470 ng/g, respectively

Table 8. Standardized (EHg3) and arithmetic mean concentrations of THg in largemouth bass fillets (*Isopterous salmoides*) (ng/g wet weight) collected in 2006 from ECP and non-ECP interior sites.

TARGET LOCATION	SAMPLING LOCATION	EHg3 ± 95th CI (mean ± 1SD, n) ng/g wet	Between-Year Change# (%) (2005 to 2006)	Cumulative EHg3
WCA1-LOX-3	LOXF4	577±53 (348±182, 20)	20.2	483
WCA-2A-F1	L39F1	247±68 (240±184, 20)	-1.2	266
WCA-2A-2-7	CA2NF	633±248 (378±637, 20)	-12.1	633
Holey Land	HOLYBC	863±86 (975±224,20)	16.6	544
Rotenberger	RotenC	NC (2) (94±21, 3)	NA	800
WCA-2A-U3	WCA2U3	NC(1) (488±149,20)	NA	732
WCA-3A-3	CA33	NA	NA	NA
Non-ECP North	CA3F1	464±57 (399±175, 20)	-19.9	505
WCA-3A-5	CA35ALT	NA	NA	NA
WCA-3A-15	CA315	NC(2) (632±129, 15*$)	NA	816
Non-ECP South	CA3F2	595±153 (350±338, 19)	NA	545
ENP P-33 Marsh	L67F1	1244±317 (1,411±744, 20)	10.1	1316

* Unable to collect 20 fish
NC Not calculated for (1) insignificant slope or (2) poor age distribution
NA Data not available
$ retrieved from archived samples
# for all samples
Note: 2006 EHg3 average = 660 ng/g
Grand mean for sites (pooled across space and time) for POR (1998 to 2006) ± 95% C1 of mean: n = 1374, 536 ± 22 ng/g; 50th and 95th percentiles for POR were 420 and 1460 ng/g, respectively

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 2006 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 (*Gambusia* sp.) collected from marsh sites in 2006 ranged from 15 monograms per gram (ng/g) at site CA2NF to 69 ng/g at site L67F1 (**Table 6** and **Figure 10**). The annual basin wide average concentration in mosquitofish collected in 2006 was 46 ng/g (**Table 6)** (for all locations, see **Figure 3**), which represents a 48 percent increase from the basin-wide mean concentration in 2005 (31 ng/g). The mean aliquot for tissue-mercury concentrations in mosquitofish for the POR (1998–2006; n = 531) was 150 ng/g. In 2006, THg levels in mosquitofish declined at two of 14 sites (**Table 6**).

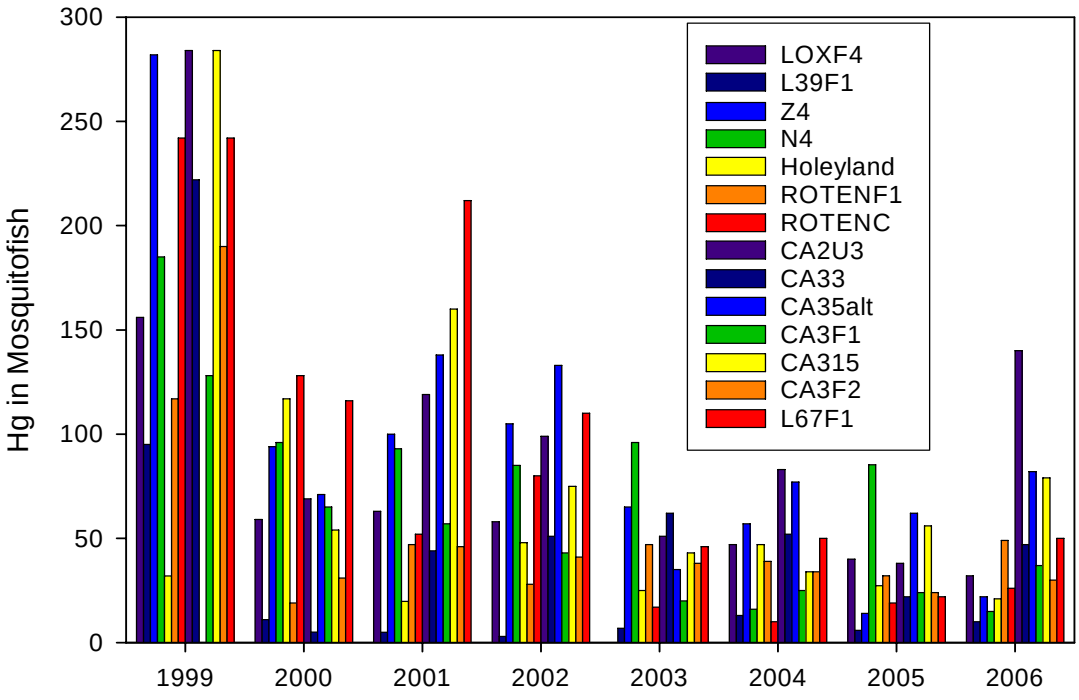

Figure 10. Mercury concentrations in mosquitofish (*Gambusia* sp.) collected at ECP and non-ECP sites for the POR (i.e., 1998–2006). Not all sites were sampled in all years (for details, see **Table 6**).

Sunfish

Mercury levels in sunfish (*Lepomis* spp.) collected from downstream sites in 2006 (n = 229) ranged from a low of 13 ng/g in a redear sunfish (*L. microlophus*) from site L39F1 to a high of 650 ng/g in a bluegill (*L. macrochirus*) from site CA33. This pattern of minimum and maximum contrasts 2005 as for this year the maximum was observed at the opposite end of the EPA at site L67F1. The grand mean of all sites in 2005 was 154 ng/g. For 2006 the grand mean is 153 ng/g indicating a 0.6 percent decrease.

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. For this year however, ANCOVA could be used to remove the observed variability in sunfish THg concentration with location due to weight (an insignificant interaction existed between location and weight; weight*location, $p = 0.1644$, $do = 12$, $f = 1.41$). After removing the impact of weight on fish THg variability the THg levels were still significantly different ($p < 0.0001$, $do = 11$, $f = 22.4$), therefore demonstrating the importance of spatial location on THg level.

As observed over the past seven years when data was pooled across sites, fish species was a significant factor in tissue mercury concentration in 2006 (Kreskas-Wallis ANOVA on Ranks; $do = 3$; $H = 54.1$; $p < 0.001$). Mercury levels were statistically lower in redear (*L. microlophus*, median = 70 ng/g) than each of the other three species (Dunn's method, $p < 0.05$): bluegill (*L. macrochirus,* median = 140 ng/g), spotted sunfish (*L. punctatus*, median = 265 ng/g), and warmouth (*L. gulosus*, median = 160 ng/g). *L. macrochirus* was also statistically lower than *L. punctatus* (Dunn's Method, $p < 0.05$). These species-specific medians were nearly similar to 2005 values. In 2006, sunfish continued to show significant spatial patterns in mercury levels (**Table 7**; **Figure 11**; $do = 11$; $H = 121$; $p < 0.001$). Fish from sites CA315 and CA35ALT contained the highest median concentrations (both 250 ng/g) and differed from all other sites (Dunn's Method, $p < 0.05$) except HOLYBC, L67F1 and WCA-2-U3.

From visual inspection of **Figure 11**, sunfish appeared to exhibit clear temporal variability in mercury burdens for most sites; however, these apparent trends were confounded by temporal differences in size or species of lipoid collected. For example, the marked decline in mercury levels for 2006 in fish from CA3F2 may be an artifact from a sample with greater numbers of redear (15 of 20 fish) as compared to previous samples. Similarly, the decline in mercury levels in fish apparent at site CA3F1 may also be due to increased numbers of redear (12 of 20). To exclude this variability due to species and size, the sunfish dataset for the POR (1998–2006) was censored to assess only bluegill ranging in size from 123 through 178 millimeters (lower and upper quartiles for all stations) for temporal trends (to reduce size-related effects further, mercury levels were normalized by dividing measured concentration by total length of the fish). This analysis showed significant differences between sites (Kreskas-Wallis ANOVA on Ranks; $do = 12$; $H = 280$; $p < 0.001$), therefore demonstrating the importance of spatial location on THg levels in bluegill. Nearly all possible paired comparisons showed significant differences (Dunn's Methods, $p < 0.05$). Sites WCA-3-5ALT (median = 1.638 ng/g/mm) and L67F1 (2.82 ng/g/mm) were both higher than all other sites. Despite the relatively close proximity of sites WCA-3-5ALT and CA3F1 (median = 0.513 ng/g/mm), sunfish THg levels were much different (**see Figure 3**).

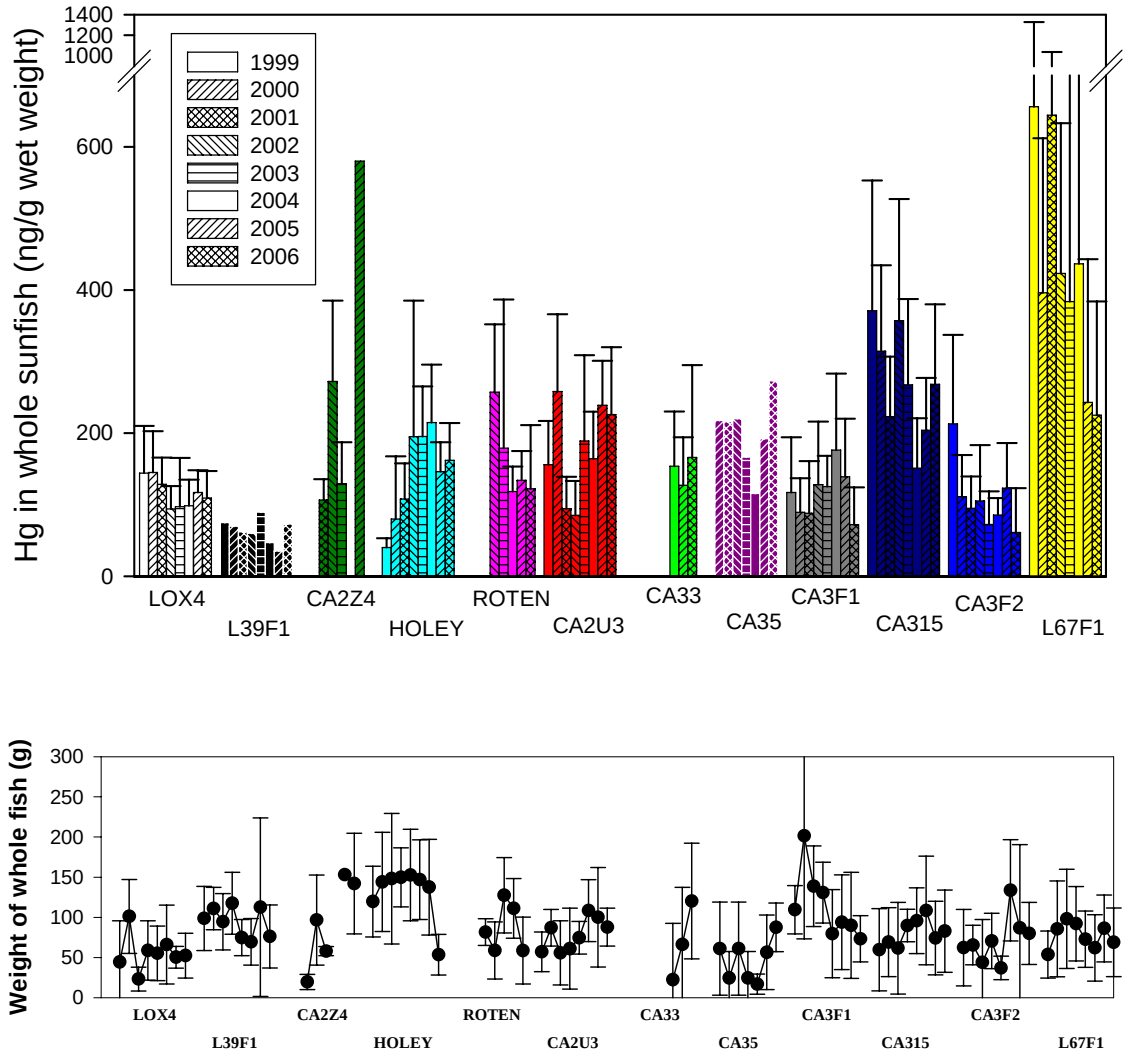

Figure 11. THg concentration (top) and weights (bottom) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the POR.

Largemouth Bass

A total of 157 largemouth bass were collected at 11 downstream sites from October through November 2006. Despite the best efforts of the FWC (who were contracted to electro-fish at these sites), bass could not be collected from sites CA33 and CA35ALT. The bass that were collected had tissue mercury concentrations ranging from a low of 64 ng/g in a one-year-old fish from site ROTENC to 3,400 ng/g in a seven-year-old fish from site L67F1. Site specific, age-standardized concentrations (EHg3) ranged from 342 ng/g at site L39F1 to 1,630 ng/g at site L67F1 (**Table 8** and **Figure 12**); however, the latter was reported for information only and contrasts somewhat with the 2005 estimates. Calculation of EHg3 was not appropriate at sites ROTENC, CA2U3, CA315, CA33, and CA35ALT 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 value was 857 ng/g in 2006, which represents a 26 percent increase over the grand mean estimated for 2005.

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**). Because of a statistically significant interaction between location and age ($f = 64.4$; $do = 10$, $p < 0.001$), ANCOVA could not be used to assess differences in mercury levels among all sites.

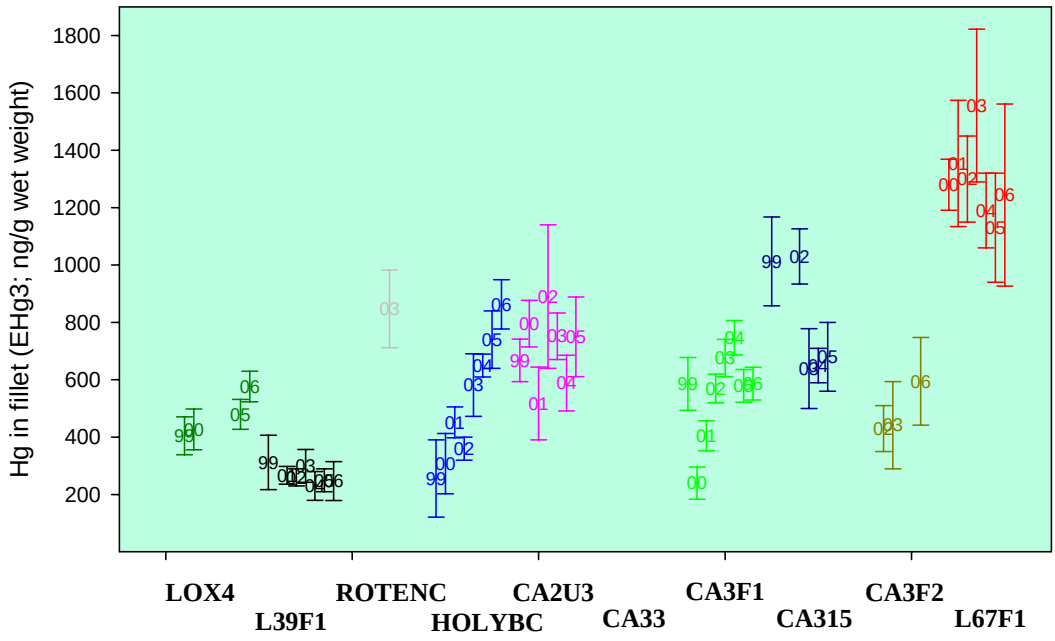

Figure 12. Age standardized (class three-year) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at downstream sites from 1999–2005. EHg3 was not calculated if regressions were not significant or if age distributions were narrow (see **Table 8**).

Based on **Figure 12,** the most obvious progressive trends of increasing mercury in fish occur at CA3F1 and the Holey Land Wildlife Management Area (Rumbold, 2005; Rumbold et al., 2006). An attempt was made to use ANCOVA to assess temporal differences in mercury levels in bass from CA3F1 due to the insignificant interaction between sample date and fish age (date*age, $f = 1.65$; $df = 5, 122$; $p = 0.150$). However, after removing the age effect on THg level, the impact of time (date) was insignificant ($f = 2.33$, $df = 1$, $p = 0.129$). At the Holey Land the effect of time on THg levels could not be evaluated due the significant interaction between age and date ($df = 141$, f value = 53.7, $p < 0.001$).

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 (Eisner, 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 3 (TL-3) and 4 (TL-4) fish, respectively, for the protection of piscivorous (fish-eating) avian and mammalian wildlife (USEPA, 1997).

In 2006, mosquitofish (considered to be at TL-2 and TL-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 the 77 ng/g criterion at all sites and approached or exceeded the 346 ng/g criterion at half of the sites (**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 x fillet THg; Lange et al., 1998) exceeded the guidance value for TL-4 fish at all but two sites (L39F1 and RotenC). 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

In early 2006 (April 30 and May 16), feather samples were collected by the District's Water Quality Monitoring Division from a total of 20 nests at the two colonies (Cypress City and L67; **Table 9**). Feather THg concentrations ranged from 0.9 μg/g in a chick from Cypress City estimated to be 15 days old to 9.2 μg/g in a chick from the L67 colony thought to be 21 days old. The overall mean concentration (both colonies pooled) was 4.49 ± 3.5 μg/g. Regression of feather mercury concentrations on bill length (i.e., as an age surrogate) were not statistically significant when the colonies were run separately or when pooled across colonies. As shown in **Table 9**, this least square mean 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 (fish eaters) 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 2007 do not appear to be at risk of toxicological effects from MeHg.

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

YEAR	JW1	L67	Cypress City	Alley North
1994	21±6 (25±8,9)	16±4 (N/A)	NA	NA
1995	14 ± 3 (N/A ± 8)	16 ± 6 (16 ± 6,14)	NA	NA
1999	7 ± 1 (4 ± 2,13)	NC (4 ± 2,20)	NA	NA
2000	7 ± 1 (3 ± 2,10)	NC (3 ± 1,10)	NA	NA
2001	Failed to initiate nesting	NC (7 ± 3,13)	NA	NA
2002	Colony abandoned	NC (2 ± 0.5,6)	NA	NA
2003	Failed to initiate nesting	NC (5 ± 2,3)	NC (6 ± 2,15)	NA
2004	Failed to initiate nesting	4 ± 2 (1 ± 1,10)	5 ± 2 (2 ± 1,10)	NA
2005	NS	Failed to initiate nesting	NS	NC (4 ± 2,3)
2006	NS	NC (5 ± 2,6)	NS	NC (3 ± 2,8)
2007	NS	NC (6.7± 3.7,10)	NC (2.2 ± 1,10)	NS

* Data from Fredrick et al. (1997)
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, 28-29 days in 2006, and 19 days old in 2007

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 Florida Department of State's Electronic Databases 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 2006 SFER – Volume I, Chapter 6).

OPPORTUNITIES FOR OPTIMIZING THE MONITORING NETWORK

Following discussions between the District and the 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 the 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 be 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 waters 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 datasets have now been established. To preserve these long-term sites, the District has submitted a monitoring plan for approval that eliminates sampling sites that have not successfully produced fish since 1998 (and thereby makes the alternate sites the new 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 1,735 unfiltered water samples (data from STA and HGLE projects) collected and analyzed for THg between 1997 and 2006 (and which met all quality controls), 74 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.
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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Attachment:

Performance Evaluation Study of the Analysis of Total Mercury and Methylmercury in Sediment

HSW Engineering, Inc., July 2007

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Performance Evaluation Study of the Analysis of Total Mercury and Methylmercury in Sediment

Summary Report

July 2007

Contract No. STS060574
Work Order No. WO03

Prepared for:

South Florida Water Management District
3301 Gun Club Road
West Palm Beach, Florida 33406

Prepared by:

HSW Engineering, Inc.
3820 Northdale Blvd, Suite 210-B
Tampa, Florida 33624
(813) 968-7722

Introduction

The South Florida Water Management District (SFWMD, or the District) routinely collects sediment samples for mercury monitoring and sends the samples to contract laboratories for analysis of total mercury (THg) and methylmercury (MeHg). To assess the ability of the District's contract laboratories to generate analytical data for THg and MeHg of acceptable quality, the District recently conducted a performance evaluation (PE) study of mercury in sediment involving three analytical laboratories: Battelle Marine Sciences Laboratory (Battelle), Florida Department of Environmental Protection laboratory (FDEP), and Frontier Geosciences (Frontier). Both Battelle and Frontier are located in Seattle, Washington. The FDEP laboratory is located in Tallahassee, Florida.

Two sediment standards were used for the PE study: (1) a standard reference material (IAEA405) purchased from an outside vendor, and (2) a PE standard (STAC33) created from a sample collected in the field by District personnel from an established sampling location for an on-going SFWMD project. This report provides a brief description of how the PE samples were created and distributed, a description of the analytical methods and results reported by each of the three laboratories, and an assessment of individual laboratory performance and the extent of comparability of data among the three laboratories, using routine laboratory evaluation criteria as well as statistical analyses (Appendix A).

PE Sample Preparation, Verification, and Distribution

An independent third party (ERA of Colorado), under contract to the District, was responsible for homogenizing sample STAC33 and for creating individual subsamples of STAC33 and IAEA405. Upon receipt of these subsamples from ERA, the District shipped three subsamples of IAEA405 and three subsamples of STAC33, with preparation dates of February 5, 2007, to each of the three participating laboratories.

To verify the uniformity of the samples submitted to the laboratories and the stability of the material throughout the time period of the study, the District's laboratory performed a pre-

test homogeneity study before sending the samples to the laboratories and a post-test stability study after receiving the analytical results from the three laboratories. These studies were performed for THg for the purchased standard (IAEA405) and homogenized field sample (STAC33). The procedures and results regarding this study have been documented (SFWMD 2007). Based on these results, SFWMD concluded the samples used for the study were homogenous and the samples and concentrations of THg were stable during the study time period.

Laboratory Results

Battelle and Frontier analyzed both sets of sediment samples for THg by cold vapor atomic fluorescence (CVAF), while FDEP analyzed both sets of sediment samples for THg by cold vapor atomic absorption (CVAA) using a method based on EPA Method 7471A (Table 1a). Each of the three laboratories analyzed both sets of sediment samples for MeHg by CVAF using modified versions of EPA Method 1630 (Table 1b and Appendix B).

Each laboratory analyzed each subsample for both THg and MeHg. Thus, a total of 12 results were reported by each laboratory, with four mean results (C_{mean}) and four standard deviations (s_{lab}) calculated for each laboratory (Table 2 and Appendix C).

Performance Evaluation Methods

Laboratory performance was assessed by determining the *percent recovery* (%R), which allows an assessment of analytical accuracy by comparison to an objective standard, the *percent relative standard deviation* (%RSD), which allows an assessment of intra-laboratory precision, and the *Z score* (Z), which allows an assessment of inter-laboratory performance (Table 3). These calculated values are defined as follows.

$$(1) \qquad \%R = [C_{mean} / C_{assigned}] \times 100$$

where C_{mean} is the mean or average concentration of each set of three results for THg and MeHg in IAEA405 and STAC33 and $C_{assigned}$ is the reference or "true" value. In the case of IAEA405, $C_{assigned}$ is the certified value of THg (810

micrograms per kilogram [ug/kg]) or MeHg (5.49 ug/kg) provided by the vendor. In the case of STAC33, $C_{assigned}$ is the average of nine replicate analyses for THg (94 ug/kg) performed by SFWMD as part of the pre-test homogeneity study. Because the District did not perform any pre-test analyses of STAC33 for MeHg, the consensus value of 1.4 ug/kg (the average of all nine results reported by the three participating laboratories) was used as $C_{assigned}$.

(2) $\%RSD = [s_{lab} / C_{mean}] \times 100$

where s_{lab} is the standard deviation of the three subsample results for THg or MeHg reported by a given laboratory for samples IAEA405 and STAC33 and C_{mean} is as previously defined.

(3) $Z = [C_{mean} - C_{assigned}] / s_{group}$

where s_{group} is the standard deviation of the three C_{mean} results reported by each laboratory for THg or MeHg in samples IAEA405 and STAC33 and $C_{assigned}$ is as previously defined. The results of a world-wide intercomparison exercise for IAEA405 also were used to generate alternative Z scores for each of the three participating laboratories for IAEA405, using standard deviations (s_{IAEA}) calculated from the results reported for THg in IAEA405 by 60 laboratories and for MeHg in IAEA405 by 14 laboratories.

Conclusions in this report regarding these performance measures as calculated for each of the three laboratories are based on method performance criteria, EPA validation guidelines, NELAC PE acceptance criteria, and generally accepted standards of good laboratory performance. For example, EPA Method 1630, which is the basis for the methods used by each of the three laboratories for analysis of MeHg (i.e., CVAF), indicates that matrix spike/matrix spike duplicate (MS/MSD) recoveries should be 65-135% and that recoveries of calibration verification standards (which would be absent of matrix effects) should be 85-115%. EPA validation guidelines consider MS/MSD recoveries of as low as 30% to be acceptable under certain conditions (i.e., the sample used for spiking is sufficiently similar in matrix to the

unspiked samples in the batch for the recoveries to be applicable and target analytes are detected in the unspiked samples); thus, a lower recovery limit of 45% was considered to be a reasonable minimum for acceptance of positive findings for this PE study. EPA validation guidelines also give an upper limit of 30%RSD associated with instrument calibrations, so 30% was selected as the upper limit for precision for the homogeneous subsamples that comprised this study. The Z score limits for acceptable performance ($-3 \leq Z \leq +3$) are based on NELAC requirements for laboratory certification and assumptions about the statistical dispersion of random errors.

Laboratory performance was further assessed by statistical analyses, including box-whisker plots, one-sample t-tests, and one-way analysis of variance (ANOVA) (Appendix A). The nine pre-test replicates and nine post-test replicates performed by SFWMD were included in these analyses, together with the results from the three participating laboratories. The box-whisker plot summarizes five statistics (median, upper and lower quartiles, and minimum and maximum data values) in a single chart to convey information about data symmetry, skewness (or extent of asymmetry), and outliers, as well as its differences as compared with other datasets. The one-sample t-test provides a technique to investigate whether or not the sample mean is different from a specified value. The one-way ANOVA, an extension of the independent group t-test, is used to compare the means of more than two independent groups to determine whether there is evidence at least one pair of means is not equal. Both the t-test and ANOVA were conducted for the IAEA405 results, while only the ANOVA was carried out for the STAC33 results (due to the absence of a certified value for $C_{assigned}$).

To determine which means are different from others, an additional multiple comparison test can be performed when a statistically significant difference is indicated by the one-way ANOVA. Bonferroni and Tukey are two frequently used pairwise methods among several available comparison options. When the number of comparisons is large, the Tukey method may be more sensitive in detecting differences, while the Bonferroni method may be more sensitive when the number of comparisons is small (SPSS 1999). In this report, both methods were used. The statistical outputs from these analyses have not been included in this report but are available upon request.

The level of significance (α) for the statistical analyses used in this report is 0.05 (or equivalently, 5 %). Thus, if a test of significance gives a probability value (*p*) lower than the α-level, the null hypothesis (i.e., the hypothesis that there is no difference between or among means) is rejected.

Results and Discussion

Although the lengths of time from sample creation to sample preparation and analysis differed for each laboratory (Table 2), the District's post-test stability study indicates holding times are not a factor in the differences in reported results (SFWMD 2007). Based on analytical quality control (QC) data included in each laboratory's data report, there is no evidence of analytical bias, loss of precision, or contamination (Appendix C).

Based on %Rs, %RSDs, and Z scores, each of the three laboratories performed acceptably (Table 3 and Figures 1a through 4b). Recoveries ranged from 67% (reported by Frontier for MeHg in IAEA405 and considered "acceptable") to 124% (reported by Battelle for THg in STAC33 and considered "good"), with the majority of reported means concluded to be "very good." There is no apparent directional bias to the recoveries except for the recovery of MeHg reported by Frontier for IAEA405. Laboratory precision also was determined to be "good" or "very good," with the %RSD less than 20% in all instances and exceeding 10% in only two instances (both involving low level detections of MeHg for which greater variability would be expected). Z scores ranged from "acceptable" to "very good."

For THg in STAC33, the box-whisker plots indicate Battelle reported higher concentrations than those reported by Frontier and SFWMD, with its minimum value the same as those from FDEP. The three FDEP THg results have an identical value of 110 ug/kg that is likely due to the laboratory's rounding of the results to two significant digits. The lack of common overlap among the box-whisker plots indicates differences may exist among these laboratories' results. This observation was verified by the *p*-value (< 0.001 at $\alpha = 0.05$) associated with the F test in the ANOVA analysis (Appendix A-1). Multiple comparisons using both the Tukey and Bonferroni methods (Table 4) indicate that, at the significance level of 0.05 with the *p*-value > 0.05, the means reported by Battelle and FDEP are similar, as are the means

of the SFWMD pre- and post-test analyses; however, the mean of Frontier is different from those of the other laboratories.

For MeHg in STAC33, results from Battelle, FDEP, and Frontier were compared (SFWMD did not analyze STAC33 for MeHg). The MeHg results from the three laboratories are different from one another in terms of their median, value range, and minimum and maximum values, as seen in the box-whisker plots (Appendix A-2). FDEP reported higher concentrations, and Frontier reported lower concentrations. Significant difference exists among the three means of MeHg from the three laboratories ($p < 0.001$ at $\alpha = 0.05$). Multiple comparisons indicate that the means of Battelle and Frontier are similar to each other, but that both are different from the mean reported by FDEP (Table 4).

Without a reference/standard value for either THg or MeHg for STAC33, the one-way ANOVA analysis discussed above can provide information on the differences among the means provided from different laboratories, but not which laboratories produced more accurate data. IAEA405 samples have reference values of 810 µg/kg for THg and 5.49 µg/kg for MeHg (IAEA 2000); therefore, the one-sample t-test was used to evaluate the accuracy of laboratory performance.

The box-whisker plots (Appendix A-3) indicate that SFWMD's pre- and post-test analytical results for THg are reported at a higher concentration than those of the three participating laboratories. Two data points which lie more than one and a half times the length of the box from the upper end of the box were identified for SFWMD's results (890 µg/kg THg for the pre-test and 875 µg/kg THg for the post-test). Additionally, no overlap of value ranges was observed among the results reported by Battelle, FDEP, and Frontier. The one-sample t-test indicates both Frontier and Battelle generated means closer to the reference value of 810 µg/kg ($p > 0.05$ at $\alpha = 0.05$), while the other laboratories' means are significantly different from this reference value ($p < 0.05$ at $\alpha = 0.05$). The multiple comparisons in the ANOVA (Table 4) indicate that Battelle and FDEP generated similar means, FDEP produced a similar mean to that reported by Frontier, and that the means generated by SFWMD differ from those reported by the other laboratories ($p < 0.05$ at $\alpha = 0.05$).

Similar analyses also were conducted for the IAEA405 MeHg analytical results (Appendix A-4). Battelle and FDEP reported MeHg results at concentrations higher than Frontier (as can be observed in the box-whisker plots). The one-sample t-test indicates Battelle and FDEP generated more accurate MeHg results ($p > 0.05$ at $\alpha = 0.05$), with means closer to the test value of 5.49 µg/kg than that of Frontier, which reported a much lower mean MeHg result than the test value ($p < 0.05$ at $\alpha = 0.05$). The one-way ANOVA test indicates that differences among the means exist ($p < 0.05$ at $\alpha = 0.05$). The multiple comparisons indicate that Battelle has a similar mean for MeHg in IAEA405 to that reported by FDEP, while both means are different from the mean reported for MeHg in IAEA405 by Frontier.

Conclusions

Based on the results of this PE study, each of the three laboratories is capable of analyzing THg and MeHg in a sediment matrix and producing data of a quality ranging from "acceptable" to "very good" as these terms are commonly understood in the environmental industry. One point of potential concern is the low recovery of MeHg (67%) reported by Frontier for IAEA405.

From the statistical analysis of the STAC33 THg sample results, Battelle produced results similar to those of FDEP, while the results for Frontier and SFWMD were different from the results for Battelle and FDEP and from each other. From the statistical analysis of the STAC33 MeHg sample results, Battelle generated a mean similar to that reported by Frontier, and FDEP generated a mean result with a higher concentration than reported by either Battelle or Frontier. Using the reference THg and MeHg values for IAEA405, an evaluation of laboratory accuracy was conducted through the one-sample t test. From the analysis of THg in IAEA405, both Battelle and Frontier produced better mean results in comparison to the reference THg value than did FDEP, while Battelle and FDEP produced mean results closer to the reference MeHg value than did Frontier. Overall, the Battelle laboratory demonstrated more consistency in terms of laboratory results, similarity with other laboratories, and accuracy toward the reference values for both THg and MeHg in both STAC33 and IAEA405.

References

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International Atomic Energy Agency. December 2000. World-Wide Intercomparison Exercise for the Determination of Trace Elements and Methylmercury in Estuarine Sediment Sample IAEA-405.

National Environmental Laboratory Accreditation Conference Standard. 2003.

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SPSS Inc. SPSS Base 10.0 Applications Guide. 1999.

TABLES

Table 1a. Summary of Methods Used by the Participating Laboratories in the Analysis of Sediment Samples for Total Mercury

Lab	Preparation			Analysis			
	Lab Method ID	Basis for Lab Method	Preparation Technique	Lab Method ID	Basis for Lab Method	Detection Technique	MDL (ug/kg)
Battelle	MSL-I-016-07	EPA 245.5, EPA 7471A, NOS ORCA 130 [a]	Aqua regia (HCl - HNO3 mixture)	MSL-I-016-07	EPA 245.5, EPA 7471A	CVAA	0.911
FDEP	HG-020-5	EPA 245.5	H_2O_2, HNO_3, $KMnO_4$, $K_2S_2O_8$	HG-008-3.13	EPA 7471A	CVAA	0.1563 [b]
Frontier	FGS-066.5	EPA 1631	Aqua regia (HCl - HNO_3 mixture)	FGS-069-04	EPA 1631	CVAF	0.3 [c]

Table 1b. Summary of Methods Used by the Participating Laboratories in the Analysis of Sediment Samples for Methylmercury

Lab	Preparation			Analysis			
	Lab Method ID	Basis for Lab Method	Preparation Technique	Lab Method ID	Basis for Lab Method	Detection Technique	MDL (ug/kg)
Battelle	MSL-I-015-07	Bloom et al 1997 [d]	KBr in MeOH, $CuSO_4$, CH_2Cl_2; 1% $NaBEt_4$	MSL-I-015-07	EPA 1630M	CVAF	0.0156
FDEP	HG-003-2.6	EPA 1630M	KOH in MeOH; 2% $NaBEt_4$	HG-003-2.6	EPA 1630M	CVAF	0.050
Frontier	FGS-045.3	Bloom et al 1997 [d]	Acidic bromide slurry, CH_2Cl_2; KOH in MeOH; $NaBEt_4$	FGS-070.2	EPA 1630M	CVAF	0.25 [e]

MDL method detection limit
CVAA cold vapor atomic absorption
CVAF cold vapor atomic fluorescence

a NOAA Technical Memorandum NOS ORCA 130 "Sampling and Analytical Methods of the National Status and Trends Program Mussel Watch Project: 1993-1996 Update."

b MDL not provided in laboratory report. Estimate of 0.1563 ug/kg obtained by dividing the PQL of 0.6250 ug/L (for 1 gram dry weight of sediment in 40 mL of water) given in the method by factor of four.

c MDL not provided in laboratory report. Estimated MDL of 0.3 ug/kg obtained from the method.

d Bloom, N., Colman, J., and Barber, L. 1997. Artifact Formation of Methylmercury during Aqueous Distillation and Alternative Techniques for the Extraction of Methylmercury from Environmental Samples. Fresenius J. Anal. Chem 358:371-377.

e MDL not provided in laboratory report. Estimated MDL of 0.25 ug/kg obtained by dividing the PQL of 1 ug/kg given in the method by a factor of four.

Table 2. Summary of Laboratory Analytical Results for Total Mercury (THg) and Methylmercury (MeHg)

Sample ID	Analyte	Assigned Value ($C_{assigned}$ in ug/kg)	Lab	Method		Holding Time (days)		Reported Result (µg/kg)	Mean Result (C_{mean} in ug/kg)	Standard Deviation (s_{lab})
				Prep	Analysis	Prep	Analysis			
STAC33	Total Mercury	94	Battelle	MSL-I-016-07	MSL-I-016-07	28	31	110 122 119	117	6.24
			FDEP	HG-020-5	HG-008-3.13	7	8	110 A 110 110	110	0.0
			Frontier	FGS-066.5	FGS-069-04	14	25	86.3 89.4 83.1	86.3	3.15
	Methylmercury	1.4	Battelle	MSL-I-015-07	MSL-I-015-07	21	22	1.11 1.26 1.08	1.15	0.0964
			FDEP	HG-003-2.6	HG-003-2.6	8	11	2.3 I 2.0 I 2.1 I	2.1	0.15
			Frontier	FGS-045.3	FGS-070.2	14	15	1.07 0.999 0.868	0.979	0.102
IAEA405	Total Mercury	810	Battelle	MSL-I-016-07	MSL-I-016-07	28	31	814 812 830	819	9.87
			FDEP	HG-020-5	HG-008-3.13	7	8	780 770 790	780	10
			Frontier	FGS-066.5	FGS-069-04	14	25	808 819 807	811	6.66
	Methylmercury	5.49	Battelle	MSL-I-015-07	MSL-I-015-07	21	22	4.85 5.12 6.86	5.61	1.09
			FDEP	HG-003-2.6	HG-003-2.6	8	11	5.7 6.2 6.3	6.1	0.32
			Frontier	FGS-045.3	FGS-070.2	14	15	3.41 3.88 3.78	3.69	0.248

All results are in micrograms per kilogram (ug/kg) on a dry weight basis.

A Value reported is the mean of two or more determinations

I The reported value is between the laboratory method detection limit (MDL) and the laboratory practical quantitation limit (PQL) and is an estimate.

-- Not determined / Not reported

The assigned values of THg and MeHg for standard IAEA405 are the certified reference values provided by the vendor. The assigned value of THg for sample STAC33 (94 ug/kg) is the average of nine replicate analyses of this sample performed by the District's laboratory as part of the pre-test homogeneity study. The assigned value of MeHg for sample STAC33 (1.4 ug/kg) is the consensus value (i.e., average of nine results reported by the three laboratories).

Table 3. Summary of Laboratory Performance in the Analysis Sediment Samples for Total Mercury (THg) and Methylmercury (MeHg)3

Sample ID	Analyte	Assigned Value ($C_{assigned}$ in ug/kg)	Lab	Mean Result (C_{mean} in ug/kg)	Standard Deviation (s_{lab})	Standard Deviation (s_{group})	Standard Deviation (s_{IAEA})	Percent Recovery (R%)		Relative Standard Deviation (%RSD)		Z Score			
												Based on s_{group}		Based on s_{IAEA}	
STAC33	Total Mercury	94	Battelle	117	6.2	16	--	124	Good	5.3	Very Good	1.4	Good	--	--
			FDEP	110	0.0			117	Good	0.0	Very Good	0.99	Very Good	--	--
			Frontier	86.3	3.2			92	Very Good	3.7	Very Good	-0.48	Very Good	--	--
	Methylmercury	1.4	Battelle	1.15	0.10	0.62	--	82	Good	8.4	Very Good	-0.40	Very Good	--	--
			FDEP	2.1	0.15			152	Acceptable	7.2	Very Good	1.18	Good	--	--
			Frontier	0.979	0.10			70	Acceptable	10.5	Good	-0.68	Very Good	--	--
IAEA405	Total Mercury	810	Battelle	819	9.9	21	139	101	Very Good	1.2	Very Good	0.42	Very Good	0.06	Very Good
			FDEP	780	10.0			96	Very Good	1.3	Very Good	-1.5	Good	-0.22	Very Good
			Frontier	811	6.7			100	Very Good	0.8	Very Good	0.065	Very Good	0.01	Very Good
	Methylmercury	5.49	Battelle	5.61	1.1	1.3	0.83	102	Very Good	19.4	Good	0.10	Very Good	0.14	Very Good
			FDEP	6.1	0.35			111	Very Good	5.7	Very Good	0.46	Very Good	0.70	Very Good
			Frontier	3.69	0.25			67	Acceptable	6.7	Very Good	-1.4	Good	-2.2	Acceptable

All results are in micrograms per kilogram (ug/kg) on a dry weight basis.
For STAC33, $C_{assigned}$ is the verified value reported by SFWMD from the homogeneity study. For IAEA405, $C_{assigned}$ is the reference value certified by the vendor.
A verified value for methylmercury in STAC33 was not determined.
C_{mean} is the average of each set of three results reported by each laboratory.
s_{group} is the standard deviation of the mean results reported by the three participating laboratories for each of the four targeted analytes.
s_{IAEA} is the standard deviation of the means reported for IAEA405 by 60 laboratories for total mercury and by 14 laboratories for methylmercury as part of the IAEA405 intercomparison exercise.

$\%R = [C_{mean} / C_{assigned}] \times 100$

85% ≤ %R ≤ 115%	Very Good
70% ≤ %R < 85% and 115% ≤ %R < 130%	Good
45% ≤ %R < 70% and 130% ≤ %R < 155%	Acceptable
%R < 45% or %R > 155%	Unacceptable

%R performance criteria based on those specified in the methods used by the participating laboratories, EPA data validation guidelines, industry standards, and professional judgment.

$\%RSD_{lab} = [s_{lab} / C_{mean}] \times 100$

0% < %RSD ≤ +10%	Very Good
10% < %RSD ≤ +20%	Good
20% < %RSD ≤ +30%	Acceptable
%RSD > +30%	Unacceptable

%RSD performance criteria based on EPA data validation guidelines, industry standards, and professional judgment.

Z score = $(C_{mean} - C_{assigned}) / s$ (where s = s_{group} or s_{IAEA})

$-1 \leq Z \leq 1$	Very Good
$-2 \leq Z \leq 2$ and $-1 > Z > 1$	Good
$-3 \leq Z \leq 3$ and $-2 > Z > 2$	Acceptable
$-3 > Z > 3$	Unacceptable

Z score performance criteria based on assumptions of random error and statistical dispersion and NELAC acceptance criteria.

Table 4. Summary of Multiple Comparison Results using Bonferroni and Tukey Methods in the One-Way ANOVA Test

Sample ID	Analyte	Similarity [a]
STAC33	THg	Battelle and FDEP; SFWMD pre- and post-tests
	MeHg	Battelle and Frontier
IAEA405	THg	Battelle and FDEP; FDEP and Frontier
	MeHg	Battelle and FDEP

a Similarity is based on the significance level at $\alpha = 0.05$ with $p < 0.05$.

FIGURES

Figure 1a. Summary of Reported Lab Means versus Assigned Values - Total Mercury (THg)

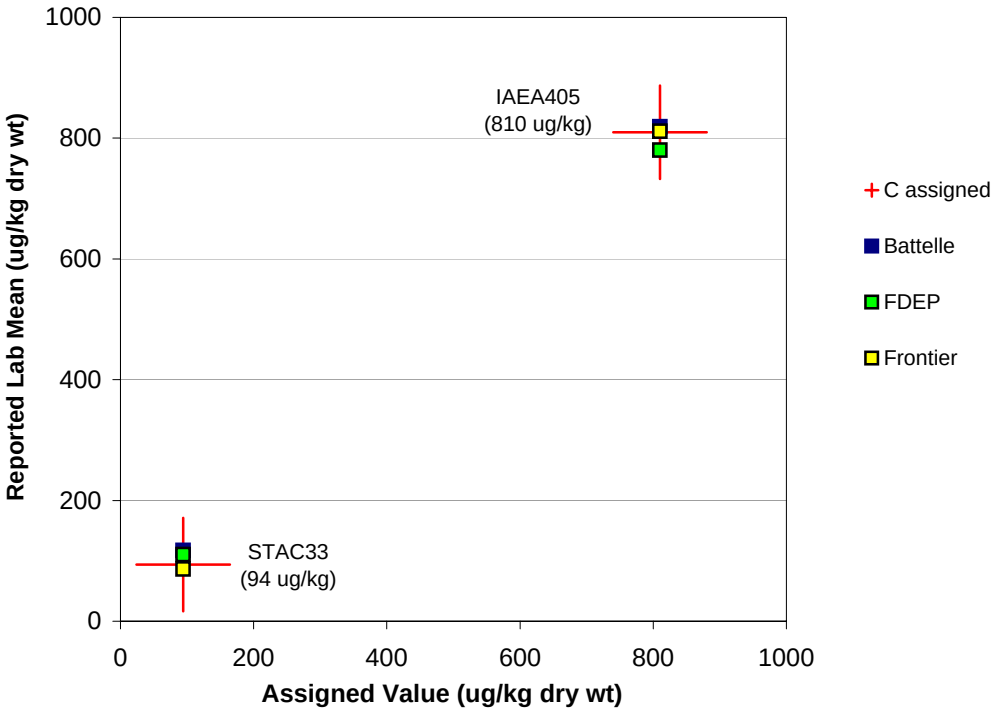


Figure 1b. Summary of Reported Lab Means versus Assigned Values - Methylmercury (MeHg)

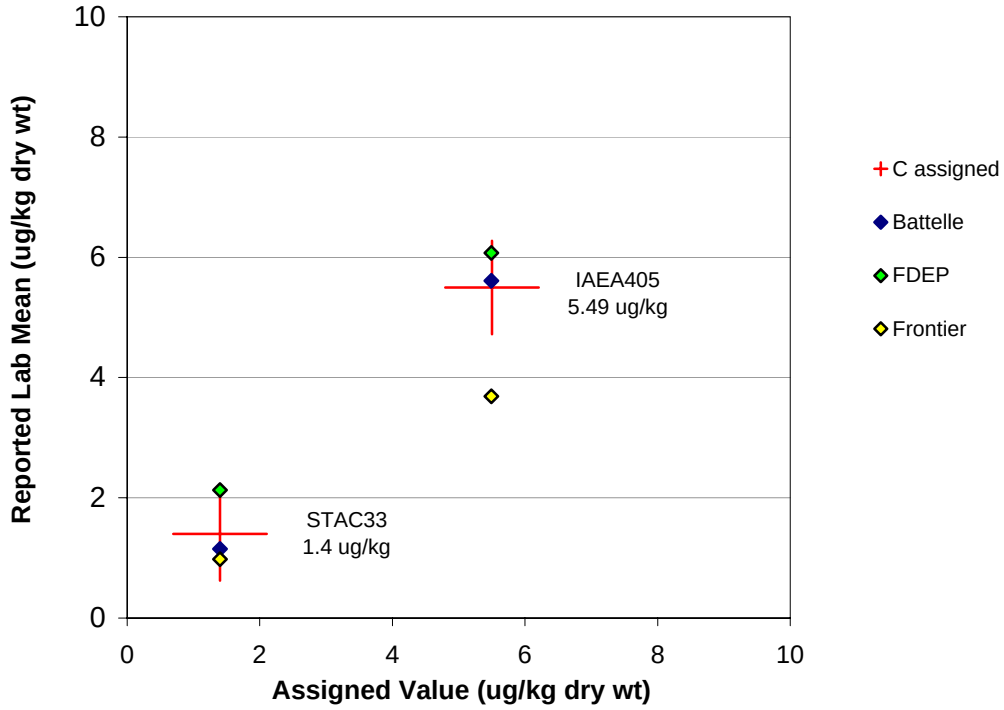


Figure 2a. Comparison of Laboratory %RSDs for Analyses of Individual Subsamples - Total Mercury (THg)

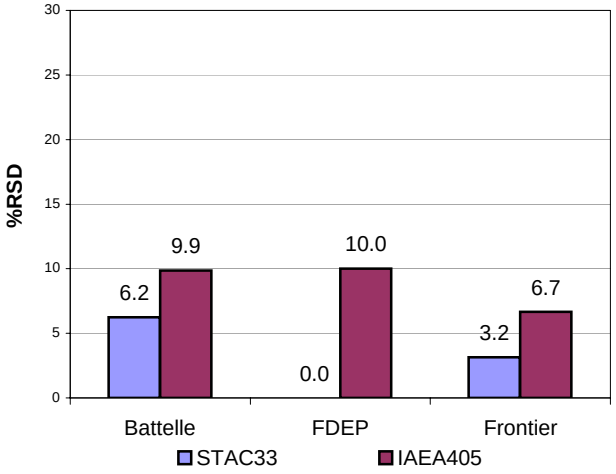


Figure 2b. Comparison of Laboratory %RSDs for Analyses of Individual Subsamples - Methylmercury (MeHg)

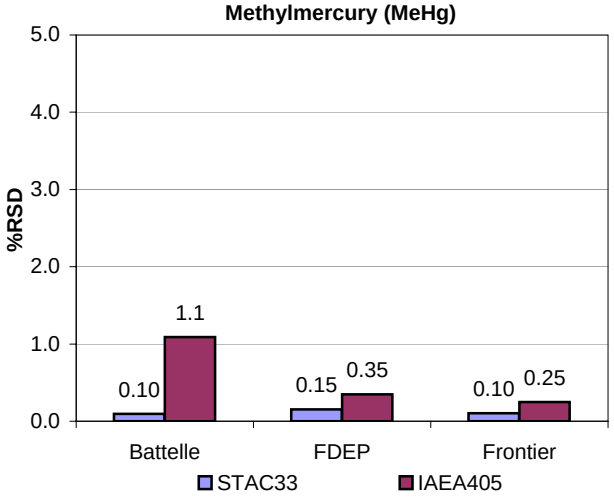


Figure 3a. Comparison of Z Scores (based on s_{group}) - Total Mercury (THg)

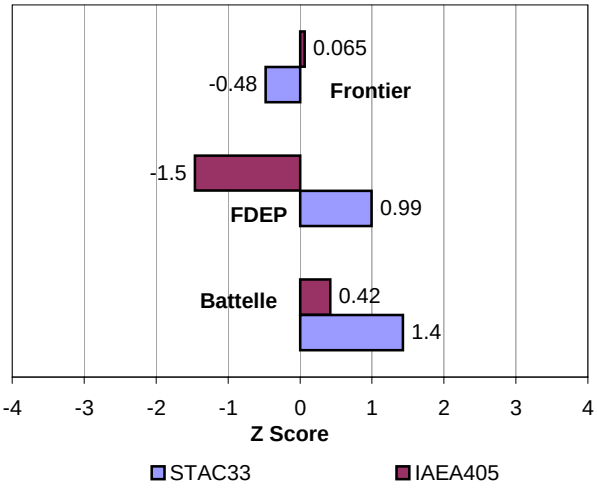


Figure 3b. Comparison of Z Scores (based on s_{group}) - Methylmercury (MeHg)

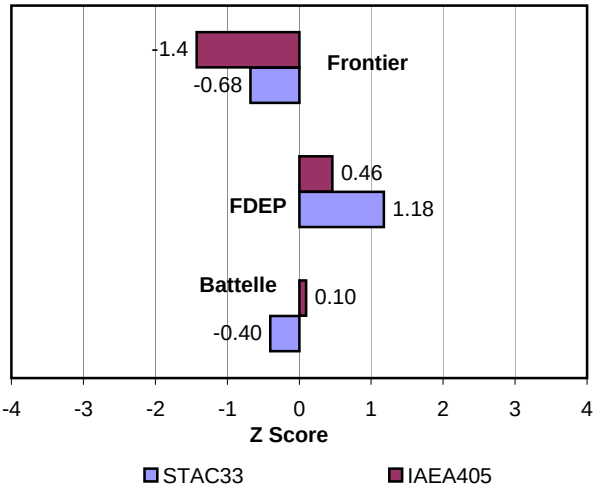


Figure 4a. Comparison of Z Scores (based on s_{IAEA}) - Total Mercury (THg)

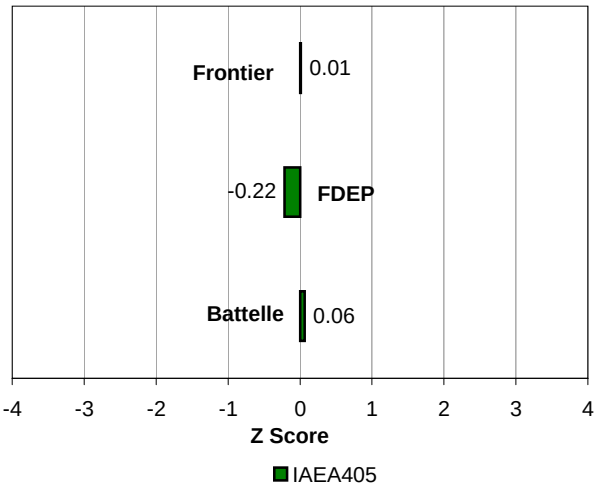
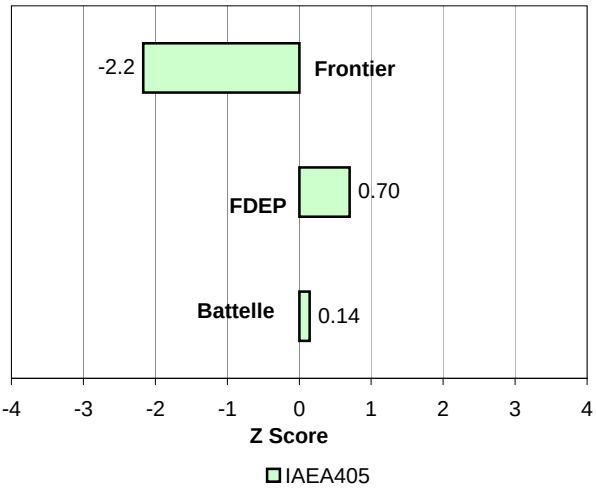


Figure 4b. Comparison of Z Scores (based on s_{IAEA}) - Methylmercury (MeHg)



Appendices A-C:
Available Upon Request