Appendix 2B-5: Annual Permit Compliance Monitoring Report for Mercury in Downstream Receiving Waters of the Everglades Protection Area

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SUMMARY

This appendix summarizes data from compliance monitoring of mercury influx and bioaccumulation in the downstream receiving waters of the Stormwater Treatment Areas (STAs) for Water Year 2003 (WY2003) (May 1, 2002 through April 30, 2003).

The key findings presented in this appendix are as follows:

1. Concentrations of total mercury (THg) in rainfall collected during WY2003 were similar to levels reported for the period of record at all three monitoring stations (ENR Project, the Florida Power and Light's Andytown substation, and the Everglades National Park's Baird Research Center). As observed previously, rainfall volumes and THg concentrations increased in late summer/early fall 2002; consequently, atmospheric wet deposition flux of THg also increased during these months (i.e., the third and fourth quarter). Owing to a combination of elevated concentration and the high annual rainfall in South Florida, wet THg deposition to the Everglades remains substantially greater than any other region monitored by the National Atmospheric Deposition Program's Mercury Deposition Network. Collectively, the results reported in this appendix for wet deposition flux of THg in comparison with monitoring of surface water at non-ECP structures continue to show that the major source of mercury to the Everglades is from atmospheric deposition.

2. Mercury monitoring at non-Everglades Construction Project (non-ECP) water control structures during the fourth quarter recorded a THg concentration of 23.7 nanograms per liter (ng/L) at S-5A. This was the first exceedance of the Florida Class III numerical water quality standard for THg (12 ng/L) at a non-ECP structure. With the exception of S-5A, THg concentrations (i.e., not volume-weighted) at other structures were generally similar to levels reported for the period of record. More importantly, methylmercury (MeHg) concentrations were greatly reduced compared to elevated MeHg concentrations observed in the third and fourth quarters of 2000. As previously observed, seasonal average concentrations of MeHg were highest during the third quarter of 2002, at the height of the wet season.

3. The 2002 basinwide median concentration of Hg in mosquitofish collected from downstream marsh sites was 70 nanograms per gram (ng/g), representing an 11-percent increase from the 2001 basinwide median concentration. Given the short life span of mosquitofish, the long-term significance of short-term temporal changes in mercury levels must be interpreted cautiously.

4. THg concentrations in sunfish samples collected from marsh sites in 2002 (n = 222) averaged 196 ng/g, but ranged as high as 950 ng/g in a bluegill sample from L67F1. The basinwide median concentration was 150 ng/g in 2002, which represents a 36-percent increase from the previous year. Interannual differences in tissue Hg concentrations were significant in sunfish at several sites, with three sites exhibiting an increase and two sites exhibiting a decrease in 2002. It is difficult to determine the cause of these short-term temporal events based on the data available from this monitoring program.

5. Largemouth bass collected from downstream sites had a median THg concentration of 390 ng/g, which was identical to the 2001 median concentration. However, it is important to note that bass caught in 2002 were much younger than in previous years (median age of bass was 1.8 yrs in 2002, 2.8 yrs in 2001, 2.8 yrs old in 2000, 2.8 yrs old in 1999, and 2.9 yrs old in 1998). The grand mean of site-specific, age-standardized concentrations (expected in a three-year-old bass, EHg3) was 655 ng/g in 2002 (based on the 8 sites where it was appropriate to calculate an EHg3), which represents a 9-percent increase over 2001. The Florida Fish and Wildlife Conservation Commission also reported slight increases in EHg3 at several sites in 2002; however, fillet-THg concentrations remained well below levels observed during the early 1990s.

6. THg concentrations in great egret nestling feathers ranged from 2.6 micrograms per gram (µg/g) to 9.8 µg/g, with an overall mean concentration (two colonies pooled) of 5.5 ±1.8 µg/g. Given the ages of nestlings sampled, THg levels in egret nestlings appear to have increased slightly in 2003 compared to 2002, are similar to 2001 levels but, most importantly, continue to be much lower than 1994 levels. Mean THg concentration in egret eggs was 0.38 µg/g in 2003. Although egg-THg concentrations have varied since 1999 (appearing to increase slightly in 2001, then decreasing again in 2002), among-year differences were not statistically significant. Nevertheless, egg-THg concentrations were substantially lower than levels reported for eggs collected in 1993.

7. Based on the U.S. Fish and Wildlife Service and the U.S. Environmental Protection Agency guidance values, certain Everglades populations of piscivorous avian and mammalian wildlife continue to be at risk from adverse effects due to mercury exposure depending on the foraging area. However, population-level toxic effects from MeHg exposure have not been demonstrated in any of the fish-eating Everglades wildlife populations studied over the last decade. Nevertheless, there is sufficient inferential evidence of negative effects to the individual to warrant concern, at least at the level of reasonable maximum exposure (Spalding et al., 1994; Sundlof et al., 1994; Beyer et al., 1997; Frederick et al., 1997, Bouton et al. 1999, Heniz, in prep). Furthermore, the lack of unambiguous epidemiological evidence of population-level effects of MeHg toxicosis may reflect the inability of the study methods used to date to detect more subtle effects in the field (i.e., behavioral teratology; Nocera & Taylor, 1998).

INTRODUCTION

This appendix is the annual permit compliance monitoring report for mercury in the downstream receiving waters of the Everglades Protection Area (EPA). This report summarizes the mercury-related reporting requirements of the Florida Department of Environmental Protection (FDEP) National Pollution Discharge Elimination System (NPDES) Permit (FL0177962-001) and the FDEP Everglades Forever Act (EFA) Permits (Chapter 373.4592, Florida Statutes [F.S.]). The latter includes permits for non-Everglades Construction Project (non-ECP) discharge structures, Stormwater Treatment Area 6 (STA-6), STA-5, STA-1W, and STA-2 (Permit Numbers 06,502590709, 262918309, 0131842, FL0177962-001, and 0126704, respectively). This report summarizes the monitoring results for Water Year 2003 (WY2003) (May 1, 2002 through April 30, 2003). For this year's reporting, the results of mercury monitoring within the STAs are presented separately in Appendix 4A-4 of the *2004 Everglades Consolidated Report*.

Following this introduction, Appendix 2B-5 consists of three main sections including (1) background, (2) summary of the Mercury Monitoring and Reporting Program, and (3) monitoring results. The background section briefly summarizes the operation of the STAs and discusses their possible impact on South Florida's mercury problem. The next section summarizes sampling and reporting requirements of the Mercury Monitoring Program. Monitoring results are then summarized and discussed. Recent results from the Mercury Monitoring and Reporting Program describe significant spatial distributions and, in some instances, among-year differences in mercury concentrations.

BACKGROUND

The STAs are treatment marshes designed to remove nutrients from stormwater runoff originating from upstream agricultural areas. The STAs are being built as part of the Everglades Construction Project (ECP). When completed, the ECP will include six STAs that will comprise about 43,000 acres of constructed wetlands. The downstream receiving waters to be restored and protected by the ECP include the South Florida Water Management District's (SFWMD or District) water management canals of the Central and Southern Florida (C&SF) Project and the interior marshes of the Everglades Protection Area (EPA). The EPA is comprised of Water Conservation Areas (WCAs) 1, 2A, 2B, 3A, and 3B, the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge), and the Everglades National Park (ENP or Park).

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

SUMMARY OF THE MERCURY MONITORING AND REPORTING PROGRAM

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

PRE-OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

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

OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

The downstream system is monitored to track changes in mercury concentrations over space and time in response to the changes in hydrology and water quality associated with the Everglades Construction Project (for site locations, see **Figures 1**, **2**, and **3**).

Rainwater

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

District Structures Surface Water

Unfiltered grab samples of surface water were collected quarterly using an ultraclean technique upstream of structures S-5A, S-10C, S-140, S-9, S-32, S-151, S-141, S-190/L-28 interceptor, S-334, and S-12D. These samples were analyzed for THg and MeHg. These sites bracket the WCAs or are major points of inflow or outflow. Monitoring of these sites is intended to capture the effect of seasonal changes in the relative contributions of rainfall and stormwater runoff contributing to water quality entering the EPA.

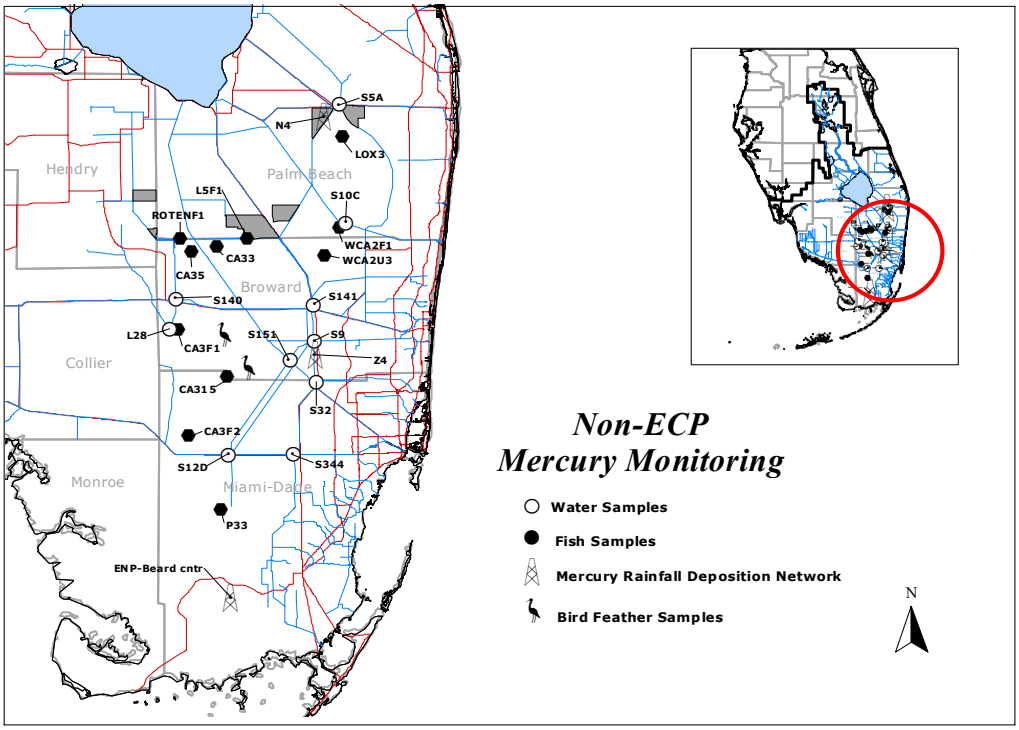

Figure 1. Map showing all non-ECP mercury (Hg) monitoring test sites. Errata: N4 and Z4 are fish collection locations in Water Conservation area 2 (WCA-2); Mercury Deposition Network (MDN) sites are ENR and Andytown, respectively.

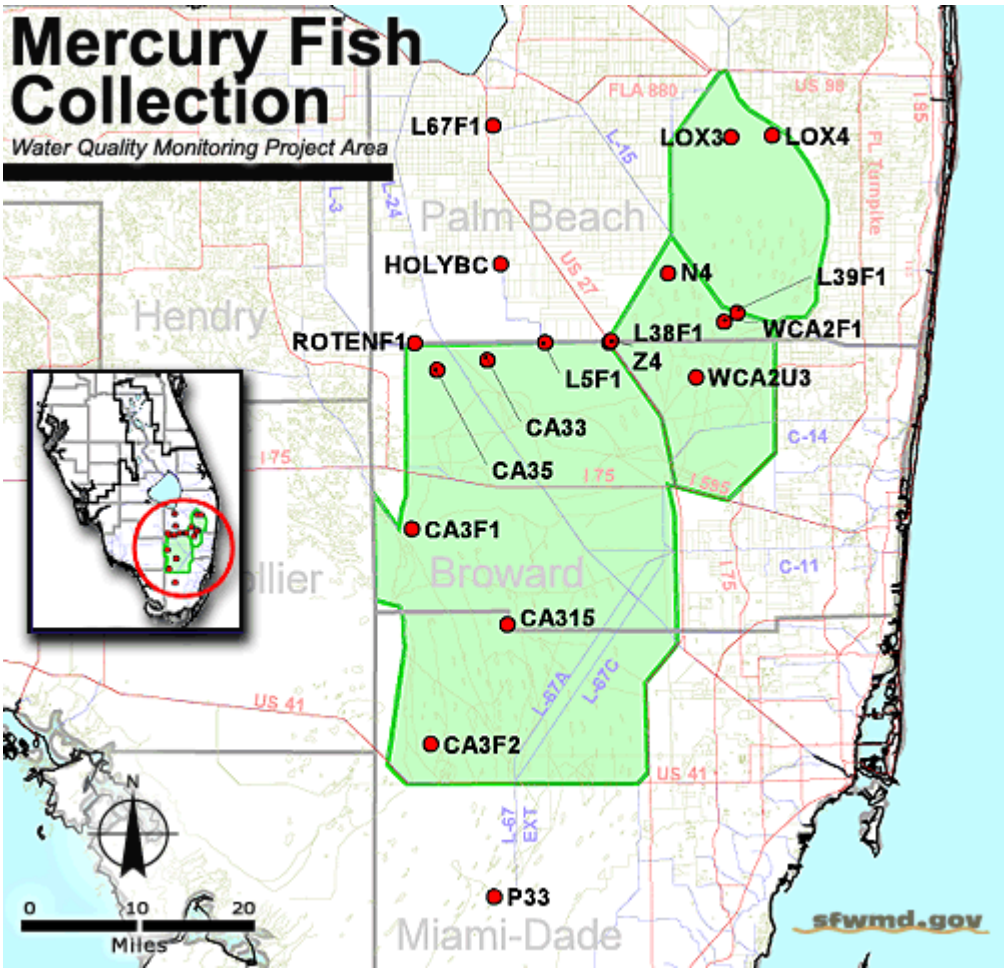

Figure 2. Map showing collection sites for monitoring Hg levels in mosquitofish, sunfish, and largemouth bass. Errata: Location of L67F1 shown in figure is incorrect; correct location is at the terminus of L67 extension adjacent to P33.

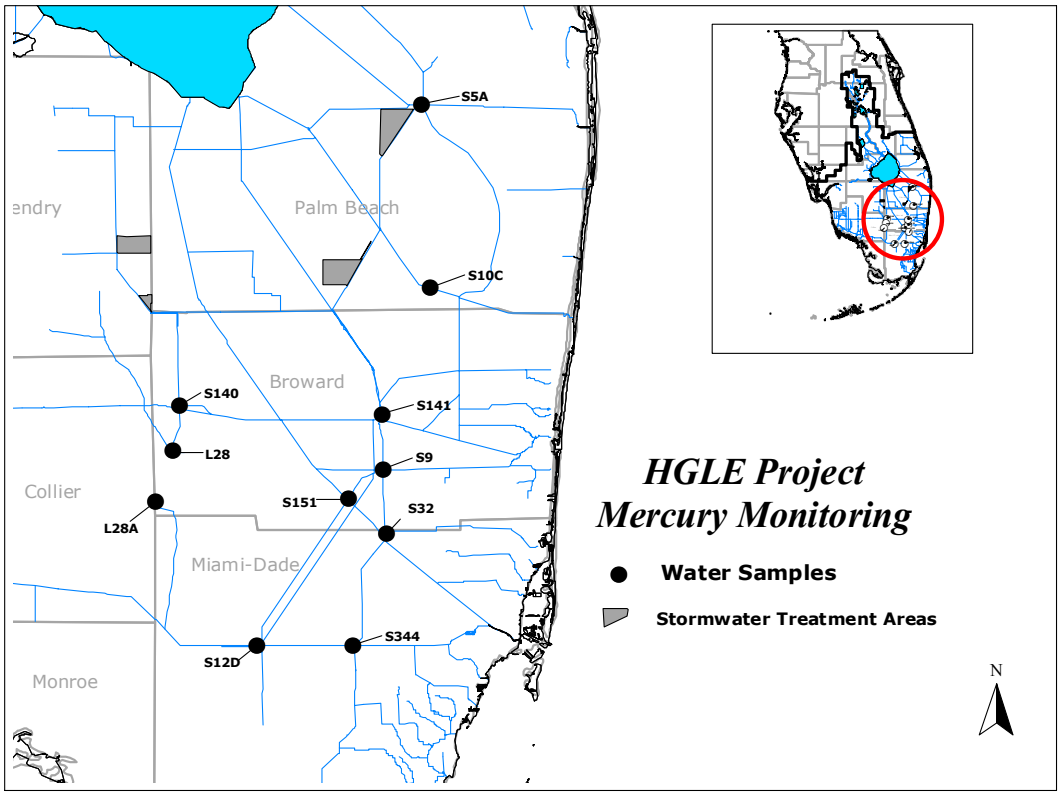

Figure 3. Map showing non-ECP structures where unfiltered surface water is collected quarterly to monitor concentrations of total mercury (THg) and methylmercury (MeHg).

Preyfish

A grab sample of between 100 and 250 mosquitofish (*Gambusia* sp.) was collected throughout the year using a dipnet at 12 downstream interior marsh sites. Subsequently, the fish were homogenized, the homogenate was subsampled in triplicate, and each subsample was analyzed for THg. (Note: On March 5, 2002, the FDEP approved a reduction in the number of replicate analyses of the homogenate from five to three; correspondence from F. Nearhoof, FDEP.) This species was selected as a representative indicator of short-term, localized changes in water quality because of its small range, short lifespan, and widespread occurrence in the Everglades.

Secondary Predator Fish

Up to 20 sunfish (*Lepomis* sp.) were collected (via electroshocking) throughout the year at 12 downstream interior marsh sites. Each fish was analyzed for THg. Because of their widespread occurrence, and because they are a preferred prey for a number of fish-eating Everglades species, sunfish were selected as an indicator of mercury exposure to wading birds and other fish-eating wildlife.

Top-predator Fish

Up to 20 largemouth bass (*Micropterus salmoides*) were collected (via electroshocking) throughout the year at 12 downstream interior marsh sites, and the fishes' muscles were analyzed for THg. Largemouth bass were selected both as an indicator of potential human exposure to mercury and because this species has been monitored at several Everglades sites since 1989.

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

Feathers

Feathers were collected throughout the year from 20 great egret (*Ardea alba*) nestlings from two different nesting colonies within WCA-3A and were analyzed for THg under appropriate state and federal permits (WX03044, WX00128a, and MB007948-0). Because MeHg bioaccumulates in top-predator fish, fish-eating birds (including wading birds) are among the most highly exposed organisms in the Everglades. It should be noted that this is a modification from the sampling scheme initially proposed, which would have involved collecting molted feathers from post-breeding adults at or in the immediate vicinity of nests or from feathers found at STAs. This modified sampling design is more consistent with protocols used in the collection of background data (Frederick et al., 1997).

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

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

QUALITY ASSURANCE AND QUALITY CONTROL MEASURES UNDER THE MERCURY MONITORING PROGRAM

The following section is an assessment of the District's Mercury Monitoring Program during WY2003 (May 1, 2002 through April 30, 2003) and, where appropriate, evaluates the data quality in terms of accuracy, precision, and completeness. This assessment is based on data quality objectives contained in the District's Quality Assurance Project Plan for the Mercury Monitoring and Reporting Program, which was approved on issuance of the permit by the FDEP on June 7, 1999.

Quality assurance (QA) and quality control (QC) are integral parts of all monitoring programs. A stringent QA/QC program is especially critical when dealing with ultra-trace concentrations of analytes in natural and human-impacted environments. Quality assurance includes design, planning, and management activities conducted prior to implementation of the project to ensure that the appropriate kinds and quantities of data will be collected with the required representativeness, accuracy, precision, reliability, and completeness. The goals of quality assurance 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. When using multiple laboratories, it is also important to establish and maintain comparability of performance and results among participating laboratories.

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

Laboratory Quality Control

Comparability of laboratory performance was ensured through compliance with the requirements in USEPA Method 1631B (Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry, 821/R-96-001), 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 01A0007846 CD-98-1600, August 1, 1998), USEPA Method 245.5 (Mercury in Sediment by Cold Vapor AAS; 600/4-79-020), USEPA Method 245.6 (Mercury in Tissues by Cold Vapor AAS, 600/4-91-010), and USEPA Method 245.7 (Mercury - CVA Fluorescence spectrometry; CD-98-Stan, February 1, 1999), which identify performance-based standards and the appropriate

levels of QA/QC. Both laboratories used by the District in the past year have some level of deviation from the original referenced methods, USEPA Method 1631B and USEPA Draft Method 1630, but remained in control of these performance-based methodologies. The District utilizes laboratories that are certified by the Florida Department of Health under the National Laboratory Accreditation Program (NELAP).

Field Quality Control Samples

A total of 717 field QC samples (e.g., field kit trip blanks, equipment blanks, field blanks, and replicate and split samples) were collected with filtered and unfiltered surface water samples at STA-1W, STA-2, STA-5, STA-6, and non-ECP structures during WY2003 (**Tables 1** and **2**). (Note: An equipment blank is now collected at the outset of the trip, and a field-cleaned equipment blank is collected at the end of the trip; field blanks have been eliminated.) These tables include the HGLE, HGOS, and HGOK projects as well as the expanded monitoring program at STA-2. This represents roughly 39 percent of the 1,841 water samples collected during this reporting period.

As reflected in **Table 1**, the frequency of occurrence of target analytes in blanks was reduced from previous years. This reduction in blank contamination likely resulted from corrective actions taken by the laboratories that included additional internal monitoring of de-ionized distilled water (DDW) systems, which generate the analyte-free water used in preparing field QC blanks systems, and by the use of disposable glass bottles. However, the frequency of MeHg detections in field kit trip blanks (**Table 1**) has been noted and is currently under review; however, the MeHg concentrations were low, resulting in relatively few data qualifiers. During WY2003, an increase in target analyte detection in both laboratory-cleaned equipment blanks and field-cleaned equipment blanks suggested a possible memory problem in sampling trains. Accordingly, all sampling trains were replaced with new, tagged trains as of August 2003. In the future, sampling trains will be tracked by the primary laboratory and will be replaced initially on an annual basis, but more frequently if annually should prove insufficient to correct this problem.

Field staff collected blank QC samples on containers (e.g., vials and bags used to ship solids) and processing equipment (e.g., blender, grinder, Polytron©, and cutting boards). During WY2003, seven container/processing blanks were collected under the fish monitoring program (i.e., HGFS project); none were found to have THg concentrations above the detection limit (0.1 nanograms per liter [ng/L]). Two additional container/processing blanks were collected for the mixing blender, and amber bottles were used to process and ship sediment samples (i.e., ST6D). Both samples contained detectable THg concentrations (0.22 and 0.24 ng/L THg). However, these levels were considered to be an insignificant contribution for sediments that invariably have levels much greater than 1 nanogram per gram (ng/g) (Note: the de-ionized [DI] water used as blank water was not from a Hg-clean lab, and inorganic Hg is a common laboratory contaminant at ultra-trace levels.) Neither of the QC blank samples contained detectable concentrations of MeHg (MDL was 0.018 ng/L). Accordingly, no soil samples were invalidated due to processing equipment or storage container contamination.

As shown in **Table 2**, the median relative standard deviation (RSD) among replicate water samples (RS) was 8 percent for both THg and MeHg.

Table 1. Frequency of occurrence and mean concentration (ng/L) of THg and MeHg in field quality control (FQC) blanks collected with unfiltered surface water samples from STA-1W, STA-2, STA-5, STA-6, and non-ECP structures. Note: Method detection limits (MDLs) are 0.1 ng THg/L and 0.022 ng MeHg/L.

FQC*	THG						MeHg					
	n**	Collection frequency	n>MDL	ng/L	V‡ flagged	% flagged	n**	Collection frequency	n>MDL	ng/L	V‡ flagged	% flagged
FKPB	70	9%	1	0.19	0		70	9%	7	0.04	2	3%
EB	85	11%	14	2.64	10	12%	86	11%	29	0.04	7	8%
EB filtered	26	21%	4	0.64	4	15%	26	21%	12	0.04	2	8%
FCEB	82	10%	12	0.57	6	7%	83	11%	16	0.04	3	4%
FCEB filtered	4	3%	0		0		4	3%	3	0.06	1	25%
FB	1	0%	0				1	0%	0			

*FKTB - Field Trip Prep Blank, EB - lab-cleaned equipment, FCEB - Field-cleaned equipment blank collected at the end of sampling, FB - field blank.

** Total number (n) of surface water samples collected under these projects during the water-year was 809 THg, 126 THg dissolved, 780 MeHg and, 126 MeHg dissolved.

‡ Indicates that the analyte was detected in the method blank.

Table 2. Relative standard deviation (RSD) among replicate unfiltered surface water samples collected at STA-1, STA-2, STA-5, and STA-6 non-ECP structures (includes HGLE, HGOK, and HGOS projects).

Analyte	N	RSD		
		Mean	**Median**	**Maximum**
THg	39	10%	8%	70%
MeHg	41	14%	8%	52%

Variability of Mosquitofish Composite Samples

To monitor spatial and temporal patterns in mercury residues in small-bodied fishes, individual mosquitofish (between 100 and 250 individual fish) were collected at various locations in the STAs and ECP and non-ECP marshes. These individuals were then composited for each site. Composite sampling can increase sensitivity (i.e., 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 sub-sampled in quintuplicate and each sub-sample analyzed for THg. Based on the apparent degree of homogenization, as evidenced by the low RSD among aliquots reported in the *2002 Everglades Consolidated Report*, the District revised its Standard Operation Procedures (SOP), after consultation with and approval from the FDEP. The revised SOP indicates that the subsampling of mosquitofish homogenates has been reduced from five to three. For WY2003, the mean RSD in THg concentrations among triplicate aliquots was 6 percent (median = 5 percent; maximum = 19 percent; n = 145).

A second disadvantage to composite sampling is that the same amount of information is not generated as when samples are analyzed individually. Because samples are physically averaged, no variance estimate for the population is generated and, consequently, uncertainty is introduced regarding the representativeness of the sample in describing the population. This also hampers statistical comparisons. To assess the representativeness of composite samples, two field duplicate (FD) mosquitofish composites were collected during WY2003, i.e., a second set of 100–250 individuals were collected at the site and composited as a second sample. The relative percent difference (RPD) between composite means was 2 percent and 39 percent. However, unlike abiotic media that may change little over the time period of replicate sample collection, dipnetting mosquitofish likely disperses the local population. Consequently, the resampled population may not represent a true replicate of the first sample.

Interlaboratory Comparability

To ensure further comparability (i.e., reproducibility) between this and other ongoing mercury sampling initiatives, split samples of surface water were submitted to the secondary laboratory (Frontier Geoscience, Inc. [FGS]) for independent analysis of THg and MeHg. It should be noted that this laboratory also generated all the pre-ECP soil and water data for the STAs and the non-ECP structures, respectively. However, the primary laboratory (FDEP) generated all the baseline fish data.

Water

The RPD between laboratories for six split samples ranged from 9 percent to 30 percent for THg (**Figure 4a**; mean concentration was 1.383 ng/L for FDEP and 1.348 ng/L for FGS), with no statistically significant (consistent) bias (paired t-test; df = 5, t = 0.348, p = 0.74).

By comparison, ultra-trace MeHg concentrations in the six splits exhibited an RPD ranging from 13 percent to 111 percent (**Figure 4a**). The resulting mean concentration, 0.083 ng/L, was identical for both laboratories. The difference between laboratories was not statistically significant (paired t-test, df = 5, t = 0.02, p < 0.986), although the sample size was small.

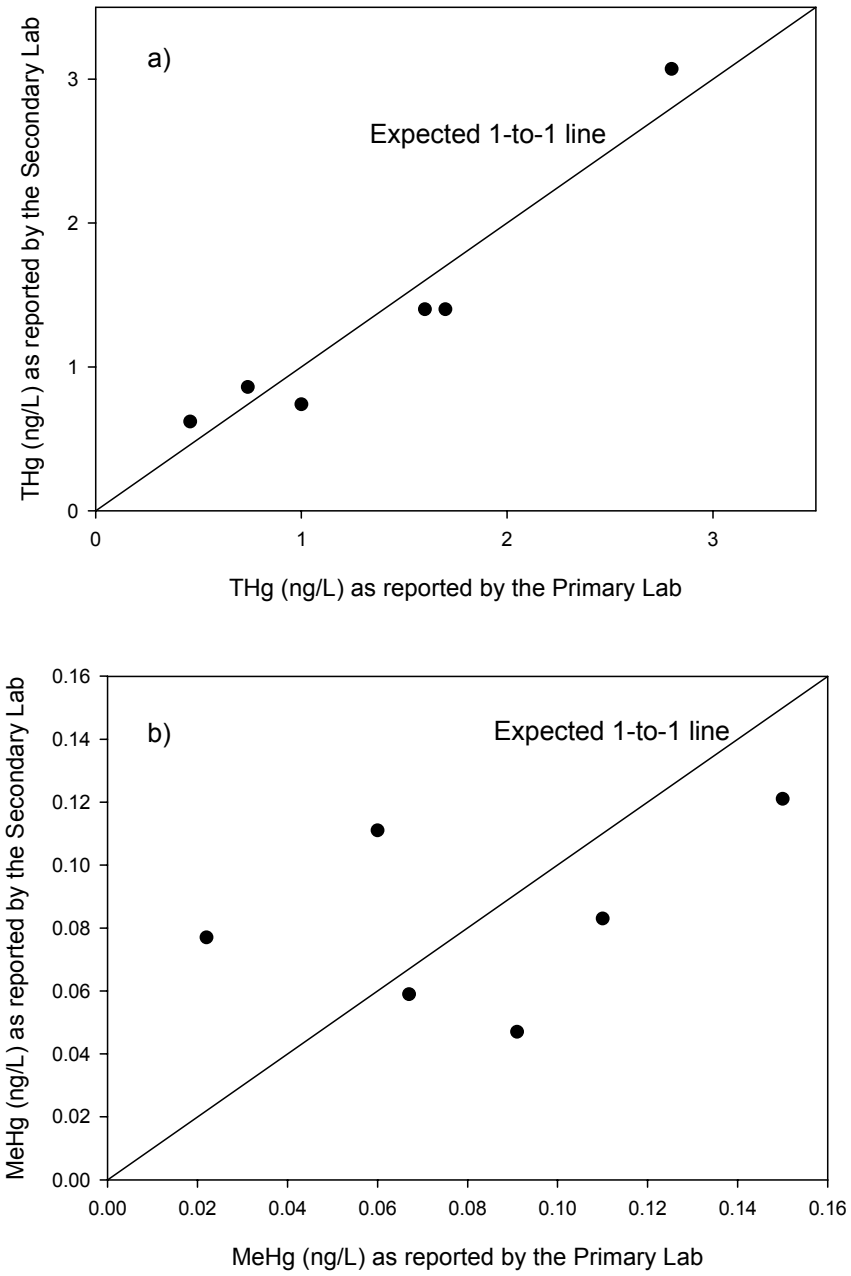

Figure 4a. Interlaboratory comparison of (a) THg and (b) MeHg determination in surface water.

These findings are consistent with results of the Everglades Mercury Round Robin (EMRR) Inter-Laboratory Comparison Program conducted by FDEP. Both laboratories scored well for MeHg (based on a scale of 1 to 5, FDEP scored 3.67 and FGS scored 4.33); FGS scored slightly lower (2.33) than FDEP (4.33) for THg (Niu and Tintle, 2003).

Fish

Split samples in 3 out of 143 mosquitofish composites collected during WY2003 (i.e., 2 percent of the fish) were sent to the secondary laboratory (FGS, Inc.) for independent analysis; the RPDs between splits (ranging in concentrations from 0.001 mg/kg to 0.088 mg/kg) were 15, 23, and 24 percent (**Figure 4b**).

Split samples in 151 out of 934 large-bodied fishes (i.e., 16 percent of whole sunfish homogenates and fillets of largemouth bass) collected during WY2003 were sent to the secondary laboratory (FGS, Inc.) for independent analysis. It should be noted that, upon request, the secondary laboratory reviewed results of samples 1002386Z and 1002387Z. Errors were found that caused the revision of the former and qualification of the latter (i.e., 1002387Z was considered suspect).

Distributions of the two datasets were nearly identical with a 25^{th} percentile at 0.07 mg/g and 75^{th} percentile at 0.28 mg/g. Concentration of THg in the splits were highly correlated (Pearson Product Moment correlation, $r = 0.98$, $p < 0.001$; **Figure 4c**), with an average RPD of 17 percent ($n = 150$, median equaled 14 percent; maximum was 63 percent). This difference between laboratories was not statistically significant (Wilcoxon Signed Rank Test, $n = 150$, $W = -1153$, $p = 0.13$).

Bird Tissues

Split samples of egg ($n = 1$) and feather ($n = 1$) material were sent (blind) to the primary laboratory for duplicate analysis; the RPD was 0 percent between egg splits and 12 percent between feather splits.

STATISTICAL METHODS

Monitoring Hg concentrations in aquatic animals provides several advantages. However, interpretability of residue levels in animals can sometimes prove problematic due to the confounding influences of the age or species of the collected animal. For comparative purposes, special procedures are used to normalize the data. Standardization to size, age, or lipid content is a common practice (Wren and MacCrimmon, 1986; Hakanson, 1980). To be consistent with the reporting protocol used by the Florida Fish and Wildlife Conservation Commission (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 against age (for details, see Lange et al., 1999). It should be noted that to adjust for the month of collection, otolith ages were first converted to decimal ages using protocols developed by Lange et al. (1999). Sunfish were not aged; consequently, age normalization was not available. Instead, arithmetic means were reported. However, efforts were made to estimate a least square mean (LSM) THg concentration based on the weight of the fish. Additionally, the distribution of the different species of *Lepomis,* including warmouth (*L. gulosus)*, spotted sunfish (*L. punctatus*), bluegill (*L. macrochirus*), and redear sunfish (*L. microlophus*), collected during electroshocking was also considered to be a potential confounding influence on THg concentrations prior to each

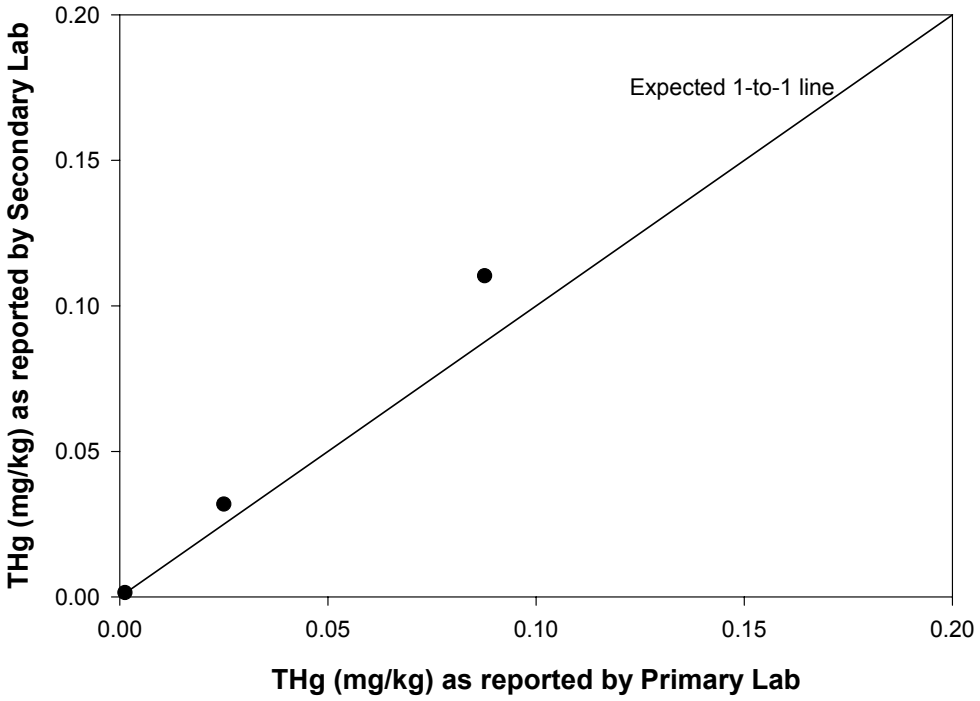

Figure 4b. Interlaboratory comparison in THg determination in mosquitofish.

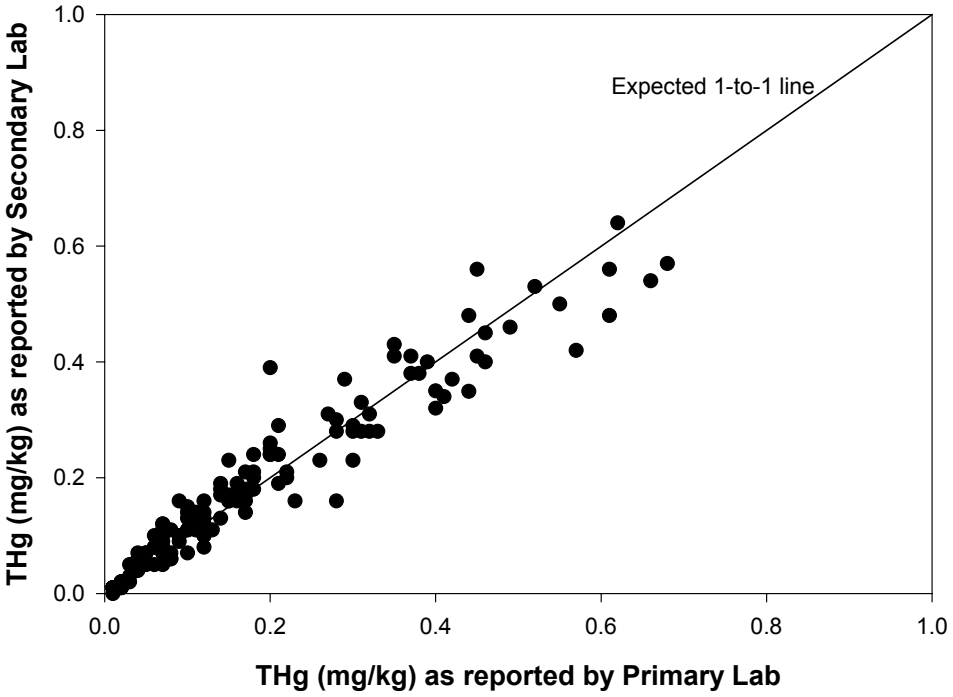

Figure 4c. Interlaboratory comparison in THg determination in large-bodied fishes (i.e., sunfish and largemouth bass).

comparison. To be consistent with the reporting protocol of Frederick et al. (1997; see also Sepulveda et al., 1999), THg concentrations in nestling feathers were similarly standardized for each site and were expressed as LSM for chicks with a 7.1 cm bill.

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

MONITORING RESULTS

RAINFALL: NATIONAL ATMOSPHERIC DEPOSITION PROGRAM, MERCURY DEPOSITION NETWORK

Samples of bulk rainfall were collected weekly under the protocols of the National Atmospheric Deposition Program's (NADP) Mercury Deposition Network (MDN) at the ENR Project, the Florida Power and Light's Andytown substation, and the Baird Research Center in Everglades National Park (**Figure 1**). For more information on MDN and to retrieve raw data, refer to the NADP's Website at http://nadp.sws.uiuc.edu/mdn.

As presented in **Table 3** and **Figure 6,** atmospheric deposition of THg to South Florida was highly variable both temporally and spatially. As shown in **Figure 6**, THg concentrations in precipitation were substantially higher during the summer months, possibly due to seasonal, tall, convective thunderclouds that can scavenge particulate Hg and water soluble reactive gaseous mercury (RGM) from the middle and upper troposphere. This is consistent with observations of Guentzel (1997) during the FAMS. Because both THg concentrations and rainfall volumes generally increase during the summer, the latter by a factor of 2 to 3, THg wet deposition typically increases fivefold to eightfold during the wet season (**Figure 5**). As reflected in **Table 3**, the volume-weighted average THg concentrations were slightly lower in 2002 at the ENR and ENP stations, as compared to 2001. In contrast, THg concentrations at Andytown increased in 2002 and were again elevated compared to the other two stations. With the exception of a few stations in the Great Lakes region and an unusually elevated concentration at a New Mexico station (28.4 ng/L), Florida has some of the highest THg concentrations in the MDN (http://nadp.sws.uiuc.edu/mdn/maps).

Wet deposition (mass/unit area-unit time), which is a function of both concentration and rainfall, remained relatively constant at the ENP but declined at Andytown and the ENR. At the

latter site, the decline was substantial (decreased from 21 $\mu g/m^2$ in 2001 to 10.4 $\mu g/m^2$ in 2002). However, problems with the MDN collector may partially account for this apparent decline; rain capture at this site was low compared to a nearby independent gauge. MDN managers have been provided supplemental data from this independent gauge for which to correct current estimates. Owing to a combination of elevated concentration and the high annual rainfall in South Florida, wet THg deposition flux to the Everglades is substantially greater than any other region of the MDN (http://nadp.sws.uiuc.edu/mdn/maps). (Note: There are often discrepancies between the Belfort rain gauges by MDN and the tipping-bucket rain gauges preferred by the District, even when there are no equipment malfunctions. When constructing mass budgets for THg, preference should be given to the Thiessen-weighted average rain depth developed by the District for a particular water body or treatment system water budget.)

Collectively, the results reported in this appendix for wet deposition flux of THg in comparison with monitoring of surface water at non-ECP structures (discussed in the next section) continued to show that the major source of mercury to the Everglades is from the air. This is consistent with previous assessments by both the FDEP (Atkeson, Online at http://www.dep.state.fl.us) and the USEPA (USEPA, 1998). Dry deposition flux, likely adds significantly to the overall atmospheric input (Keeler and Lindberg, 2001; Atkeson et al., 2002).

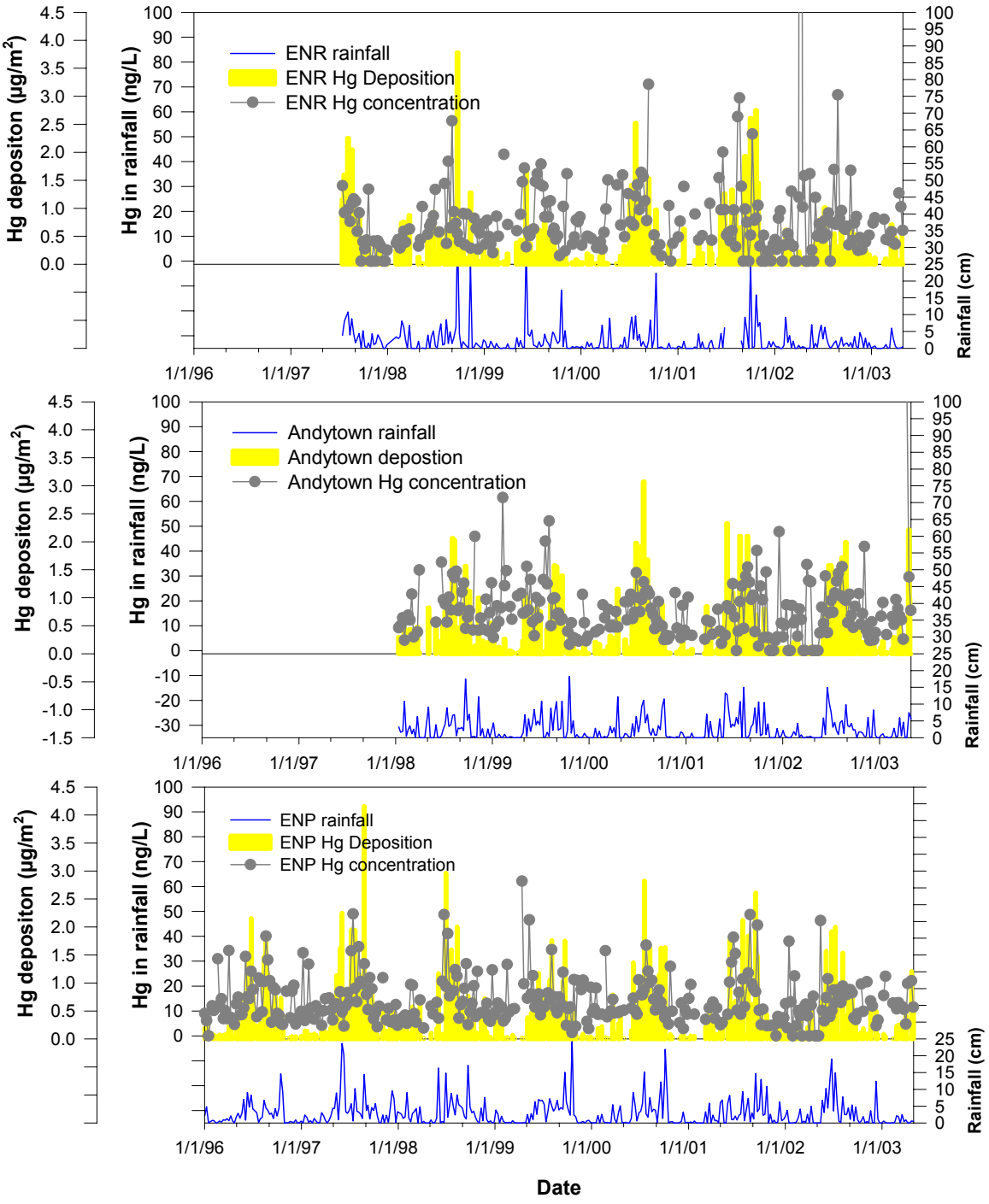

Figure 5. Time series of rainfall, rainfall Hg concentrations, and Hg rainfall deposition at MDN sites located at the ENR Project, Andytown, and ENP Baird Research Center. Note: 2002 rainfall and deposition data for ENR should be considered preliminary and subject to change.

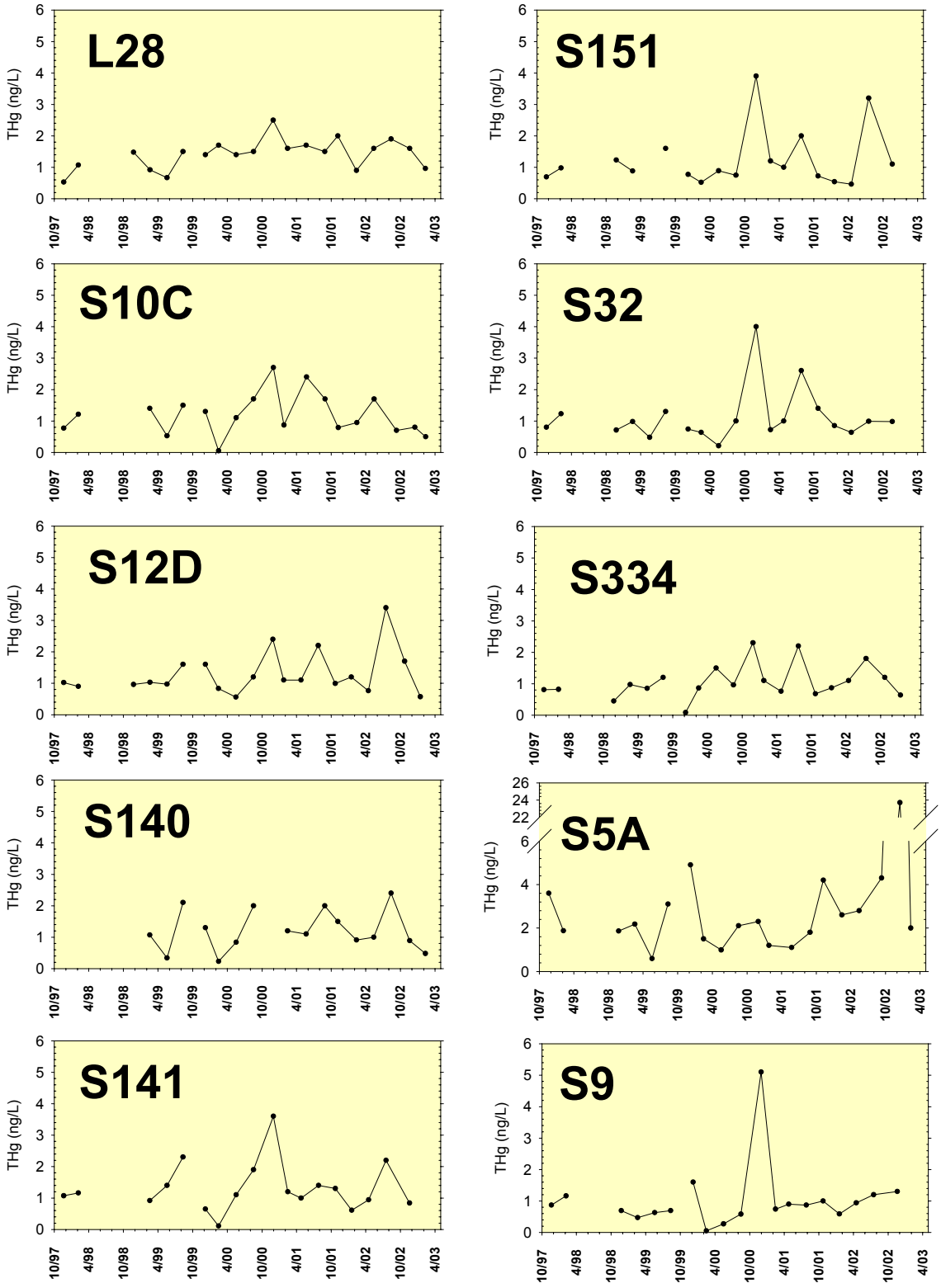

Figure 6. Concentrations of THg in unfiltered surface waters at 10 non-ECP structures for the period of record (i.e., 1997–2003). Note: Break in y-axis (THg concentration) in S-5A graph.

Table 3. Volume-weighted, biweekly mean bulk rainfall THg concentration data (ng/L) from the compliance sites of the MDN in WY2003. Note: Annual point estimates are based on calendar year.

Week ending	ENR (FL34)	Andytown (FL04)	ENP (FL11)
5/7/2002	0.0	0.0	0.0
5/21/2002	4.8	7.0	10.4
6/4/2002	25.6	11.0	13.1
6/18/2002	14.4	7.8	11.7
7/2/2002	10.7	17.4	11.6
7/16/2002	14.4	19.8	14.7
7/30/2002	19.3	28.7	14.4
8/13/2002	31.8	28.3	19.2
8/27/2002	15.5	20.3	17.7
9/10/2002	19.4	16.5	17.5
9/24/2002	13.0	12.5	6.6
10/8/2002	11.7	17.8	8.9
10/22/2002	33.4	16.5	12.4
11/5/2002	15.6	19.6	21.7
11/19/2002	9.3	6.4	14.0
12/3/2002	4.8	5.9	12.0
12/17/2002	7.4	4.6	4.2
12/31/2002	10.4	0.0	0.0
1/14/2003	16.5	16.8	17.6
1/28/2003	0.0	6.4	0.0
2/12/2003	0.0	0.0	13.1
2/25/2003	10.0	11.0	10.9
3/11/2003	8.1	18.4	13.3
3/25/2003	10.4	13.7	11.6
4/8/2003	6.8	4.6	4.8
4/22/2003	24.7	29.7	22.1
Volume-wt. concentration (ng/L)			
1997*	NA	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†	12.1	14.0	12.1
Deposition Annual (µg/m2)			
1997*	NA	NA	27.2
1998*	18.4	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†	10.4**	17.9	18.1

* Adapted from NADP / MDN Program Office Report by C. Sweet, http://nadp.sws.uiuc.edu/mdn/maps/

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

** Problem with capture efficiency of MDN-collector; MDN Managers provided supplement data - estimates will likely be revised.

SURFACE WATER AT NON-ECP STRUCTURES

Table 4 and **Figures 6** and **7** summarize monitoring results of unfiltered THg and MeHg in surface water samples collected quarterly at non-ECP structures (**Figure 3**). The maximum THg concentration observed during WY2003 was 23.7 ng/L and occurred at S-5A during the fourth quarter of 2002 (**Figure 6**). Thus, unlike previous years, there was an apparent exceedance of the Florida Class III water quality standard for THg (12 ng/L) at the non-ECP structures monitored. However, as noted by the analytical laboratory, the sample had a significant amount of suspended particulate matter, which likely contributed to the elevated THg concentration. Additionally, there was no corresponding jump in MeHg (therefore, the percent MeHg in this sample was low), supporting the hypothesis that this apparent exceedance was a transient phenomenon associated with unusually high turbidity. The maximum MeHg concentration observed during WY2003 at a non-ECP structure was 0.36 ng/L and occurred at L-28 during the third quarter of 2002 (**Table 4**, **Figure 7**). Currently, Florida has no Class III numerical water quality standard for MeHg.

With the exception of the exceedance discussed above, concentrations of THg were generally similar to or lower than cumulative averages (**Table 4**; Note that the concentrations are not volume-weighted in this table). Exceptions to this generalization were sites S12D and S1541, which had higher annual averages. More importantly, concentrations of MeHg observed during WY2003 were similar to WY2002 and much reduced compared to the spikes observed in the third and fourth quarters of WY2001. Seasonal average concentrations of THg were highest during the fourth quarter (primarily due to the spike at S-5A; otherwise, it would have been highest during the third quarter); average concentrations of MeHg were highest during the third quarter at the height of the wet season (**Table 4**).

FISH FROM ECP AND NON-ECP INTERIOR MARSHES

Results from monitoring downstream interior marsh mosquitofish (*Gambusia* sp.), sunfish (*Lepomis* spp.), and largemouth bass (*Micropterus salmoides*) are summarized in **Tables 5** through **7** respectively. It should be noted that values for individual, large-bodied fish can be found at the District's Website at http://www.sfwmd.gov/org/ema/dbhydro/index.html. Fish were collected from a total of 12 downstream interior marsh sites (**Figures 1** and **2**). Where fish could not be collected after good-faith efforts, collection sites defaulted to nearby canals where fish were more plentiful and the same water source was being sampled. Mercury levels in largemouth bass at three of these sites, LOX4 (WCA-1-GFC4), CA2U3 (WCA-2A-U3), and CA315 (WCA-3A-15), were monitored by the FWC prior to initiation of the ECP (period of record extends back to 1993).

As further discussed, fishes collected in WY2003 showed both spatial and temporal patterns in tissue Hg concentrations. In keeping with the primary objective of this monitoring program, the focus will be on temporal changes in mercury concentration in fish tissues to assess possible adverse effects from the construction of the ECP and the operation of the STAs. Nevertheless, spatial patterns of tissue Hg concentrations are important, particularly where there has been a variation from background conditions (i.e., pre-ECP conditions established by the FWC). Therefore, spatial patterns will be reviewed in detail only where there have been changes over time (i.e., interaction between treatment effects).

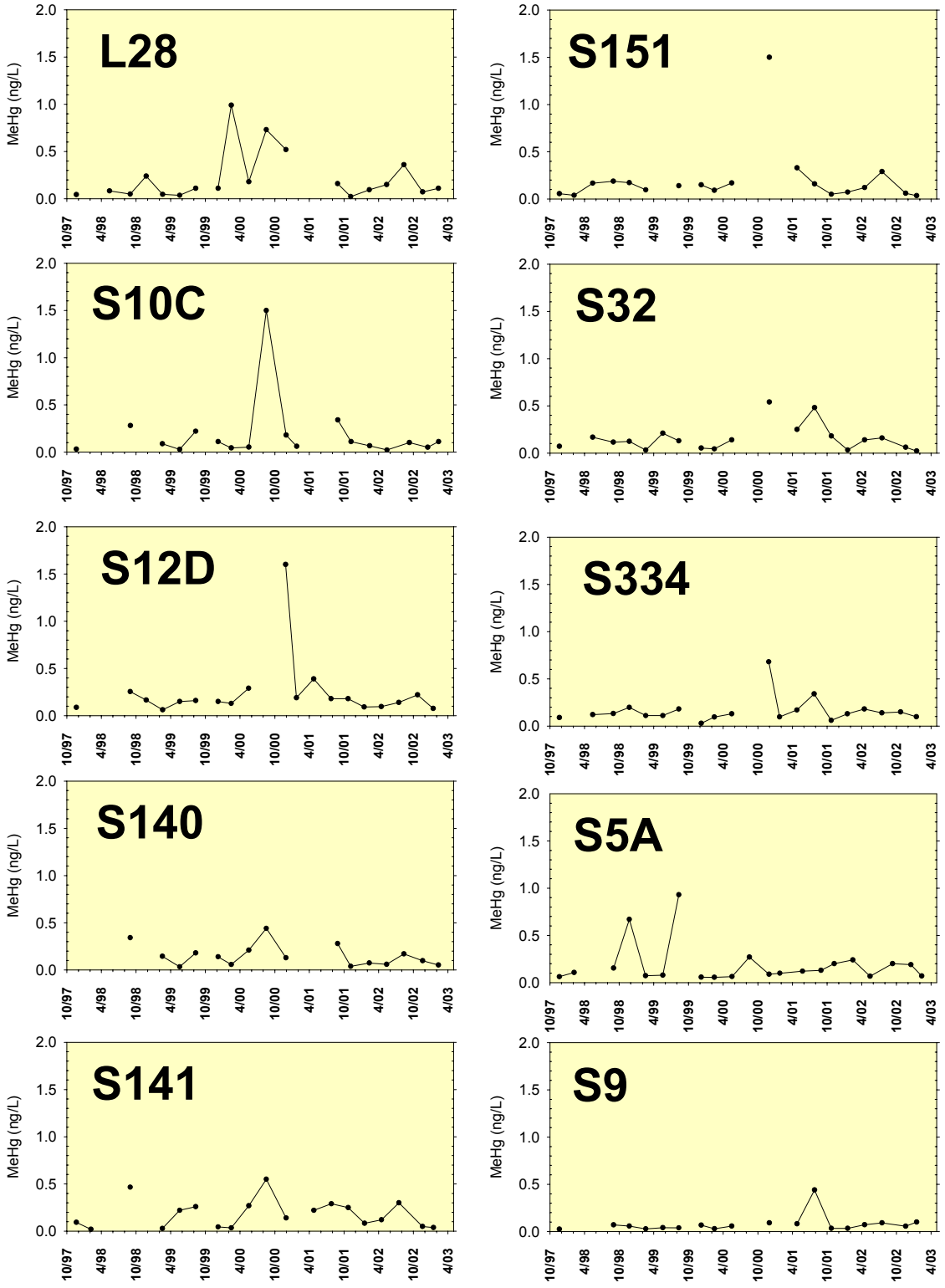

Figure 7. Concentrations of MeHg in unfiltered surface waters at 10 non-ECP structures for the period of record (i.e., 1997–2003).

Table 4. Concentrations of THg and MeHg (ng/L) in non-ECP structure surface waters in WY2003. Note: Due to shifts in scheduling within the quarter, sampling may have occurred outside the water year.

Structure	Quarter	THg ng/L	remark **	WQS*	MeHg ng/L	remark **	% MeHg
L28	2nd Quarter	1.60		<WQS	0.150		9%
	3rd Quarter	1.90		<WQS	0.360		19%
	4th Quarter	1.60		<WQS	0.072	I	5%
	1st Quarter	0.96	A	<WQS	0.110		11%
	Average[1] last 4 qt.	1.52			0.173		11%
	cumulative avg[1].	1.42			0.219		15%
S10C	2nd Quarter	1.70		<WQS	0.022	U	1%
	3rd Quarter	0.70		<WQS	0.100		14%
	4th Quarter	0.80		<WQS	0.051	I	6%
	1st Quarter	0.50		<WQS	0.110		22%
	Average last 4 qt.	0.93			0.071		11%
	cumulative avg.	1.19			0.188		19%
S12D	2nd Quarter	0.76		<WQS	0.096		13%
	3rd Quarter	3.40		<WQS	0.140		4%
	4th Quarter	1.70		<WQS	0.220		13%
	1st Quarter	0.57		<WQS	0.076	I	13%
	Average last 4 qt.	1.61			0.133		11%
	cumulative avg.	1.18			0.216		24%
S140	2nd Quarter	1.00		<WQS	0.060	I	6%
	3rd Quarter	2.40		<WQS	0.170		7%
	4th Quarter	0.89		<WQS	0.098		11%
	1st Quarter	0.48		<WQS	0.052	I	11%
	Average last 4 qt.	1.19			0.095		9%
	cumulative avg.	1.21			0.153		13%
S141	2nd Quarter	0.94		<WQS	0.120		13%
	3rd Quarter	2.20		<WQS	0.300		14%
	4th Quarter	0.84		<WQS	0.050	I	6%
	1st Quarter	0.68	V	<WQS	0.039	I	
	Average last 4 qt.	1.33			0.127		11%
	cumulative avg.	1.31			0.183		14%
S151	2nd Quarter	0.46		<WQS	0.120		26%
	3rd Quarter	3.20	A	<WQS	0.290		9%
	4th Quarter	1.10		<WQS	0.060	I	5%
	1st Quarter	0.47	A,V	<WQS	0.035	I	
	Average last 4 qt.	1.59			0.126		14%
	cumulative avg.	1.25			0.205		15%
S32	2nd Quarter	0.64	A	<WQS	0.140		22%
	3rd Quarter	0.99		<WQS	0.160		16%
	4th Quarter	0.98		<WQS	0.061	I	6%
	1st Quarter	0.55	V	<WQS	0.022	U	
	Average last 4 qt.	0.87			0.096		15%
	cumulative avg.	1.12			0.155		18%

Table 4. Continued.

Structure	Quarter	THg ng/L	remark**	WQS*	MeHg ng/L	remark**	% MeHg
S334	2nd Quarter	1.10		<WQS	0.180		16%
	3rd Quarter	1.80		<WQS	0.140		8%
	4th Quarter	1.20		<WQS	0.150		13%
	1st Quarter	0.64		<WQS	0.098		15%
	Average last 4 qt.	1.19			0.142		13%
	cumulative avg.	1.05			0.162		17%
S5A	2nd Quarter	2.80		<WQS	0.067	I	2%
	3rd Quarter	4.30		<WQS	0.200		5%
	4th Quarter	23.70		>WQS	0.190		1%
	1st Quarter	2.00		<WQS	0.070	I	4%
	Average last 4 qt.	8.20			0.132		3%
	Cumulative avg.	3.43			0.187		8.5%
S9	2nd Quarter	0.94		<WQS	0.071	I	8%
	3rd Quarter	1.20		<WQS	0.091		8%
	4th Quarter	1.30		<WQS	0.056	I	4%
	1st Quarter	0.23	I,V	<WQS	0.100		
	Average last 4 qt.	1.15			0.080		6%
	Cumulative avg.	1.03			0.078		13%
	Ann. avg[1]. 02-2	1.19	±0.7(10)[¶]		0.10	±0.1 (10)	12%
	Ann. avg. 02-3	2.21	±1.1 (10)		0.19	±0.1 (10)	10%
	Ann. avg. 02-4	3.41	±7.1 (10)		0.10	±0.1 (10)	7%
	Ann. avg. 03-1	0.86	±0.6 (6)		0.07	±0.0 (10)	13%
	Cum. avg[1]. 1st Q	0.98	±0.5 (55)		0.10	±0.1 (47)	14%
	Cum. avg. 2nd Q	1.00	±0.5 (39)		0.14	±0.1 (40)	18%
	Cum. avg. 3rd Q	1.77	±0.8 (40)		0.28	±0.2 (45)	17%
	Cum. avg. 4th Q	1.90	±3.1 (58)		0.18	±0.3 (59)	14%

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

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

1 Averages were not volume-weighted.

[¶] Value in parenthesis, i.e., (n), is number of unqualified values used to calculate mean ±1SD.

Table 5. Mean concentrations of THg in mosquitofish composites (*Gambusia* sp.) (ng/g wet weight) collected in WY2003 from downstream sites. Value represents a mean of 3 analyses.

Location	THg (ng/g)	Between-yr. change (%)	Cum. average
LOX4	58	-8%	83
CA2 F1	3	-39%	22
CA27 Alt (Z4)	70	-16%	77
CA27 Alt (N4)	96	-48%	141
Holey Land (North canal)	25	-48%	48
Rotenberger Alt. (RotenF1)	103	97%	131
Rotenberger Fish Camp (RotenFC)	58	NA	58
CA2U3	88	-31%	126
CA33 Alt (L5F1)	52	33%	80
CA35alt2	133	-4	116
Non-ECP North (CA3F1; end of L-28)	43	-24%	73
CA315	75	-53%	138
Non ECP South (CA3F2)	42	-10%	65
L67F1	110	NA	156
P33	210	-1%	181
annual mean	78	-17%	

NA = data not available due to the absence of fish at the site.
Grandmean of site means for POR (1998-02) ±95%CI: n=64, 102±18

Table 6. Mean concentrations (±1 SD; ng/g wet weight) of THg in sunfish (*Lepomis* spp.) collected in WY2003 from marshes within the EPA downstream of the STAs.

Target location	Sampling Location	Mean THg ng/g (±1SD, n)	Between-yr. change (%)	Mean for fish collected 1998-2002
WCA1-LOX3	LOX4	94 (±32, 20)	-27%	146
WCA-2A F1	L39F1	60 (±59, 20)	-3%	74
WCA-2A 2-7	Z4	272 (±113,13)	157%	152
Holey Land	Holey Land	195 (±190, 20)	81%	92
Rotenberger	RotenFC1	257 (±95,10)	NA	257
WCA-2A U3	CA2U3	85 (±48, 20)	-10%	140
WCA-3A 3	L5F1	160 (±69, 19)	256%	90
WCA-3A 5	Alt. 2 site	220 (±98, 20)	2%	218
Non-ECP North	CA3F1	128 (± 88, 20)	45%	121
WCA-3A 15	CA315	357 (±170, 20)	60%	328
Non-ECP South	CA3F2	105 (±78, 20)	11%	157
ENP P33 Marsh	L67F1	423 (±210, 20)	-34%	493
ENP P33 Marsh	P33 Marsh	NA		414
Average		196	19%	

1 Unable to collect 20 fish from each site.
NA = data not available due to the absence of fish at the site.
Grandmean of site means for POR (1998-02) ±95%CI: n=57, 195±39

Table 7. Standardized (EHg3) and arithmetic mean concentrations of THg in largemouth bass fillets (*Micropterus salmoides*) (ng/g wet weight) collected in WY2003 from ECP and non-ECP interior marsh sites.

Target Location	Sampling Location	EHg3 ± 95th CI (mean ±1SD, n) ng/g wet	Between-yr. Change (%)	Consumption advisory exceeded*	Cum. Mean for EHg3
CA1-LOX3	LOX4	NC (2) (191±48, 7)	NA	No	501
CA2-F1	L39F1	260±30 (268±101, 20)	-3%	No	280
CA2-7	Z4	NC (2) (570±14, 2)	NA	Likely	NA
Holeyland	HOLYBC	360±40 (347±158, 20)	-21%	No	333
Rotenberger1		NC (2) (NA, 0)	NA	NA	NA
CA2-U3	CA2U3	890±250 (549±288, 20)	72%	Yes	679
CA3-3	L5F1	400 ±70 (368±141, 20)	NA	No	415
CA3-5	CA3-5	NC (2) (NA, 0)	NA	NA	NA
Non-ECP North	CA3F1	570±50 (481±194, 19)	41%	Yes	450
CA3-15	CA3-15	1,030 ±96 (649±394, 22)	NA	Yes	1,022
Non-ECP South	CA3F2	430 ±80 (212±105, 20)	NA	No	430
ENP-P33	ENP-P33	NC (2) (NA , 0)	NA	NA	NA
ENP-P33	L67F1	1,300±150 (1,130±483, 20)	-4%	Yes	1,276

* Florida limited fish consumption advisory threshold is 500 ng/g in 3-yr-old bass.
1 Unable to collect fish from site.
NC - not calculated for: (1) insignificant slope or (2) if poor age distribution. NA - not available.
Grandmean of site EHg3 for POR +95%CI: n = 32, 591 ±116

Mosquitofish

THg concentrations in mosquitofish (*Gambusia* sp.) collected from marsh sites in WY2003 ranged from 3 ng/g at site CA2F1 to 210 ng/g at site P33 (**Table 5**). It is noteworthy that the minimum and maximum concentrations were also observed at these same two sites in WY2002. The WY2003 basinwide median concentration was 70 ng/g (**Table 5**; for locations, see **Figure 2**), which represents an 11-percent increase from the WY2002 basinwide median concentration (Note: When annual arithmetic means were compared, a 17-percent decrease was observed from 2001 to 2002, as shown in **Table 5**.) In WY2003, all sites except for two (RotenF1 and L5F1) showed a decline (negative, between-year change) in THg in mosquitofish (**Table 5**). Mosquitofish at most sites exhibited a dramatic increase in 1999 following a drydown and reflooding, decreasing substantially in 2000 but rebounding (increasing) in 2001 (**Figure 8**). This among-year difference in mercury concentration in mosquitofish was statistically significant (ANOVA; df = 4,68; F = 12.6; $p < 0.001$), with 1999 levels (pooled across sites) differing from 2000 (Tukey HSD, $p < 0.05$). Comparisons between other years, including WY2003, were not significant ($p > 0.05$). When pooled over time, levels of THg in mosquitofish differs among sites (Kruskal-Wallis One Way Analysis of Variance on Ranks; H = 28.1, df = 17, p = 0.04); however, post-hoc pair-wise comparisons (Dunn's Method) were not significant ($p > 0.05$). This lack of significance was likely attributable to the extreme variability among years at each of the sites (**Figure 8**).

Sunfish

THg concentration in sunfish (*Lepomis* spp.) collected from marsh sites in WY2003 (n = 222) averaged 196 ng/g, but ranged as high as 950 ng/g in a bluegill from L67F1 (**Table 6**). The basinwide median concentration was 150 ng/g in WY2003, which represents a 36-percent increase from the previous year. However, as discussed below, caution should be exercised when interpreting these basinwide concentrations. For a given site, the between-year percent change in Hg levels from WY2002 to WY2003 ranged from a 256-percent increase at L5F1 to a 34-percent decrease at L67F1 (**Table 6**, **Figure 9**).

Interannual differences in tissue Hg concentration in sunfish were statistically significant at several sites, with three sites exhibiting increases and two sites exhibiting a decrease in WY2003. However, results must be interpreted with caution due to differences in sizes and species of collected sunfish. Although there are statistical methods to address confounding factors, such as age or weight, addressing species differences is more problematic, particularly when it is one of two possible confounding factors (i.e., weight, species, or both). Statistical analyses of the sunfish data sets were also hampered or prevented because THg concentration, weights, or both often failed assumptions of normality and equal variance.

As discussed in the *2003 Everglades Consolidated Report* (Rumbold and Fink, 2003), attempts to use ANCOVA to evaluate patterns of mercury concentrations in sunfish using weight as a covariate were often inappropriate because weight/concentration relationships were inconsistent (i.e., slopes were either not significant or were not parallel each year). The lack of a strong concentration/size relationship likely resulted from interspecies differences (i.e., among the different *Lepomis* spp.) in growth and bioaccumulation factors (which are likely a function of diet). Species was a significant factor in tissue Hg concentration in sunfish caught in WY2003 (Kruskal-Wallis ANOVA on Ranks, df = 3, H=57.7, $p < 0.001$); THg was less concentrated in *L. microlophus* (redear, median 68 ng/g) than each of the other three species (Dunn's method, $p < 0.05$), e.g., *L. punctatus* (spotted sunfish, median = 280 ng/g), *L. gulosus* (warmouth, median = 215 ng/g), and *L. macrochirus* (bluegill, median = 170 ng/g). When pooled across sites,

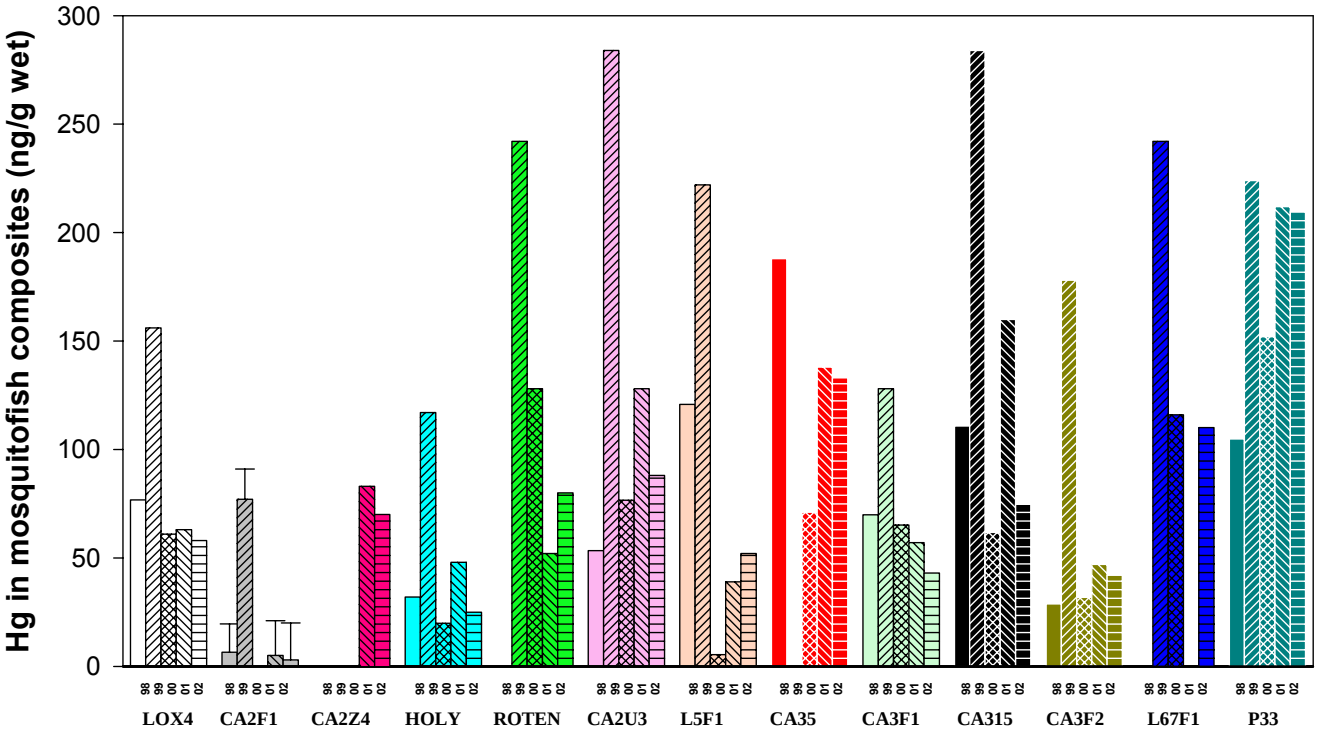

Figure 8. Hg concentrations in mosquitofish (*Gambusia* sp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2003). Not all sites were sampled in all years (for details, see **Table 5**).

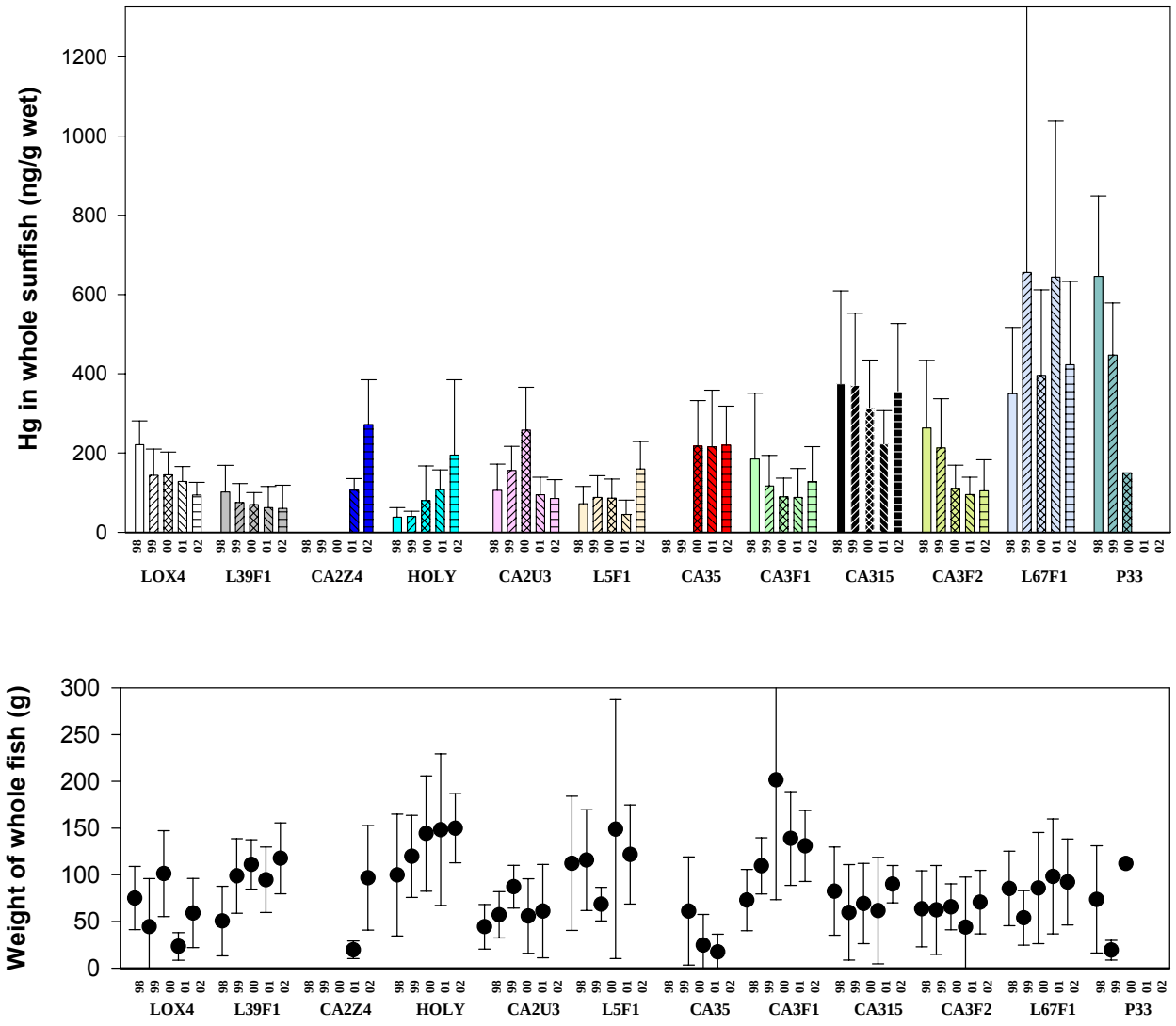

Figure 9. THg concentration (a) and weights (b) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the period of record (i.e., 1998–2003).

the difference between bluegill and spotted sunfish was also statistically significant ($p < 0.05$); other paired comparisons were not significant. However, some of these interspecies differences can vary over time and space. For example, it is noteworthy that the increase reported last year in the basinwide average THg concentration in bluegill (pooled across sites; Rumbold and Fink, 2003), with resulting mean concentration in bluegill greater than that of warmouth, was not repeated in WY2003. As another example, in WY2003, warmouth at sites WCA3A15 and L67F1 contained much higher Hg levels than spotted sunfish (as well as the other two species). The difference was statistically significant at the former site ($df = 3$, $H = 32.6$, $p < 0.001$; Dunn's post hoc test $p < 0.05$) but not the latter ($p > 0.05$). Similarly, when pooled over time, bluegill at site L67F1 also had unusually high levels of Hg (440 ng/g) relative to spotted sunfish (270 ng/g). Therefore, caution should be exercised when interpreting basinwide point estimates because they may simply reflect where samples were collected in a given year, i.e., if a greater number of bluegill were collected in 2002 from known hotspots for bluegill, such as L67F1, then the basinwide estimate for bluegill would increase.

As in previous ECRs, among-year differences in tissue Hg and sunfish weights were assessed at each location using a one-way ANOVA (i.e., parametric tests on raw or transformed data or non-parametric tests, if assumptions were violated; **Figure 9**), with qualitative consideration given to possible influences from among-year differences in collected species. Fishes at site Z4 exhibited between-year differences in mercury levels (Mann-Whitney Rank sum test, $df = 13, 20$, $T = 343.5$, $p < 0.001$), with much higher levels occurring in 2002 than 2001. However, fish size also differed between years ($df = 13, 20$, $T = 346$, $p < 0.001$), and this likely confounded any potential change in exposure (i.e., median weight was 68 g in 2002 and only 18.5 g in 2001).

Sunfish collected at the Holey Land Water Management Area (WMA) site also exhibited among-year variability ($df = 4$, $H = 59.4$, $p < 0.001$), with statistically significant pair-wise comparisons between 2002 (median = 140 ng/g) and 2000 (median = 59 ng/g), 1999 (median = 38 ng/g), and 1998 (median = 30 ng/g). Although 2002 Hg levels did not differ significantly from 2001, the 2001 Hg levels (84 ng/g) were also significantly higher than 1998 and 1999. Thus, there appears to be a continuous, progressive increase in mercury levels from one year to the next (**Figure 9**). Although fish size was variable at the Holey Land, it did not differ significantly and it did not increase in a monotonic fashion. However, differences in species of sunfish collected over time at the Holey Land may, in part, explain a temporal trend in mercury levels. Redear sunfish were caught in higher proportions in 1998 (78 percent for redear) and 1999 (85 percent for redear) compared to later years (about 50 percent for redear and 50 percent for bluegill) and, for the reasons stated above, this may explain the lower average mercury levels observed in those earlier years. Sunfish at L5F1 also contained greater concentrations of mercury in 2002 compared to each of the four previous years ($df = 4, 93$; $F = 13.3$; $p < 0.001$; post-hoc Tukey HSD). However, neither species caught nor fish size appeared to account for the difference in mercury (with the possible exception of fish caught in 2000, which were almost half the size of the fish caught in 2002).

Alternatively, mercury levels appeared to decline in concentration in sunfish from the LOX4 and CA3F2 sites (**Figure 9**). Although statistically significant ($df = 4$, $H = 35.3$, $p < 0.001$), the decline at LOX4 was likely an artifact from only three fish being caught in 1998, all of which were warmouth. Additionally, LOX4 sunfish in 2002 were also much smaller (i.e., half the size) than fish collected in 1998 and 2000 (**Figure 9**) and, consequently, it was difficult to evaluate the potential annual difference in mercury. The apparent decline at CA3F2 was also statistically significant ($df = 4$, $H = 26.5$, $p < 0.001$); however, the proportion of spotted sunfish and warmouth collected was much higher in 1998 (70 percent) and 1999 (80 percent) in comparison to 2002 (30 percent).

Largemouth Bass

A total of 170 largemouth bass (*Micropterus salmoides*) were collected at 10 out of 12 sites in WY2003. The average tissue Hg concentration in these bass was 490 ± 376 ng/g; the median concentration was 390 ng/g, which was identical to the 2001 median concentration. However, it is important to note that the bass caught in 2002 were much younger than previous years (median age of bass was 1.8 years old in 2002, 2.8 years old in 2001, 2.8 years old in 2000, 2.8 years old in 1999, and 2.9 years old in 1998). The grand mean of site-specific age-standardized concentrations (expected for a three-year-old bass, EHg3) was 655 ng/g in 2002 (based on the 8 sites where it was appropriate to calculate an EHg3), which represents a 9-percent increase over the 600 ng/g estimated for 2001. However, similar to sunfish, caution should be exercised when interpreting basinwide point estimates for bass, because they may simply reflect numbers of bass collected at each site in a given year.

Largemouth bass exhibited spatial patterns in tissue Hg concentrations similar to those observed in sunfish, with higher levels generally being found at the southern sites (**Table 7, Figure 10**). For instance, as observed over the past four years, highest tissue Hg concentrations in both sunfish and bass occurred at L67F1 in 2002. In 2002, bass at L67F1 had significantly greater tissue Hg concentrations than fish from either the well-known MeHg "hot spot", CA315, or CA2U3 (ANCOVA, df = 2,58; $F = 11.5$, $p < 0.001$; Tukey HSD, $p < 0.05$). Of equal interest, tissue Hg concentrations at the latter two sites did not differ significantly ($p > 0.05$).

Over the past four years, within-site temporal patterns in Hg levels in bass (i.e., EHg3, **Figure 10**) were generally consistent with temporal patterns observed in co-occurring sunfish (**Figure 9**). However, there were some key differences between the two trophic levels in 2002. This was best exemplified at the Holey Land and CA2U3 sites (ironically, in 2001, these two sites exemplified trophic similarities). As discussed above, sunfish from the Holey Land appeared to be exhibiting an increasing trend in Hg over time. By comparison, largemouth bass from the Holey Land showed no general trends in Hg from 1998 to 2000, a significant increase in 2001, followed by a decrease in 2002. The Hg levels in largemouth bass from the Holey Land in 2002 did not differ significantly from concentrations observed during the first three years of monitoring (ANCOVA; df = 4,93; $F = 7.74$, $p < 0.001$; Tukey HSD, $p > 0.05$). At CA2U3, Hg levels were relatively stable in sunfish over the last two years (**Figure 9**). By comparison, Hg levels increased significantly in largemouth bass at CA2U3 in 2002 compared to 2001 (i.e., 72 percent increase) and compared to 1998 (df = 4,93; $F = 11.7$, $p < 0.001$; Tukey HSD, $p < 0.05$). Any explanation for inconsistency between these trophic levels would be speculative at this point. However, based on the inherent difficulties in the interpretation of sunfish (see discussion in previous section), greater weight should be given to temporal patterns in largemouth bass.

Tissue Hg concentrations did not differ among years at L67F1 (df = 3, 73; $F = 0.81$, $p = 0.49$). It is also noteworthy that tissue Hg concentrations did not differ between years at CA315 (i.e., sufficient bass were collected only in years 1999 and 2002; df = 1, 40; $F = 0.23$, $p = 0.64$). The CA3F1 and L5F1 data sets did not meet the criteria for ANCOVA, i.e., interaction between age/year was significant (df = 4, 89, $F = 2.49$, $p < 0.05$; df = 2, 54, $F = 3.5$, $p < 0.05$). Caution must be exercised when interpreting the bass results. Although age distributions were satisfactory and EHg3 values were calculated for sites that exhibited a significant regression, several data sets (e.g., sites CA2U3, L5F1, and L39F1) were either not normally distributed or had unequal variance and, therefore, EHg3 values should be considered tentative.

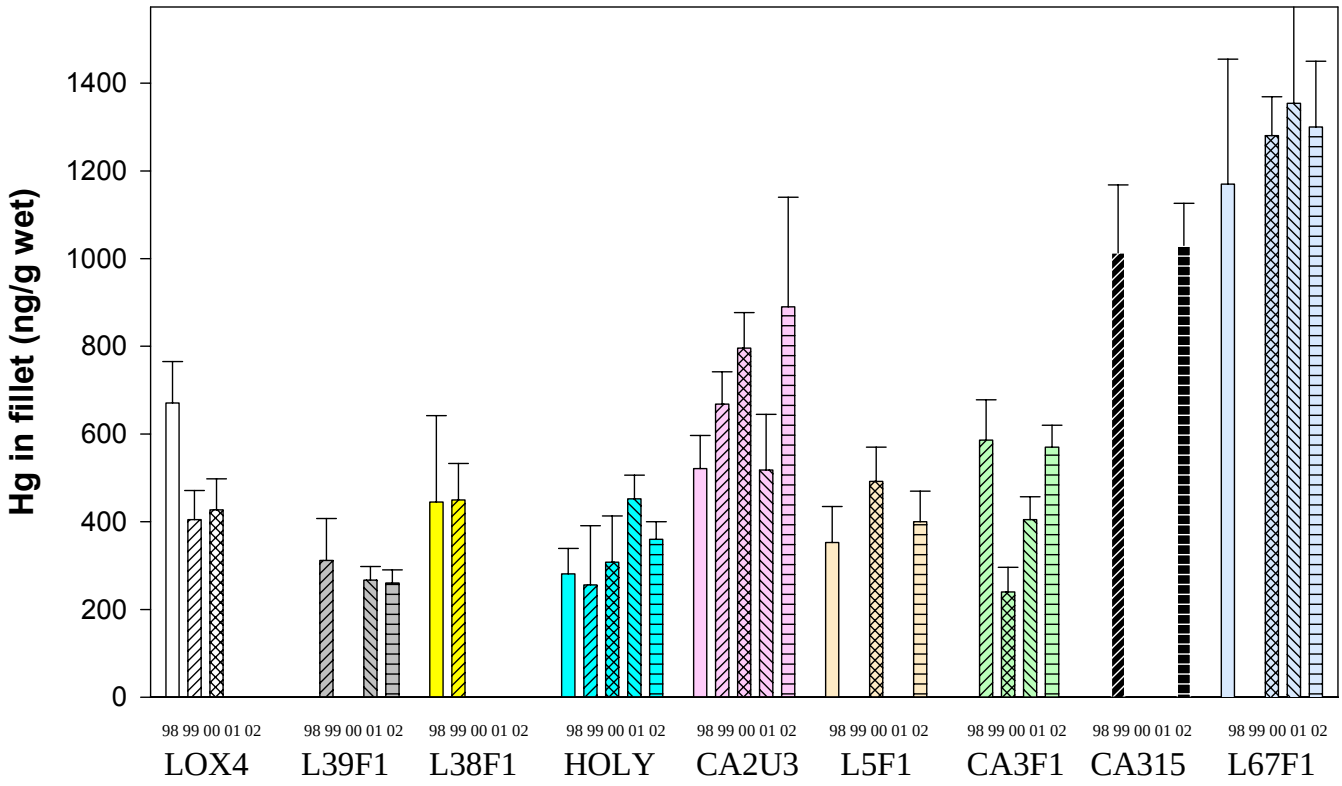

Figure 10. Standardized age (class 3 years) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at ECP and non-ECP sites for period of record (i.e., 1998–2002). EHg3 was not calculated if regressions were not significant or if age distributions were narrow.

It is important to note that although the FWC also reported slight increases in EHg3 at several sites in 2002, fillet-THg concentrations remained well below levels observed during the early 1990s (T. Lange, FWC, personal communication).

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 THg in prey species (Eisler, 1987). In the Mercury Study Report to Congress, the USEPA proposed 77 ng/g and 346 ng/g for trophic level (TL) 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997).

8. In 2002, mosquitofish (considered to be at trophic level 2 to 3, depending on age; Loftus et al., 1998) at 6 out of 15 downstream sites had THg concentrations exceeding either the USFWS or USEPA criterion (i.e., approximately 40 percent of the monitored sites, **Table 5**). Sunfish, which are at TL 3 (*L. gulosus* at TL 4; Loftus et al., 1998), at 11 out of 12 sites (92 percent) contained mean THg concentrations exceeding one or both of the predator protection criteria in 2002 (**Table 6**). This finding is significant because sunfish represent the preferred prey item of many fish-eating species in the Everglades as previously noted. Consequently, sunfish represent the best measure of potential upper-trophic-level exposure to THg. After adjusting arithmetic mean THg concentrations in largemouth bass fillets (**Table 7**) to whole-body concentrations (where, whole-body THg concentration = 0.69 x fillet THg; Lange et al., 1998), largemouth bass at 4 out of 10 sites (40 percent) also exceeded the guidance value for TL 4 fish. However, caution must be exercised in the latter assessment because largemouth bass are considered to be at TL 5 (Loftus et al., 1998). Based on these guidance values, it appears that certain Everglades populations of piscivorous avian and mammalian wildlife continue to be at risk of adverse effects from mercury exposure depending on where they forage. However, population-level toxic effects from MeHg exposure have not been demonstrated in any of the fish-eating Everglades wildlife populations studied over the last decade. Nevertheless, there is sufficient inferential evidence of negative effects to the individual to warrant concern, at least at the level of reasonable maximum exposure (Spalding et al., 1994; Sundlof et al., 1994; Beyer et al., 1997; Frederick et al., 1997, Bouton et al., 1999; Heniz, in prep). Furthermore, the lack of unambiguous epidemiological evidence of population-level effects of MeHg toxicosis may reflect the inability of the study methods used to date to detect more subtle effects in the field (i.e., behavioral teratology; Nocera & Taylor, 1998).

WADING BIRD FEATHERS FROM ECP INTERIOR MARSHES

To evaluate temporal trends, results from the District's program to monitor mercury bioaccumulation in wading birds were compared to results from similar collections made by Frederick et al. (1997; later published by Sepulveda et al., 1999) in 1994 and 1995. In accordance with USACE permit 199404532, Condition 8b.2, these results were found to be representative of background mercury concentrations in Everglades wading birds (FTN Associates, 1999). The study by Frederick et al. (1997) involved monitoring THg in feathers of the great egret nestlings at various Everglades colonies. The District's monitoring program focuses on two egret colonies, designated as JW1 and L67, which are located in WCA-3A. These two colonies consistently showed the highest THg concentrations during background studies (Frederick et al., 1997; FTN Associates, 1999; Sepulveda et al., 1999).

In WY2003, conditions were not optimal for wading bird nesting in central and southern WCA-3A. The JW1 and L67 colonies were first visited on February 19, 2003. At that time, L67

was found to be active and ten eggs (i.e., one egg each from 10 nests) were collected for THg determination (see discussion below). In contrast, JW1 was again found to be inactive (refer to the *2000 Everglades Consolidated Report*). Accordingly, an alternate colony located several miles east of JW1, designated as "Cypress City", was visited on March 6, 2003. Cypress City was found to be active and ten eggs were collected from this location. Chicks were also present in several nests at Cypress City during the first sampling event. When the Cypress City colony was revisited for feather collection on April 1, 2003, nests were found to have been abandoned with many dead chicks remaining; several live chicks were located and sampled for feathers (see below). In addition, six dead chicks were salvaged for organ-tissue analysis (sample results are currently pending), as well as feather samples. The L67 colony was revisited for feather sampling on April 7, 2003. Again, many nests were found abandoned and, consequently, a full sample set was not collected (see discussion below). Other investigators reported similar colony abandonment for great egrets (possibly as high as 75 percent), wood storks, and white ibises in WCA-3A (D. Gawlik, personal communication). In an attempt to collect additional feathers to complete the sample set, a final visit was made to the L67 colony on April 28, 2003 but no great egret chicks were found.

In WY2003, 15 feather samples were collected from the Cypress City colony (9 from living nestlings and 6 salvaged from dead chicks), and 7 feather samples were collected from the L67 colony (5 nestlings and 2 salvaged adult plumes found on the ground or in the nest). Regrettably, L67 feathers samples were lost in transit. Accordingly, archive material of the nestling feather samples was sent to the laboratory for analysis. However, insufficient material of P14784-3 remained for analysis, and the sample mass for P14784-1 was analyzed although it was exceptionally small (0.0020 g; T. Chandrasekhar, personal communication). Therefore, the resulting value for the P14784-1 sample (10 µg/g dry weight) is considered suspect. No archive material was available for the lost adult plumes.

Feather THg concentrations ranged from 2.6 µg/g to 9.8 µg/g dry weight (excluding the suspect value discussed above), with an overall mean concentration (two colonies pooled) of 5.5 ±1.8 µg/g. However, caution must again be used when interpreting these results because the THg concentrations in nestling feathers are often dependent on the duration of exposure and, thus, the age of the bird. Regression and standardization of feather Hg concentrations in 2003 (two colonies pooled) based on bill length (i.e., age surrogate) were not statistically significant ($df = 1, 16$; $F = 0.16$; $p = 0.7$). (Note: regression was also attempted on Cypress City only and was also nonsignificant.) Attempts to standardize feather THg for 1999 through 2001 at the L67 colony were also not statistically significant (**Table 8**). (Note: Regressions were significant at JW1.) This lack of significant regressions (i.e., concentration does not show a statistically significant increase with age) has been interpreted as an indication that exposure at L67 had been reduced to a level such that growth dilution overwhelmed daily intake. Nevertheless, temporal trends can be assessed qualitatively. On average, nestlings sampled in 2003 were 13 days old (i.e., based on an average bill length of 4.4 cm and the relationship developed by P. Frederick), which is the same age as birds sampled in 2002, three days younger than chicks sampled in 1994, and only two days younger than chicks sampled in 2001. Given these ages, THg levels in great egret nestlings appear to have increased slightly in 2003 compared to 2002 (**Table 8**). The 2003 levels are similar to those observed in 2001, but most importantly, continue to be much lower than the 1994 levels. The interpretation that mercury exposure to great egrets was reduced in 2003, as compared to 1994 as well as 2001, was strengthened by the results of egret egg collections.

Table 8. Standardized least square mean of THg (µg/g dry weight) for a chick with a 7.1 cm bill (arithmetic mean concentration±1SD, n) in growing scapular feathers collected annually from great egret nestlings (2 to 3 weeks old) at the JW1 and L67 colonies.

Colony	1994 *1	1995 *	1999	2000	2001	2002	2003
JW1	21.12 ± 6.1 (25.0 ±7.9, 9)	14.51±3.31 (NA, 8)	7.18 ±1.14 (4.0 ±2.2, 13)	6.9 ±1.3 (3.4 ±1.9, 10)	Failed to initiate nesting	Colony abandoned	Failed to initiate nesting
L67	16.29 ± 4.53 (NA, 27)	15.51 ±6.16 (15.9 ±6.16, 14)	NC (3.6 ±1.5, 20)	NC (3.2 ±1.4, 10)	NC (7.0 ±3, 13)	NC (2.1 ±0.5, 6)	NC (5.1 ±2, 3)
Cypress City							NC (5.6 ±2, 15)

* Data from Frederick et al. (1997).

[1] Concentrations standardized to a bill length of 5.6 cm.

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

Estimated mean age of sampled nestling, based on bill length, was 16 days in 1994, 24 days in 1995, 15 days in 1999, 16 days in 2000, 15 days in 2001 and 13 days in 2002 and 2003.

In addition to collecting feather samples for compliance with the aforementioned federal and state permits, District staff have also collected egret eggs to support an ecological risk assessment of MeHg (Rumbold, 2000) and to better assess spatial and temporal trends in wading bird exposure (for details, refer to Rumbold et al., 2001). As mentioned above, the District continued to collect egret eggs in 2003. The mean THg concentration was 0.37 µg/g (±0.21; fresh weight) in eggs at L67, and 0.38 (±0.24) in eggs at Cypress City colony (**Figure 11**). This between-colony difference was not significant (df = 1, 18; F = 0.01; p = 0.9). While egg THg concentration has varied since 1999 (appearing to increase slightly in 2001, and then decrease again in 2002), among-year differences were not statistically significant at L67 (df = 4, 45; F = 1.5; p = 0.22). However, egg-THg concentrations observed in 2003 continue to be lower than the levels reported for eggs collected in 1993 (great egret eggs collected within WCA-3A in 1993 by USGS contained on average 0.46 µg/g, n = 43; D. Day, USGS, personal communication).

Egg concentration is thought to be the best predictor of MeHg risk to avian reproduction (Wolfe et al., 1998); however, embryonic sensitivity differs among species. To date, a critical egg concentration has not yet been determined for wading birds. Thompson (1996) has proposed generic benchmarks based on a literature review, with a heavy emphasis on studies of mallards. Thompson concluded that adverse effects were unlikely to occur in birds at egg THg concentrations of less than 0.5 µg/g, but toxic effects were probable at concentrations greater than 2.0 µg/g. In between these values, there was a gray area characterized by great uncertainty in terms of the probability of adverse effects. It should be noted that the mean THg concentration in egret eggs collected in 2003 was below Thompson's estimated no observed effects level (NOAEL) for *in ova* exposure. However, preliminary results of a study by USGS may suggest that Thompson's benchmark underestimates the risk to the great egret eggs.

In 2001 and 2003, the District assisted the USGS in a study to reduce uncertainty and establish a critical egg concentration for various wading bird species. To assist the USGS, the District collected 168 eggs from five species (47 great egret eggs, 29 anhinga eggs, 58 white ibis eggs, 21 tricolor heron eggs, and 13 snowy egret eggs) in 2001. In 2003, the District also collected 151 eggs from two species (100 white ibis eggs and 51 tricolor heron eggs). However, a few of the tricolor heron eggs were later identified as little blue or snowy egret eggs. In both years, the eggs were shipped live to the USGS Biological Resources Division's Patuxent Wildlife Research Center (Patuxent) in Laurel, MD, where they were incubated after being injected with MeHg. Results from the 2001 study suggested that the embryos of some species of fish-eating birds may be more sensitive to MeHg than the eggs of mallards, and that estimates of harmful levels of mercury may have to be reevaluated (Heinz et al., 2001). Preliminary results from the 2003 injections support this suggestion with statistically significant effects from the 0.4 µg/g treatment. However, the USGS researchers are still in the process of translating the toxicity of MeHg egg injections into what effects would have manifested if the female had naturally deposited that same concentration to the egg during development (G. Heinz, personal communication).

Establishing a benchmark for critical feather THg concentration has also been difficult because of observed or suspected interspecies differences in mercury sensitivity, particularly between piscivores and nonpiscivores and between freshwater birds and seabirds. This is further complicated because, unlike MeHg in eggs, MeHg bonded to keratin and sequestered in feathers no longer represents a risk to the bird. Feather THg concentration is used only as an indicator of MeHg level and possible risk in targeted organs. However, Bouton et al. (1999) and Spalding et al. (2000) recently reported results of a controlled dosing study that combined feather analysis with toxicological observations of great egrets. They dosed great egret juveniles with MeHg-containing gelatin capsules at 0.5 mg Hg/kg food (n = 5) and found subtle behavioral changes and

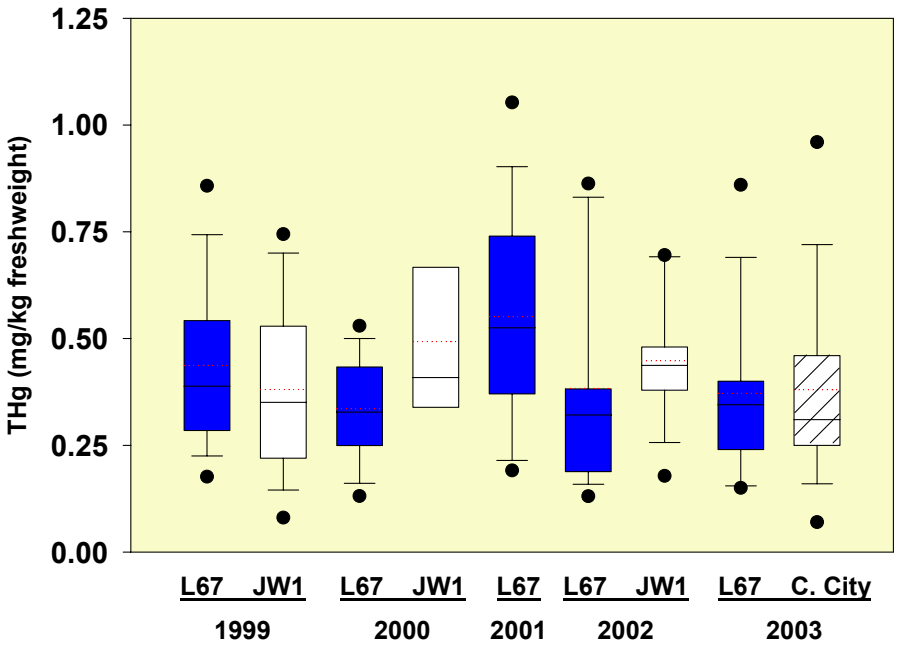

Figure 11. Boxplots of THg concentration in great egret eggs collected from colonies within WCA-3A. Note: Eggs were collected at Cypress City colony in 2003 due to inactivity at JW1 colony. Outliers that lie outside the 10th and 90th percentile are shown as filled circles.

statistically significant differences in blood chemistry, liver biochemistry, and weight index (Bouton et al., 1999; Frederick et al., 1979; Spalding et al., 2000). At five weeks, chicks in this dose group had 19 µg/g THg in feathers and showed a significant decline in packed cell volume (i.e., lowest observed effects level) (Spalding et al., 2000). For the reasons stated above, it is unlikely that levels of THg in egret nestling feathers in 2003 would have exceeded the lowest observed adverse effect benchmark established by Spalding et al. (2000).

WADING BIRD HABITAT AND FORAGING PATTERNS

Various combinations of environmental characteristics determine the suitability of an area for foraging and nesting wading birds. Among others, these characteristics include water depth, vegetation density, and densities and size distribution of the preferred prey populations. These factors have been reviewed in previous Everglades 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 that may show possible changes in wading bird habitat and foraging patterns within the Everglades basin during WY2003. Studies and monitoring programs identified during this search are discussed below.

From February through June of each year, researchers for the USACE carry out systematic reconnaissance flights (SRFs) for wading bird activity in the WCAs and Big Cypress National Preserve. Results of the 2002 SRFs are summarized in Rumbold and Fink (2003); results of the 2003 SRFs are not available at the date of this report.

In 2003, various individuals or agencies also made systematic aerial and ground surveys of nesting wading birds in South Florida. As mentioned above, preliminary information suggests that 2003 was not optimal for nesting and that many nests were abandoned following a water level reversal (i.e., increased in stage) in early March. In 2003, ibis nesting at the Alley North colony was much reduced compared to the 2002 estimates (i.e., 20,000 ibis nests were observed in 2002; D. Gawlik, personal communication). However, preliminary information suggests that no major spatial shifts occurred in Everglades colonies as a result of construction or operation of the STAs.

LITERATURE CITED

Atkeson, T., D. Axelrad, C. Pollman and J. Keeler. 2002. Integrating Atmospheric Mercury Deposition and Aquatic Cycling in the Florida Everglades. Integrated Summary. Prepared by the Florida Department of Environmental Protection for the U.S. Environmental Protection Agency Region 4. Tallahassee, FL.

Beyer, W.N., M.G. Spalding and D. Morrison. 1997. Mercury Concentrations in Feathers of Wading Birds from Florida. *Ambio*, 26: 97-100.

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

Bouton, S.N., P.C. Frederick, M.G. Spalding and H. McGill. 1999. Effects of Chronic, Low Concentration of Dietary Methylmercury on the Behavior of Juvenile Great Egrets. *J. Environ. Toxicol. Chem*., 18(9): 1934-1939.

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

FGMFWTF. 1991. *Mercury Technical Committee (MTC) Interim Report*. Florida Governor's Mercury in Fish and Wildlife Task Force, Tallahassee, FL.

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

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

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

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

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

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

Heinz, G.H., D.J. Hoffman, D.R. Murray and C.A Erwin. 2001. Using Egg Injections to Measure the Toxicity of Methylmercury to Avian Embryos. Abstract submitted for the November 2001 Annual Meeting of the Society of Environmental Toxicology and Chemistry, Philadelphia, PA.

Hughes, K.D., P.J. Ewins and K.E. Clark. 1997. A Comparison of Mercury Levels in Feathers and Eggs of Osprey (*Pandion halibuts*) in North American Great Lakes. *Arch. Environ. Contam. Toxicol*., 33: 441-452.

Hurley, J.P., D.P. Krabbenhoft, L.B. Cleckner, M.L. Olson, G.R. Aiken and P.S. Rawlik, Jr. 1998. System Controls on the Aqueous Distribution of Mercury in the Northern Florida Everglades. *Biogeochem.*, 40: 293-311.

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

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

Lange, T.R., D.A. Richard and H.E. Royals. 2000. Long-Term Trends of Mercury Bioaccumulation in Florida's Largemouth Bass. Abstract from the Annual All-Investigators Meeting: South Florida Mercury Science Program. Palm Harbor, FL.

Lange, T.R. and D.A. Richard. 2001. Long-Term Trends of Bioaccumulation of Mercury in Florida Largemouth Bass. Presented at the Annual All-Investigators Meeting: South Florida Mercury Science Program. West Palm Beach, FL.

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

Nocera, J.J. and P.D. Taylor. 1998. *In Situ* Behavioral Response of Common Loons Associated with Elevated Mercury (Hg) Exposure. *Conservation Ecology*, 2(2): 10. Online at http://www.consecol.org/vol2/iss2/art10.

Rumbold, D.G. 2000. Appendix 7.3b: Methylmercury Risk to Everglades Wading Birds: A Probabilistic Ecological Risk Assessment. G. Redfield, ed. In: 2000 Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL.

Rumbold, D.G., L. Fink. 2003. Appendix 2B-3: Annual Permit Compliance Monitoring Report for Mercury in Downstream Receiving Waters of the Everglades Protection Area. G. Redfield, ed. In: 2003 Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL.

Rumbold, D.G., L.E. Fink, K. Laine, F. Matson, S. Niemczyk and P. Rawlik. 2001. Appendix 7-9: Annual Permit Compliance Monitoring Report for Mercury in Stormwater Treatment Areas and Downstream Receiving Waters of the Everglades Protection Area. G. Redfield, ed. In: *2001 Everglades Consolidated Report*, South Florida Water Management District, West Palm Beach, FL.

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

Rumbold, D.G., S.L. Niemczyk, L.E. Fink, T. Chandrasekhar, B. Harkanson and K.A. Laine. 2001. Mercury in Eggs and Feathers of Great Egrets (*Ardea albus*) from the Florida Everglades. *Arch. Environ. Contam. Tox*., 41: 501-507.

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

Spalding, M.G., R.D. Bjork, G.V.N. Powell and S.F. Sundlof. 1994. Mercury and Cause of Death in Great White Herons. *J. Wildl. Manage*., 58: 735-739.

Sundlof, S.F., M.G. Spalding, J.D. Wentworth and C.K. Steible. 1994. Mercury in the Livers of Wading Birds (Ciconiiformes) in Southern Florida. *Arch. Environ. Contam. Toxicol*. 27: 299-305.

Sweet, C.W. 2001. Wet Deposition of Mercury in Florida, 1995 through 2000. Abstract from the USEPA Workshop on the Fate, Transport, and Transformation of Mercury in Aquatic and Terrestrial Environments. West Palm Beach, FL.

Thompson, D.R. 1996. Mercury in Birds and Terrestrial Mammals. Beyer W.N., G.H. Heinz and A.W. Redmon-Norwood, eds. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis Publishers, Boca Raton, FL.

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

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

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

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

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