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

Mark C. Gabriel, Nicole Howard and Shane Atkins

Contributors: Diane Malone, Kevin Nicholas, Brent Warner, Francine Matson, Richard Uhler, Richard Walker, Michael Wright and Yvette Hernandez

SUMMARY

This appendix summarizes data from compliance monitoring of mercury (Hg) influx and bioaccumulation in the downstream receiving waters of the Everglades Protection Area. Results displayed in this appendix for fish, sediment, bird feathers, and surface water are based on calendar year 2007 (CY2007) (January 1, 2007–December 31, 2007). Results displayed for surface water-associated quality assurance/quality control are for Water Year 2008 (WY2008) (May 1, 2007–April 30, 2008).

The key findings presented in this appendix are as follows:

1. Total annual deposition for Everglades National Park (ENP or Park) in CY2007 was 157 kilograms of mercury per year (kg-Hg/yr), which is a 15 percent increase from 2006. This figure represents the average of stations FL11, FL34 (Everglades Nutrient Removal Project or ENR), and FL97 (Western Broward County). In CY2007, annual volume-weighted maximum total mercury (THg) concentrations differed slightly among the ENR and Broward County stations; however, both stations were significantly less (20 percent) than the Park. As a result of difficulties associated with sampling handling, low precipitation, and mechanical failures, several periods were missing for CY2007 for all stations. Consequently, estimates for both the volume-weighted (wet) concentration and annual wet deposition are to be viewed with caution.
2. The THg concentration observed at non-Everglades Construction Project (non-ECP) water control structures was 10.1 nanograms per liter (ng/L) at S141 during the third quarter of 2007. 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

4.1 ng/L, which also occurred at S141 during the third quarter of CY2007. Currently, Florida has no Class III numerical water quality standard for MeHg. After more than eight years of monitoring, little indication of statistically significant temporal trends have been found in either THg or MeHg concentrations (or percent MeHg) at any of the individual structures.

3. Mosquitofish (*Gambusia holbrooki*) collected from downstream marsh sites had mercury levels ranging from 10 nanograms per gram (ng/g) at site WCA-2-F1 (Water Conservation Area 2, F1) to 206 ng/g at site WCA-2-U3 (Water Conservation Area 2, U3). The average basinwide concentration for 2007 was 71 ng/g. This average concentration level represents a 35 percent increase from the basinwide mean concentration in 2006. The grandmean for the period of record (POR) (1998–2007) over all basins is 75.7 ng/g (±5.3). From 2005 to 2007 (three sample years), several stations show statistically significant temporal increases.

4. Sunfish (*Lepomis* spp.) collected from downstream sites had mercury levels ranging from 17 ng/g at site L39F1 to 945 ng/g at site L67F1. The basinwide average concentration in sunfish was 187 ng/g, representing a 12.3 percent increase from 2006. When the dataset was controlled to only look at bluegill (*L. macrochirus*) and length-standardized mercury levels, sites CA35ALT, L67F1, and CA315 had statistically higher THg levels than all other sites. Stations HOLYBC and WCA-2-U3 show statistically significant temporal increases since the POR. The grandmean for the POR (1998–2007) over all basins is 166 ng/g (±0.2).

5. Fillets from individual largemouth bass (LMB) (*Micropterus salmoides*) collected from downstream sites had tissue mercury concentrations ranging from 64 ng/g at site L39F1 to 3,060 ng/g at site L67F1. Site-specific, age-standardized concentrations (estimated for a three-year-old bass symbolized as EHg3) ranged from 639 ng/g at site CA3F1 to 1,890 ng/g at site L67F1. Standardized mercury levels (EHg3) increased 36 percent from 2006 to 2007; however, this increase should be viewed with caution as this is an average of only three available EHg3 values (31.7–41.2 percent). The Holey Land Wildlife Management Area (Holey Land WMA) (site HOLYBC) continues to show a linear and statistically significant increase in THg levels for the POR.

6. Great egret (*Ardea alba*) feathers were collected from two nestlings at the Cypress City colony in Water Conservation Area 3A (WCA-3A) in CY2007. Feather THg concentrations ranged from 0.20 to 0.27 micrograms per gram (µg/g). Levels in 2008 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 for 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 hot spots or have shown reversing (i.e., increasing) trends in recent years, e.g., site HOLYBC in the Holey Land WMA, site CA315 in WCA-3A, and site L67F1 in the Park. From 2006 to 2007 there was an increase in mercury for 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 2007 (CY2007) (January 1, 2007–December 31, 2007), summarizing results of monitoring mercury (Hg) in the downstream receiving waters of the Everglades Protection Area (EPA). This report satisfies the mercury-related reporting requirements of the Florida Department of Environmental Protection (FDEP) Everglades Forever Act (EFA) permits [Chapter 373.4592, Florida Statutes (F.S.)], including permits for Stormwater Treatment Areas 1 West, 1 East, 2, 3/4, 5, and 6 (STA-1W, STA-1E, STA-2, STA-3/4, STA-5, and STA-6). This report includes the monitoring results in 2007 and surface water total mercury (THg) quality assurance/quality control (QA/QC) results for Water Year 2008 (May 1, 2007–April 30, 2008) (WY2008). The results of monitoring mercury within the STAs are presented separately in Appendix 5-4 of this volume.

BACKGROUND

In 1994, the Florida legislature enacted the Everglades Forever Act (Chapter 373.4592, F.S.) that established long-term water quality goals for the restoration and protection of the Everglades. To achieve these goals, the South Florida Water Management District (SFWMD or District) implemented the Everglades Construction Plan (ECP). A crucial element of the ECP was the construction of six wetlands, termed Stormwater Treatment Areas (STAs), to reduce phosphorus loading in runoff from the Everglades Agricultural Area (EAA). The original STAs were built mainly on formerly cultivated lands within the EAA and total over 20,000 hectares (approximately 50,000 acres). The downstream receiving waters to be restored and protected by the ECP are part of the Everglades Protection Area (EPA). The EPA comprises the following defined regions: the Arthur R. Marshall Loxahatchee National Wildlife Refuge, which contains Water Conservation Area 1 (WCA-1); Water Conservation Areas 2A and 2B (WCA-2A and WCA-2B); Water Conservation Areas 3A and 3B (WCA-3A and WCA-3B); and Everglades National Park (Park or ENP).

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

A key to understanding the Everglades mercury problem is recognizing that it is primarily a methylmercury (MeHg) problem, not an inorganic or elemental mercury problem. MeHg is more toxic and bioaccumulative than the inorganic or elemental form. Elsewhere in the world, industrial discharge or mine runoff (e.g., chlor-alkali plant in Lavaca Bay in Texas, New Idria Mine in California, and Idrija Mercury Mine in Slovenia) can contain total mercury (THg) concentrations much greater (in some areas three-hundredfold higher) than that found in the Everglades but, at the same time, have lower MeHg concentrations. In the Everglades, atmospheric loading has been found to be the dominant, proximate source of inorganic mercury,

with the ultimate source likely being coal-fired utility boilers (far field) and municipal and medical waste incinerators (Atkeson and Parks, 2002). After deposition, a portion of this inorganic mercury is then converted to MeHg by sulfate-reducing bacteria (SRB) in the sediments of aquatic systems (Gilmour et al., 1992; Gilmour et al., 1998; Jeremiason et al., 2006). This methylation process is extraordinarily effective in the Everglades due to the availability of sulfate, the large pool of labile dissolved organic matter, and significant mercury source input from atmospheric deposition (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 of the EPA and in the STAs (see Appendix 5-4 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 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, this 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 collecting information from the National Atmospheric Deposition Program's (NADP) Mercury Deposition Network (MDN). Under MDN protocols, bulk rainfall samples are collected weekly at the Everglades Nutrient Removal (ENR) Project (FL34), Western Broward County (referred to as Broward County station [FL97]), and Everglades National Park (FL11) 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 (FL97). Surface measurements at the Broward County station began at the end of November of 2006.

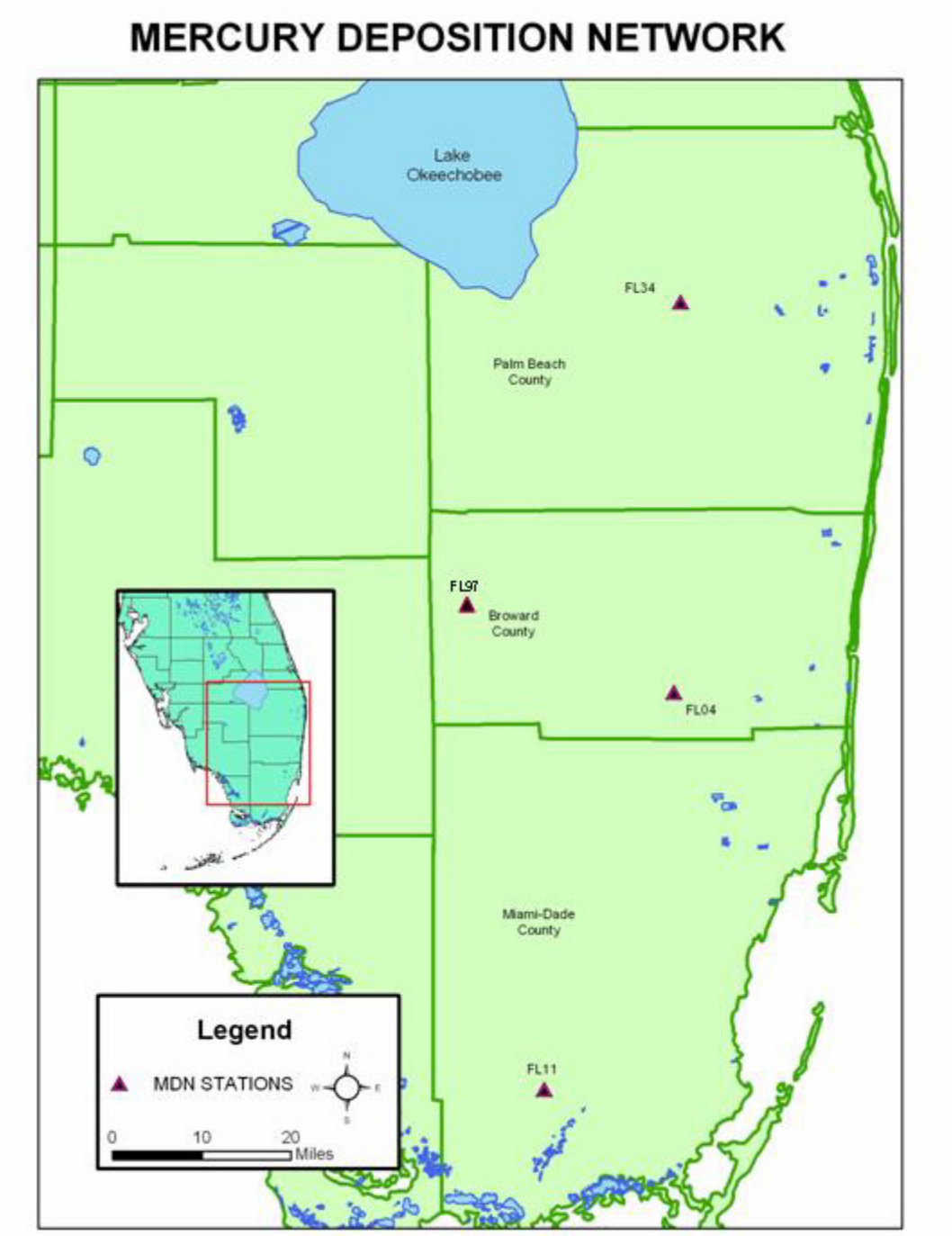

Figure 1. Map showing mercury deposition monitoring sites.

Surface Water

Unfiltered grab samples of surface water are collected quarterly using an ultraclean technique. Currently, sampling occurs upstream of structures S-9, S-10C, S-12D, S-140, S-141, S-151, and S-190/L-28 interceptor (**Figure 2**). These samples are analyzed for THg and MeHg. Throughout the course of WY2008 several changes were made to the non-Everglades Construction Project structure sampling (HGLE program). Refer to the *Optimizing the Monitoring Network* section of this appendix for details.

Preyfish

Using a dip net, a grab sample of between 100 and 250 mosquitofish (*Gambusia* spp.) are collected during single sampling events at 12 downstream interior marsh sites **(Figure 3)**. Fishes are homogenized, the homogenate is sub-sampled in triplicate, and each sub-sample is 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.) Mosquitofish was selected as a representative indicator of short-term, localized changes in water quality because of its small range, short life span, and widespread occurrence in the Everglades. Mosquitofish become sexually mature in approximately three weeks and have an average life span of only four to five months (though some individual females may live up to 1.5 years); the life span of males is shorter than females (Haake and Dean, 1983; Haynes and Cashner, 1995; Cabral and Marques, 1999). In October 2007, the District took responsibility of analyzing all fish types (mosquitofish and large-bodied fish) for THg that do not require pesticide analysis. Samples needing both mercury and pesticide analysis are analyzed by the FDEP.

Secondary Predator Fish

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

Top-Predator Fish

Using electroshocking techniques, up to 20 largemouth bass (*Micropterus salmoides*) (LMB) are also collected at the 12 downstream interior marsh sites **(Figure 3)**; the fillets are 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, LMB were selected as an indicator of potential human exposure to mercury.

Tissue concentrations in each of these three monitored fish species 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 LMB should take a greater amount of time to respond, in terms of tissue concentrations, to changes in ambient MeHg availability. Most importantly, sunfish and LMB 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.

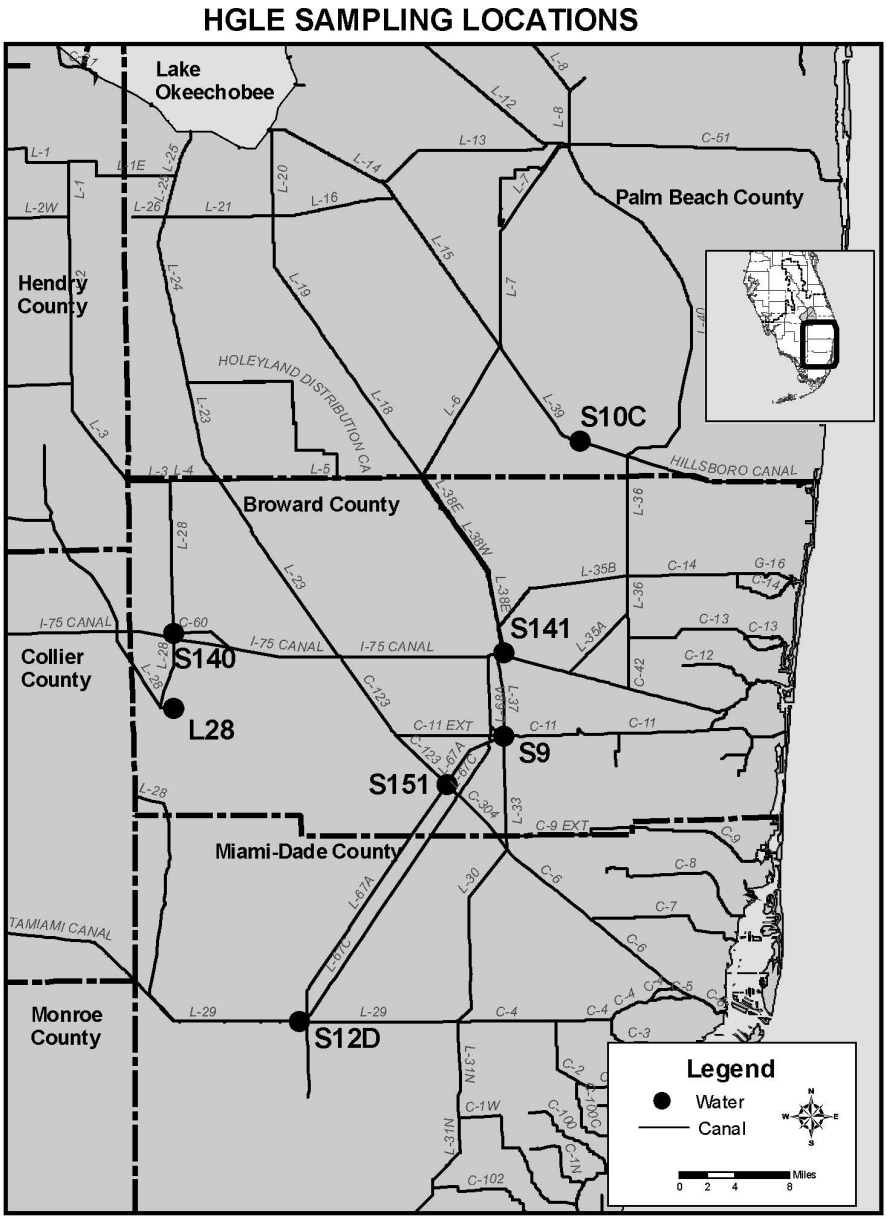

Figure 2. Map showing current non-Everglades Construction Project (non-ECP) structures (HGLE program) where unfiltered surface water is collected quarterly to monitor (per water year) concentrations of (total mercury) THg and methylmercury (MeHg).

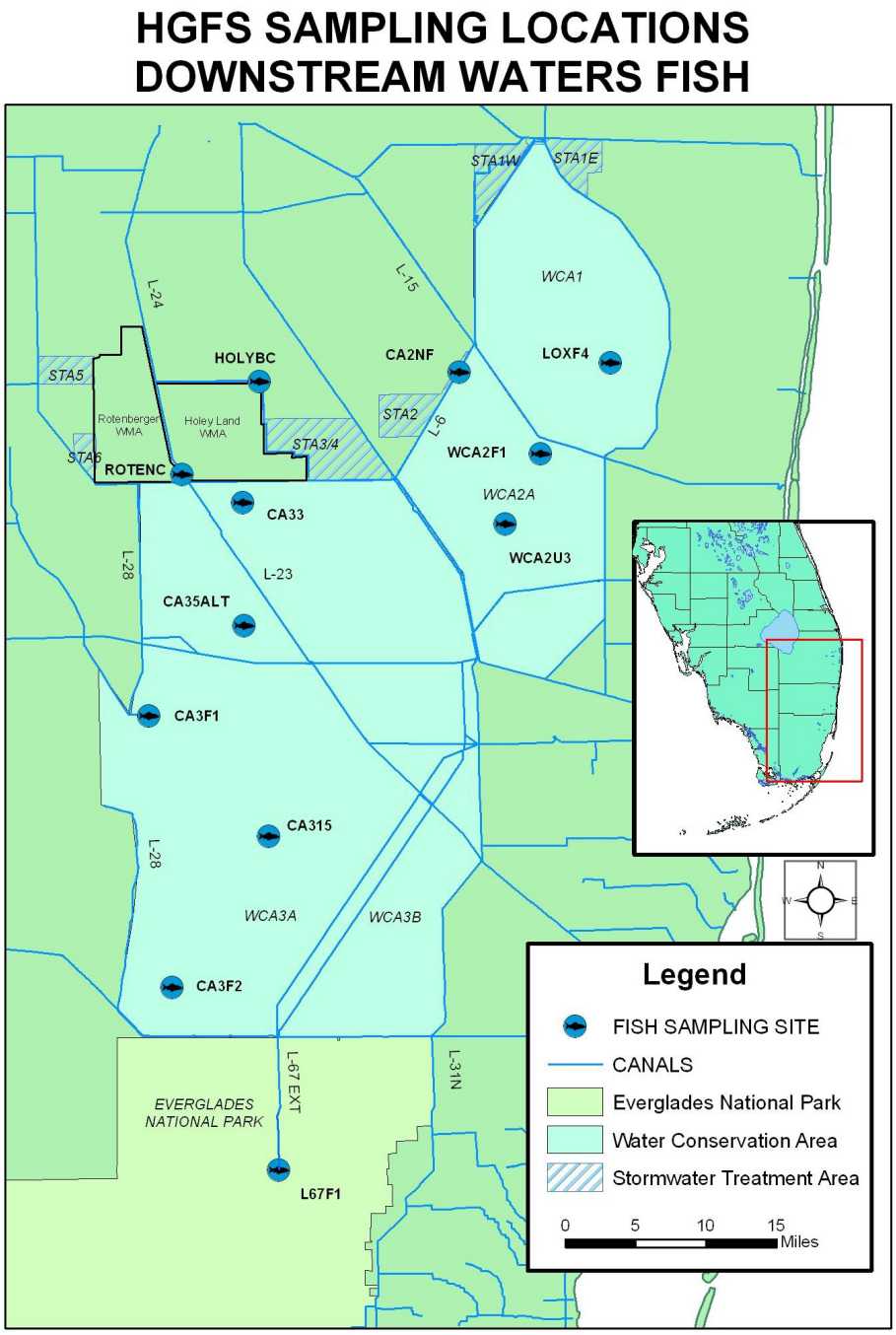

Figure 3. Map showing collection sites for monitoring THg levels in mosquitofish, sunfish, and largemouth bass.

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 not a high priority, egg collections were discontinued in 2004.

In addition to the monitoring program described above, in accordance with Condition 4.iv of the Mercury Monitoring Program, the District is required to "report changes in wading bird habitat and foraging patterns using data collected in ongoing studies conducted by the permittee and other agencies." Further details regarding rationales for sampling scheme, procedures, and data reporting requirements are in the District's Everglades Mercury Monitoring Plan revised in March 1999 (Appendix 1 of the Quality Assurance Protection Plan, June 7, 1999).

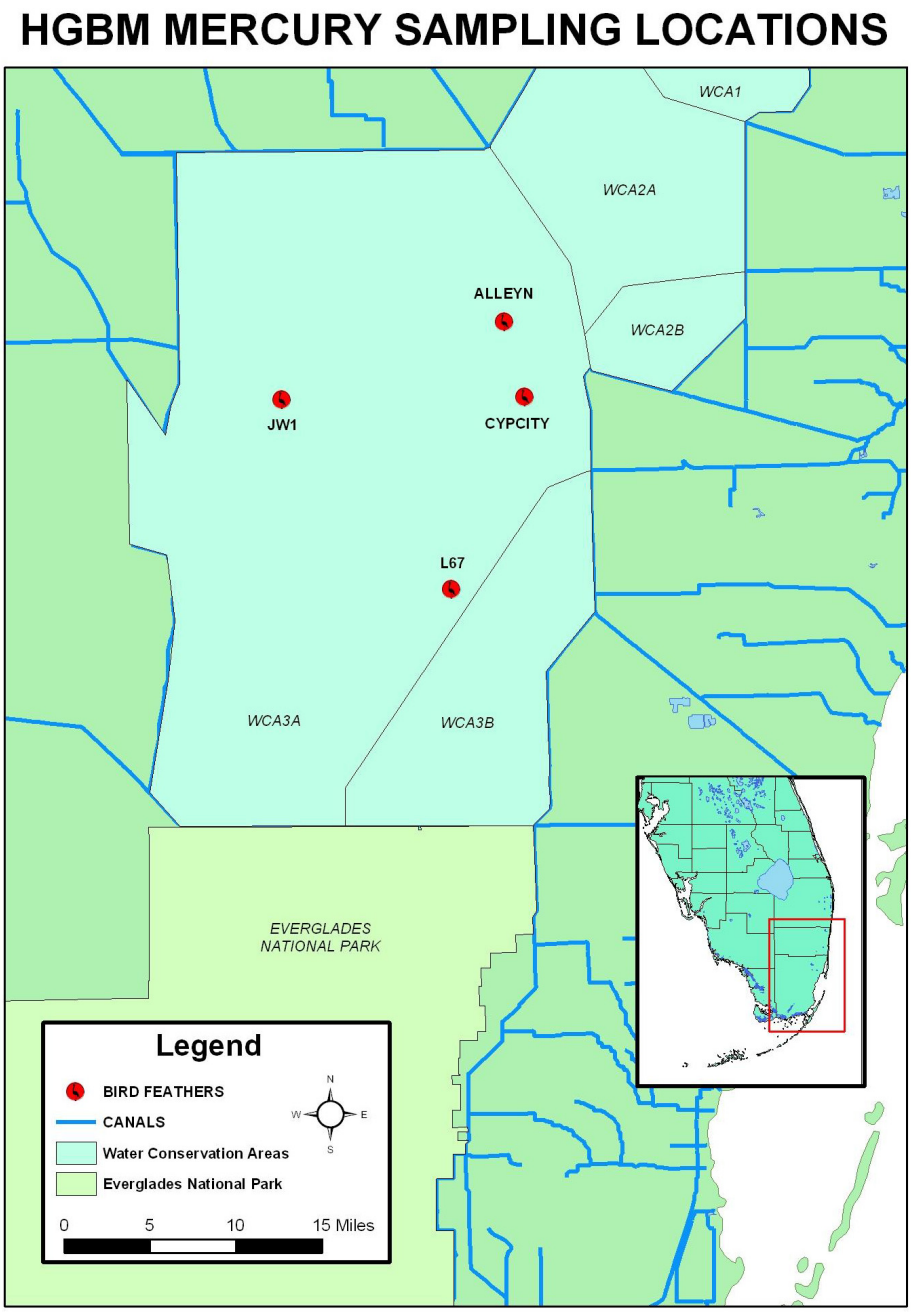

Figure 4. Map showing colonies where great egret (*Ardea alba*) nestling feathers have been collected. Although efforts are made to repeatedly collect from the same colony, colonies are sometimes inactive or abandoned, thus requiring collection at an alternate colony.

QUALITY ASSESSMENT FOR THE MERCURY MONITORING PROGRAM

This section is a quality assessment of the District's Mercury Monitoring Program during WY2008 and, an evaluation of the accuracy, precision, and completeness of the data quality where appropriate. 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 assurance measures are incorporated during the sample collection and laboratory analysis to evaluate the quality of the data. These measures give an indication of measurement error and bias (or accuracy and precision). Aside from using these results as an indication of data quality, an effective QA program must utilize these QC results to determine areas of improvement and implement corrective measures. QC measures include both internal and external checks. Typical internal QC checks include replicate measurements, internal test samples, method validation, blanks, and the use of standard reference materials. Typical external QC checks include split and blind studies, independent performance audits, and periodic proficiency examinations. Data comparability is a primary concern because mercury-related degradation of water quality is defined here as relative to baseline data generated by one or more laboratories. It is important to establish and maintain comparability of the performance and results among participating laboratories assessing the reporting units and calculations, database management processes, and interpretative procedures. Comparability of laboratory performance must be ensured if the overall goals of the Mercury Monitoring Program are to be realized.

Laboratory Quality Control

Data for this program was generated by the District and the FDEP, both of which are certified by the Florida Department of Health under the National Environmental Laboratory Accreditation Program. The following methods were utilized when analyzing samples for THg and MeHg during WY2008: FDEP–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 245.6 (Mercury in Tissues by Cold Vapor AAS [uses liquid digestion]); EPA 7471A (Mercury in solids by Cold Vapor AAS [uses liquid digestion]); District–EPA 7473 (Mercury in solids and tissues by direct thermal decomposition, amalgamation and AA [does not incorporate liquid digestion]). All of the above methods use performance-based standards

employing the appropriate levels of QA/QC required by National Environmental Laboratory Accreditation Conference, the specific reference method, and the Mercury Monitoring Program.

Field Quality Control Samples

A total of 164 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 WY2008. These field QC check samples represented approximately 34 percent of the 474 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. QC results for this water year were significantly different than WY2007. The percent flagged doubled for THg in EB and FCEB. For WY2007 and WY2008, the greatest percentages of '% Flagged' were for EB.

Table 1. Frequency of field quality control (QC) blanks from Stormwater Treatment Areas (STAs) 1 West, 1 East, 2, 3/4, 5, and 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					
FieldQC[1]	n[2]	Collection[6] Frequency %	n > MDL	Mean ng/L[3]	n V[4] Flagged	% Flagged	n[2]	Collection[6] Frequency %	n > MDL	Mean ng/L[3]	n J[5] Flagged	% Flagged
FKPB	10	4.2	1	0.11	0	0	10	4.2	0	0	1	1
EB	19	8.0	4	0.60	4	21	21	8.8	5	0.03	2	1
FCEB	20	8.5	3	0.50	1	5	20	8.4	3	0.03	1	5

[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 WY2008 was 235 THg and 237 MeHg.

[3] Mean concentration of contaminated QC samples.

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

[5] Estimated value; not accurate.

[6] Percentage of all samples collected (n = 235 for THg and n = 237 for MeHg).

Analytical and Field Sampling Precision

Field replicates are samples that have been collected 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, 56 replicate, unfiltered surface water samples (28 THg and 28 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 WY2008. **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, two replicate samples were matched with one routine sample. For WY2008, five THg relative standard deviations and two MeHg relative standard deviations were greater than the required 20 percent QA/QC precision level.

Mosquitofish Composite Samples

To monitor spatial and temporal patterns in mercury residues in small-bodied fishes, individual mosquitofish (100 to 250 individual fish) were collected at various locations in the STAs, ECP, and non-ECP marshes. These individuals were then composited for each site. Composite sampling can increase sensitivity by increasing the amount of material available for analysis, reduce inter-sample variance effects, and dramatically reduce analytical costs. However, there are disadvantages to composite sampling. Subsampling from a composite introduces uncertainty if homogenization is incomplete. Since 1999, the District has used a Polytron® homogenizer to homogenate composited mosquitofish. Until late 2001, the homogenate was sub-sampled in quintuplicate and each sub-sample analyzed for THg. Based on the apparent degree of homogenization as evidenced by the low relative standard deviation (RSD) among aliquots reported in the 2002 Everglades Consolidated Report, the District revised its Standard Operation Procedure after consultation with and approval by the FDEP, reducing subsampling of the homogenate from five to three. In 2007, replicates were further reduced from three to one homogenate. Laboratory replicates of mosquitofish were processed by the analytical laboratories and analyzed for THg. For CY2007, the mean percent RSD between replicate and routine samples for the 19 aliquots was 9.6 percent (**Table 2**). Two RSDs were greater than the required 20 percent QA/QC precision level.

Sediment Composite Samples

For CY2007, a total of six sediment samples were collected for THg and MeHg analysis (six replicates for THg and six for MeHg). These samples were collected from STA-2 and STA-6 (projects ST2D and ST6D). Routine sediment THg values for STA-2 and STA-6 ranged from 0.035 to 0.081 milligrams per kilogram (mg/kg) (n = 3) and MeHg was 0.0005 mg/kg (n = 1; all other routine samples were below detection). The average percent RSD for THg for these stations was 9.3 percent (min. = 1.5, max = 18.4). The percent RSD for MeHg for STA-6 was 26 percent, which is greater than the required 20 percent QA/QC precision level (percent RSD could not be calculated for all others because samples were below detection).

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

% Relative Standard Deviation (RSD)*					
Analyte	**n**	**Minimum**	**Maximum**	**Mean**	**Median**
†Surface Water THg	14	1.6	62	20	9.7
†Surface Water MeHg	14	0.9	56	12	7.5
‡Mosquitofish THg	19	0	38	9.6	8.6
‡Sediment THg	6	1.5	18	9.3	8.1
‡Sediment MeHg	1	§NA	§NA	§NA	§NA

$* \left(\frac{SD}{Mean} \right) x100$

§ Data unavailable due to only one RSD calculated (see S*ediment Composite Samples* section of this appendix)
† Based on Water Year 2008 (WY2008) (May 1, 2007–April 30, 2008)
‡ Based on calendar year 2007 (CY2007) (January 1, 2007–December 31, 2007)

Interlaboratory Comparability Studies

To ensure further reproducibility between ongoing mercury sampling initiatives and to evaluate the performance of contract laboratories used for mercury analysis, round-robin studies for water, fish, and sediment are routinely initiated. These studies are done by the District and contracted laboratories.

Surface Water and Fish

In late March 2008, an interlaboratory study was initiated by the FDEP for the purpose of assessing the comparability of total and MeHg analysis for several laboratories that have been or currently are contracted by the FDEP and the District. Participating laboratories received nine unknown samples of ambient water from the Everglades for analysis of THg and/or MeHg. See the attached document, Statistical Analysis and Summary of Hgrr8 Mercury Round Robin Data, at the end of this appendix. No interlaboratory comparability studies were performed for fish in CY2007.

Sediment

In CY2007, 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, the FDEP, and Frontier Geosciences. The District was also added in the comparisons but only for THg. For details on this study see the attachment titled Performance Evaluation Study of the Analysis of Total Mercury and Methylmercury in Sediment in the 2008 SFER – Volume I, Appendix 3B-1.

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 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), Hg concentrations in LMB were standardized to an expected mean concentration in three-year-old fish (EHg3) at a given site by regressing Hg on age (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 egret 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 Hg concentrations with age (LMB), 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 groups were compared using either Tukey HSD (Honestly Significant Difference; for equal-sized datasets) test or the Tukey-Kramer (for unequal-sized datasets). 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 datasets) or Dunn's Method (for unequal-sized datasets). Pearson Product moment (or the non-parametric equivalent Spearman Rank Order) was used to evaluate the relationship between two parameters. Linear regression was used 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 National Atmospheric Deposition Program (NADP) Mercury Deposition Network (MDN) at the ENR Project (i.e., STA-1W), the Baird Research Center in the Park, and the Western Broward County site (FL97) (**Figure 1**). Operation of FL97 began on November 14, 2006, following shut-down of the Andytown substation on October 17, 2006. For more information on MDN and to retrieve raw data, refer to the NADP's web site, 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 landfall or near misses of hurricanes Katrina (fourth week of August), Rita (third week of September) and Wilma (fourth week of October). In 2004, the northern most station, ENR, was most affected. In 2005, the southern station, ENP, was most significantly affected by the first two storms. During these events, the collectors recorded significant precipitation with little THg. All three collectors were non-functioning during Hurricane Wilma (2005). Therefore, among-year differences in both volume-weighted concentration and deposition must be viewed with caution. In 2007, missing samples at each station in were due to a combination of no precipitation and mechanical failure.

Notwithstanding the uncertainties caused by tropical rainfall events, 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**), likely 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 is commonly understood, as observed with several studies, e.g., Guentzel (1997); Lai et al. (2007); Selin and Jacob (2008). 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 Mercury Deposition Network (MDN) in calendar year 2007.

Week Ending	ENR (FL34)	Broward (FL97)	ENP (FL11)
1/2/2007	11.6	6.30	NA
1/9/2007	9.00	8.90	25.8
1/16/2007	8.00	8.60	12.0
1/23/2007	10.9	14.7	NA
1/30/2007	15.2	4.60	11.2
2/6/2007	6.20	8.80	6.10
2/13/2007	17.0	6.40	8.40
2/20/2007	10.2	15.7	8.20
2/27/2007	NA	NA	17.9
3/6/2007	11.9	24.6	NA
3/13/2007	NA	NA	NA
3/20/2007	12.8	NA	NA
3/27/2007	8.30	2.60	11.8
4/3/2007	NA	NA	8.90
4/10/2007	24.1	27.9	25.7
4/17/2007	10.1	9.40	9.8
4/24/2007	NA	NA	NA
5/1/2007	NA	NA	22.9
5/8/2007	19.5	10.3	10.9
5/15/2007	3.80	5.50	NA
5/22/2007	NA	NA	NA
5/29/2007	NA	1.70	NA
6/5/2007	2.50	2.90	2.40
6/12/2007	13.5	16.7	10.4
6/19/2007	13.0	12.4	13.4
6/26/2007	22.3	23.1	21.3
7/3/2007	20.1	10.	24.9
7/10/2007	9.80	52.7	14.2
7/17/2007	51.5	23.6	26.4
7/24/2007	14.8	22.6	15.2
7/31/2007	36.3	20.0	12.7
8/7/2007	21.2	20.8	13.2
8/14/2007	22.2	12.7	31.6
8/21/2007	9.10	18.4	14.5
8/28/2007	18.5	26.3	29.9
9/4/2007	NA	20.1	16.7
9/11/2007	10.7	12.0	24.2
9/18/2007	26.6	27.3	25.9
9/25/2007	12.1	8.20	16.8
10/2/2007	3.30	3.60	13.0
10/9/2007	7.40	2.60	9.80
10/16/2007	NA	32.8	NA
10/23/2007	5.70	7.00	13.2
10/30/2007	4.20	2.90	8.30
11/6/2007	2.20	1.90	4.40
11/13/2007	NA	56.3	NA
11/20/2007	11.2	NA	3.70

Table 3. Continued.

Week Ending	ENR (FL34)	Broward (FL97)	ENP (FL11)
11/27/2007	NA	14.8	NA
12/4/2007	13.1	6.30	7.80
12/11/2007	NA	11.7	15.7
12/18/2007	4.50	4.10	9.00
12/26/2007	5.80	8.70	NA
Volume-Weight Concentration (ng/L)			
1996*			14.1
1997*	18.7	NA[b]	14.7
1998*	11.4	13.8[b]	12.7
1999*	10.8	12.3[b]	11.6
2000*	13.7	15.8[b]	13.6
2001*	13.9	13.2[b]	13.1
2002*	12.3	14.2[b]	12.1
2003*	16.1	16.4[b]	16.4
2004*	13.7[a]	14.7[b]	14.7
2005*	11.7	13.7[b]	10.6
2006*	12.6	14.9[c]	12.4
2007	11.8	11.3	14.5
Deposition Annual (µg/m^2)			
1996*			17.2
1997*	32.4	NA[b]	27.2
1998*	26.1	20.1[b]	20.3
1999*	12.1	17.5[b]	17.7
2000*	14.3	18.1[b]	20.0
2001*	21.0	21.1[b]	18.0
2002*	10.3[a]	18.7[b]	18.2
2003*	17.8	28.5[b]	26.8
2004*	a	18.3[b]	18.7
2005*	11.5	14.5[b]	17.5
2006*	14.4	NA[a,c]	15.4
2007	13.5	22.3	16.8

*Adapted from 2008 SFER – Volume I

[a] Rain gauge malfunction in 2004/several trips missed because of highly active tropical season (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

[b] Data just from the Andytown station (FL04)

[c] Combination of data from the Andytown (FL04) and the Broward County stations (FL97)

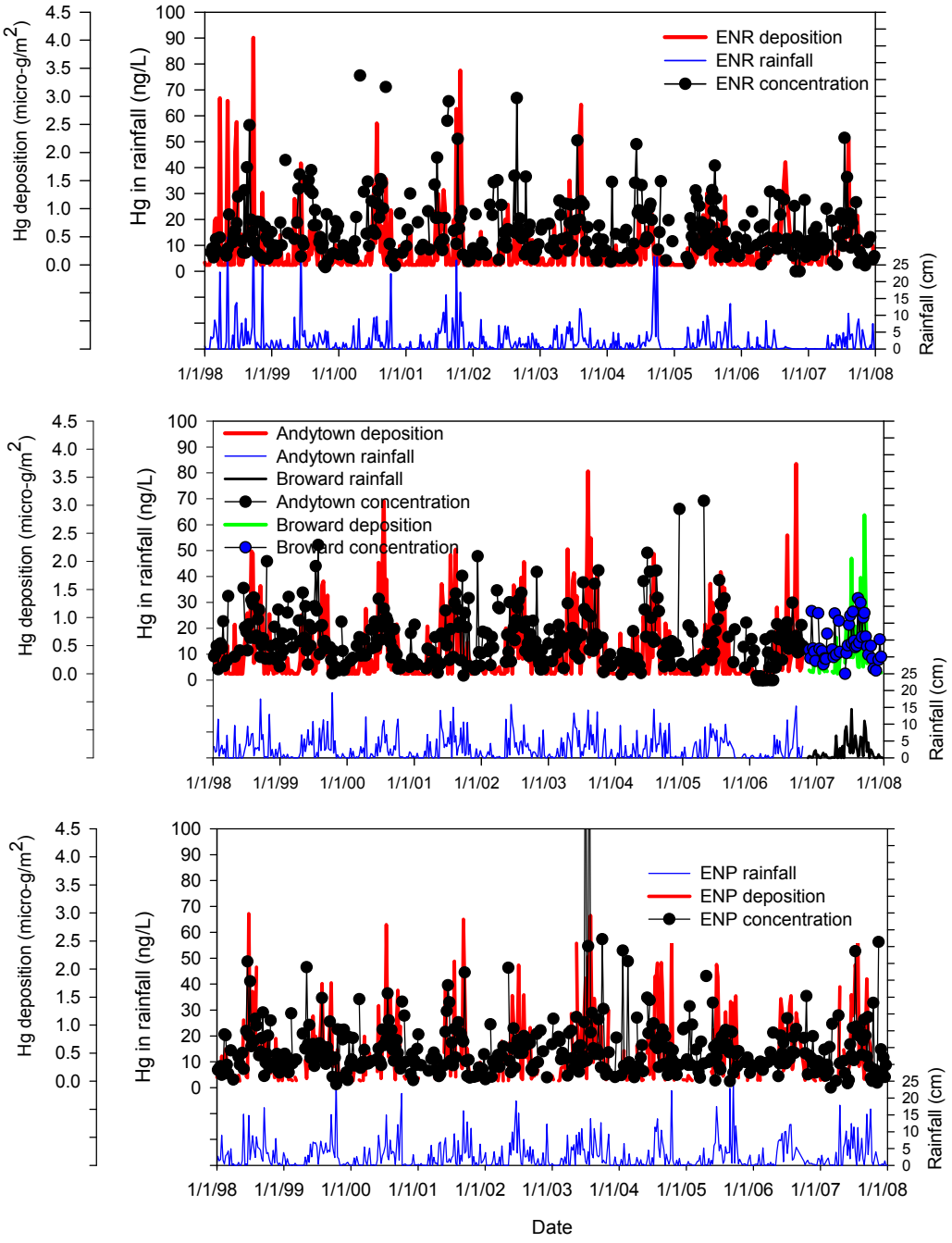

Figure 6. Time series of rainfall, rainfall Hg concentrations, and wet Hg deposition at the Everglades Nutrient Removal (ENR) Project, Andytown, Everglades National Park (ENP) Bair Research Center, and Broward County, as reported by the MDN.

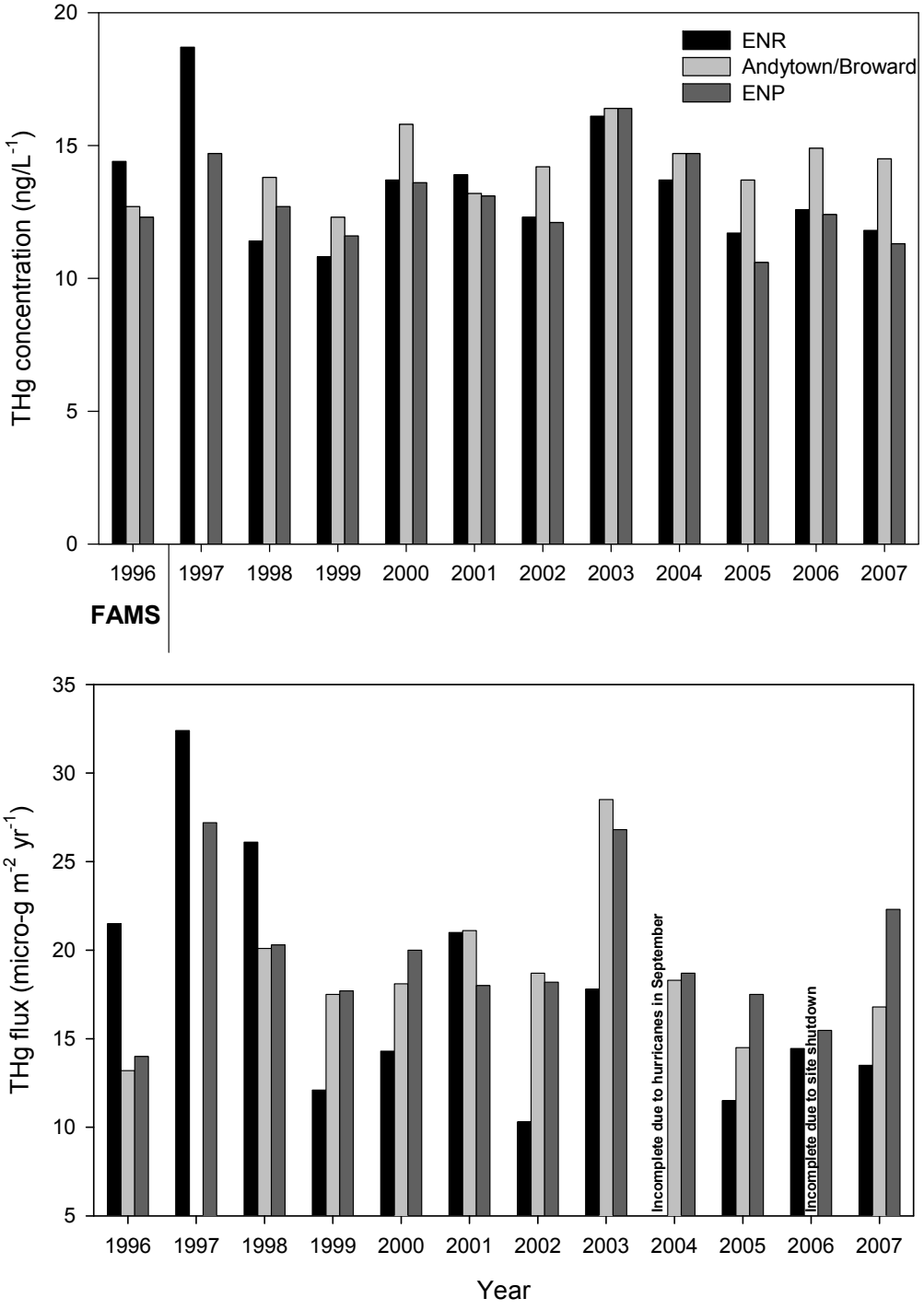

Figure 7. Time series of annual volume-weighted concentration (top) and annual THg flux (bottom) at three MDN stations (FAMS data from Guentzel et al., 2001). The Andytown site was shut down in early 2006 and replaced with Broward County site F97.

Annual volume-weighted THg concentrations differed slightly among the ENR and Broward County stations in CY2007; however, both stations were significantly less than the ENP station (**Table 3** and **Figure 7**). The higher level of rainfall at the ENP station likely contributed to greater deposition. For example, compared to the ENP, 22 to 28 percent less rainfall for the ENR and Broward County for CY2007 coincides with 7 to 25 percent lower annual volume-weighted concentration from 2006 to 2007. The Park's site rose in annual Hg deposition from 2006 to 2007 by 10 percent and the ENR site decreased by 7 percent. Annual change in Hg deposition for FL97 could not be evaluated because of its initiation in November 2006. Over the period of record (POR) from 1997 to 2007, the ENR shows an apparent gradual decrease, but the ENP demonstrates a slight increase. Temporal trends will be further discussed in the following section.

Seasonal Kendall analyses (of ranks) revealed no significant trend in monthly median THg concentrations at the ENR (1997–2007; n = 132 months; Tau = -0.034; p = 0.23) or the ENP sites (1996–2007; n = 144 months; Tau = 0.005; p = 0.95; S. Hill, SFWMD, personal communication, May 22, 2008). Trend analysis was not performed for the Broward County station as this station has only been in operation for approximately one year. The finding of no trend is consistent with a report by Nilles (2004), which found no trends in volume-weight monthly averages from the three sites in South Florida. Seasonal Kendall analysis did not show any long-term trend in the monthly deposition at the Park (n = 144; Tau = -0.03; p = 0.63) or the ENR (n = 144, Tau = -0.11, p = 0.11) (S. Hill, SFWMD, personal communication, May 22, 2008) for the 1997–2007 POR; however, as one can see by the ENR p-value, only marginally. There was a decrease in rainfall (Tau = -0.34, $p < 0.001$) at the ENR.

Based on the average deposition rates measured at the three sites, wet-only atmospheric loading of THg to the EPA (9.01×10^9 m^2) was estimated at 157 kg-Hg/yr (**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; Marsik et al., 2007). This estimate should be viewed with caution as 13 percent of all possible collections were not available due to issues associated with mechanical problems. The overall increase in atmospheric deposition from 2006 to 2007 may have been solely responsible for the ENP station as total annual rainfall increased from 1,257 millimeters (mm) to 1,861 mm. The change in annual rainfall for the ENR station was much smaller (322 mm in 2006 to 407 mm in 2007).

Table 4. Comparison of atmospheric to surface water loading to the Everglades Protection Area (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]
2007	157[g]	2.0[h]

[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 into the EPA

[b] Rumbold (2005)

[c] Sum of loads at S5A, S6, S7, and S8 over CY2005

[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

[g] Based on an average annual loading from FL34, FL11, and FL97

[h] Sum of loads at S7, S8, S150, G251, G310, G335, G300, G301, and S362 over CY2007

SURFACE WATER AT NON-ECP STRUCTURES

Table 5 and **Figures 8** and **9** summarize monitoring results for 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 10.0 ng/L at S-141 during the third 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 period of record is examined (Kruskal-Wallis ANOVA on ranks; $H = 24.7$; $df = 6$; $p < 0.0001$). Site S9 had the lowest THg concentrations compared to all other sites. The low THg concentration at S9 is likely related to typically low total suspended solids (TSS) surface water concentration at this site. In CY2007, average TSS concentration at S9 was below method detection limit (est. 1.75 mg/L). Using Dunn's method of pairwise multiple comparisons, four out of 21 comparisons displayed significant differences ($p < 0.05$) with S9 different from four of the seven sites. The four significant comparisons were between L28 and S9, S141 and S9, S140 and S9 and S10C and S9 with the former sites in each comparison having the higher median. Since 2004, six of the eight stations show an apparent progressive increase in THg (**Figure 8**).

Table 5. Concentrations of THg and MeHg (ng/L) in non-ECP structure surface waters in calendar year 2007.

Structure	Quarter	THg ng/L	remark**	WQS	MeHg ng/L	remark**	% MeHg
L-28	Jan–March	1.2		<WQS	0.22		18.3
	April–June	2.2		<WQS	0.09		4.1
	July–Sept	2.1		<WQS	0.091	§	4.3
	Oct–Dec	NA		<WQS	0.11		
	Median	2.1			0.10		4.3
	Median POR	**1.48**			**0.11**		**7.4**
S-10C	Jan–March	0.77		<WQS	0.094	§	8.3
	April–June	1.3	A	<WQS	0.03	§	2.3
	July–Sept	3.7		<WQS	0.27		7.3
	Oct–Dec	NA		<WQS	0.084	§	
	Median	1.3			0.074		7.3
	Median POR	**0.99**			**0.089**		**8.9**
S-12D	Jan–March	—		<WQS	—		
	April–June	—		<WQS	—		
	July–Sept	2.5		<WQS	0.15		6.0
	Oct–Dec	§		<WQS	§		
	Median						
	Median POR	**1.0**			**0.155**		**15.3**
S-140	Jan–March	0.85	A	<WQS	0.13		15.3
	April–June	1.2	A	<WQS	0.09		7.6
	July–Sept	4.3		<WQS	0.48		11.2
	Oct–Dec	NA		<WQS	0.09		
	Median	1.2			0.11		11.2
	Median POR	**1.15**			**0.13**		**11.3**
S-141	Jan–March	2.9		<WQS	0.22		7.6
	April–June	8.9	A	<WQS	0.21		2.4
	July–Sept	10.0		<WQS	4.1		41.0
	Oct–Dec	NA		<WQS	0.5		
	Median	8.9			0.36		7.6
	Median POR	**1.16**			**0.19**		**16.3**
S-151	Jan–March	0.91		<WQS	0.08	§	8.7
	April–June	2.3		<WQS	0.19		8.3
	July–Sept	3.8		<WQS	0.20		5.3
	Oct–Dec	NA		<WQS	0.16		
	Median	2.3			0.17		8.3
	Median POR	**1.0**			**0.15**		**15.0**

Table 5. Continued.

Structure	Quarter	THg ng/L	remark **	WQS	MeHg ng/L	remark **	% MeHg
S-9	Jan–March	0.45		<WQS	0.04	§	8.9
	April–June	1.3		<WQS	0.03	§	2.3
	July–Sept	1.9	A	<WQS	0.11		5.8
	Oct–Dec	NA		<WQS	0.03	§	
	Median	1.3			0.03		5.8
	Median POR	**0.71**			**0.06**		**3.7**
	Median Jan–March	**0.85**	(5)		**0.104**	(2)	**12.2**
	Median April–June	**1.3**	(3)		**0.09**	(3)	**7.0**
	Median July–Sep	**1.75**	(1)		**0.02**	(6)	**11.4**
	Median Oct–Dec	**3.7**	(5)		**0.11**	(4)	**3.0**

*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

— Not available due to site construction

§Not available due to project transfer; site S-12D changed to S-12A and moved to project PIN

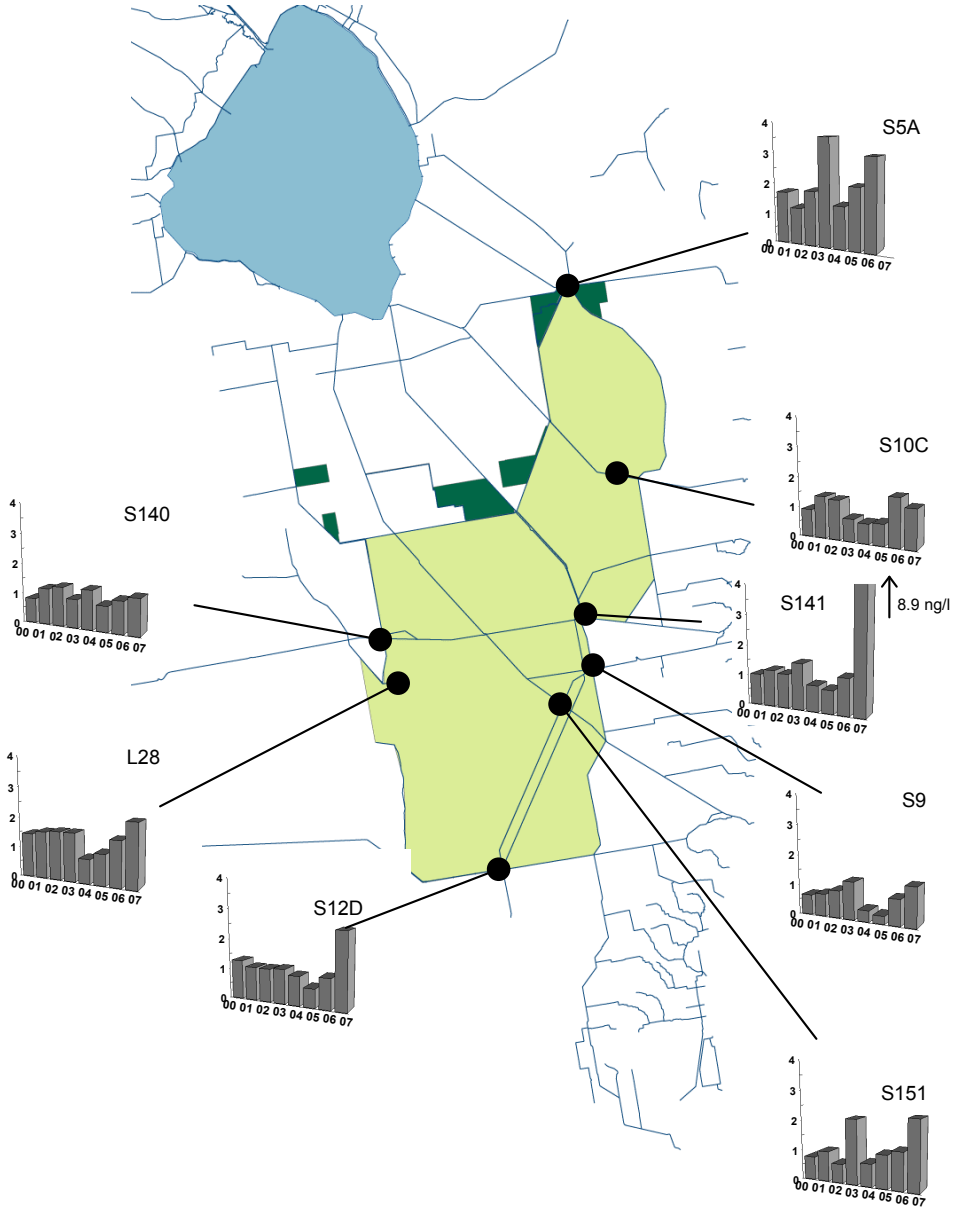

Figure 8. Annual median THg concentrations for period of record (POR) at stations sampled under project code HGLE (non-ECP sites).

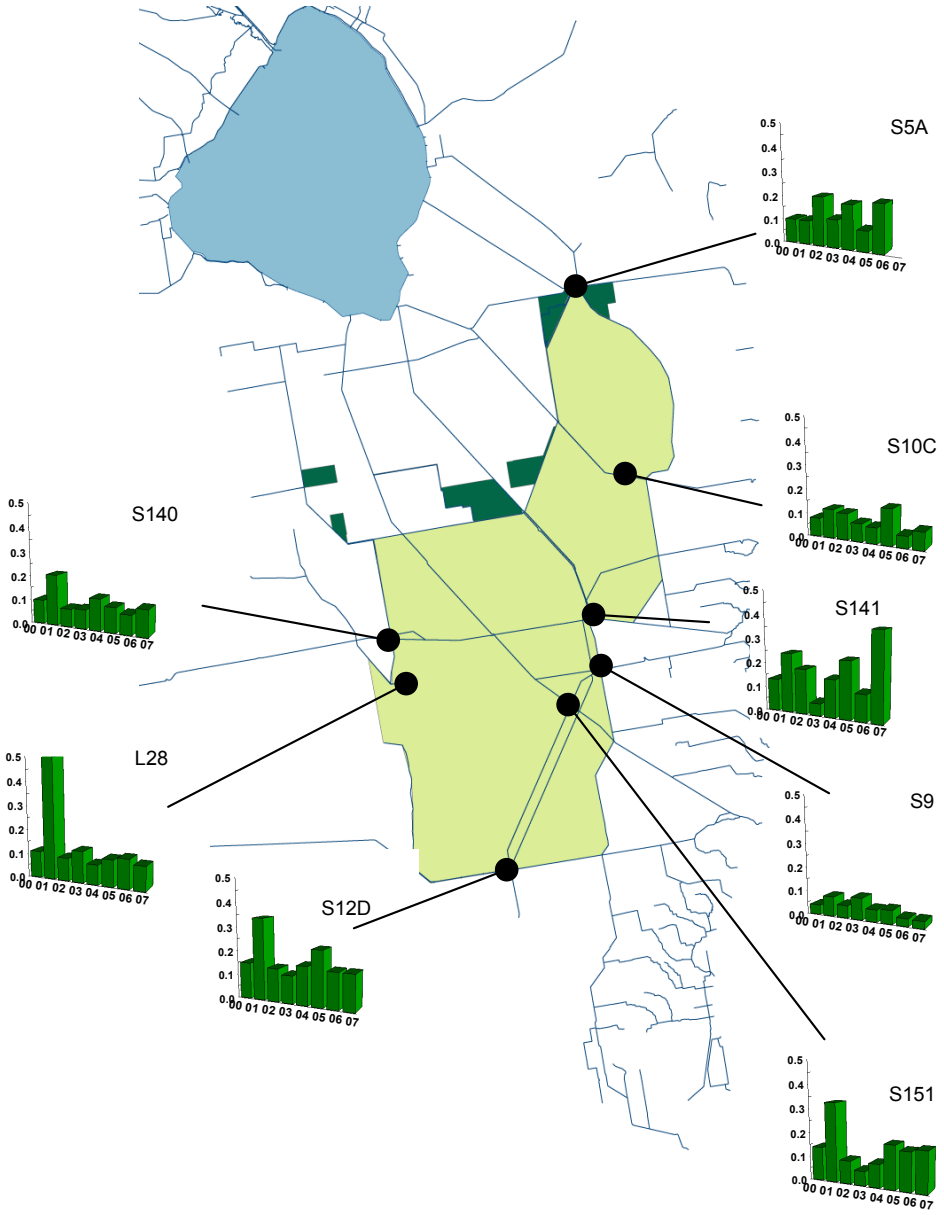

Figure 9. Annual median MeHg concentrations for POR at stations sampled under project code HGLE.

The maximum water-column MeHg concentration observed during CY2007 at a non-ECP structure was 4.1 ng/L, which occurred at S-141 (**Table 5**). This level is approximately 35 times the average MeHg concentration at all other stations. Currently, Florida has no Class III numerical water quality standard for MeHg. Over the period of record 1997–2007, MeHg varies statistically by station (Kruskal-Wallis ANOVA based on ranks; $H = 51.3$; $df = 6$; $p < 0.0001$). Pairwise comparisons showed S141 was higher than all stations (Dunn's test; $p < 0.05$) and S9 was lower than all other stations.

After more than 10 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. For these concentrations, calculated Tau values, which were based on four seasons [i.e., quarterly samples ($n \leq 38$)] ranged from -0.10 to +0.14 for THg and from -0.03 to +0.26 for MeHg (a negative Tau indicates a decreasing trend, whereas a positive Tau indicates an increasing trend). For percent MeHg, Tau values ranged from -0.1 to +0.11. None of the (significance) p values (both with and without autocorrelation correction) were significant ($p < 0.05$) (assessment by S. Hill, personal communication, July 3, 2008).

As observed in previous consolidated reports (Rumbold et al., 2006; Gabriel et al., 2007), concentrations of both THg and MeHg were generally highest during the late summer months of July–September (i.e., third quarter) of CY2007.

FISH FROM ECP AND NON-ECP INTERIOR MARSHES

Results from monitoring downstream interior marsh mosquitofish, sunfish, and LMB are summarized in **Tables 6** through **8**, respectively. Raw data for individual fish can be found through the District's web site at www.sfwmd.gov under the *What We Do*, *Environmental Monitoring*, *DBHYDRO Browser* section. Fish collections were targeted at 12 downstream marsh sites in the interior of the WCAs and 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* spp.) collected in CY2007 from downstream sites. Value presents a mean of three analyses.

Location	THg (ng/g)	Between-Year Change (%) (2006 to 2007)*	Cumulative Average (ng/g)
LOXF4	56.6	53.8	68.3
CA2F1 (L39F1)	10.0	27.2	27.3
CA27 Alt (Z4)	63.0	94.3	92.2
CA2NF	14.0	-13.3	88.6
Holey Land WMA (North canal)	59.0	18.5	47.0
Rotenberger Alt. (RotenF1)	126.0	130.9	88.7
Rotenberger rim canal (RotenC)	68.3	5.35	51.4
WCA2U3	203.6	34.9	118.7
CA33	57.3	19.1	64.8
CA35ALT	66.0	-21.9	90.1
Non-ECP North (CA3F1; end of L-28)	74.6	67.3	55.7
CA315	57.6	-32.0	95.1
Non ECP South (CA3F2)	52.0	51.8	43.0
L67F1	87.3	48.3	119.1
Annual mean	**71.0**	**34.6**	**75.0**

*[(2007–2006)/Average]*100

NA – Data not available

Note: Grandmean for period of record (POR) (1998 to 2007; aliquots pooled across time and space) ± 95% C.I. of mean: n = 524; 75.7 ± 5.3 ng/g; 50^{th}, 75^{th}, and 90^{th} percentiles for POR were 57.2, 98.4, and 160 ng/g, respectively

Table 7. Mean concentrations (±1 SD; ng/g wet weight) of THg in sunfish collected in CY2007 from interior EPA marshes downstream of the STAs.

Target Location	Sampling Location	Mean THg ng/g (± 1SD, n)	Between-Year Change (%) (2006 to 2007)[$]	Grandmean (1998 to 2007) (ng/g)
WCA1-LOX3	LOXF4*	150 (±66,15*)	31.7	127
WCA-2A F1	L39F1	37 (±22,20)	-65.5	66.4
WCA-2A 2-7	Z4	NA	NA	180
	CA2NF	104 (±112,4*)	39.1	76.0
Holey Land	Holey Land	297 (±52,20)	58.8	150
Rotenberger	RotenC (canal)	NA*	NA	153
WCA-2A U3	WCA2U3	242 (±103,20)	6.8	176
WCA-3A 3	CA33	207 (±68,20)	22.0	146
WCA-3A 5	CA35ALT	178 (±131,20)	-42.1	195
Non-ECP North	CA3F1	113 (±79,20)	44.3	122
WCA-3A 15	CA315	231 (±99,20)	-14.8	283
Non-ECP South	CA3F2	71 (±62,20)	15.2	119
ENP P33 Marsh	L67F1	336 (±211,20)	39.6	412
Average		**187**	**12.3**	

*Unable to collect 20 fish

$ [(2007–2006)/mean]*100

NA – Data not available

Note: Grandmean of sites (pooled across space and time) for period of record (POR) (1998 to 2007) ± 95% C.I. of mean: n = 1,908, 166 ± 0.2 ng/g; 50th, 75th, and 90th percentiles for POR were 120, 210, and 327 ng/g, respectively

Table 8. Standardized (EHg3) and arithmetic mean concentrations of THg in largemouth bass fillets (*Micropterus salmoides*) (ng/g wet weight) collected in CY2007 from ECP and non-ECP interior marsh sites.

Target Location	Sampling Location	EHg3 ± 95th C.I. (mean ± 1SD, n) ng/g wet	Between-Year Change (%) (2006 to 2007)	Cumulative EHg3
WCA1-LOX3	LOXF4	NC(1) (354 ±139, 20)	NA	483
WCA-2A F1	L39F1	NC(1) (172 ±146, 11*)	NA	265
WCA-2A 2-7	CA2NF	NC(2) (363±156, 9*)	NA	477
Holey Land	HOLYBC	866±39.4 (831±121,20)	34.7	573
Rotenberger	RotenC	NC (2) (489,1*)	NA	809
WCA-2A U3	WCA2U3	997±104.4 (649±305,23)	NA	743
WCA-3A 3	CA33	NA	NA	NA
Non-ECP North	CA3F1	639±44.7 (350±126, 20)	31.7	505
WCA-3A 5	CA35ALT	NA	NA	NA
WCA-3A 15	CA315	1156±195.9 (495±423, 16*)	NA	828
Non-ECP South	CA3F2	NC(1) (200±93, 20)	NA	546
ENP P33 Marsh	L67F1	1890±134.9 (2,029±426, 20)	41.2	1348
ENP P33 Marsh	L67F1	1890±134.9 (2,029±426, 20)	41.2	1348

*Unable to collect 20 fish
NC – Not calculated for (1) insignificant slope or (2) poor age distribution
NA – Data not available
2007 EHg3 average = 1,109 ng/g
Note: Grandmean for sites (pooled across space and time) for period of record (POR) (1998 to 2007) ± 95% C.I. of mean: n = 1,534, 548 ± 22 ng/g; 50th, 75th, and 90th percentiles for POR were 420, 682, and 1,041 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 hydrologic and habitat restoration has occurred so that chances of finding fish year-to-year are high. Although this level of restoration may take a number of years at certain sites (e.g., sites WCA-2-F1, WCA-3-3, and WCA-3-5), waiting until fish are present consistently will prevent alternating collections between the two sites and the concomitant disruption of data continuity.

Fishes collected in CY2007 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 significant changes over time.

Mosquitofish

Mercury levels in mosquitofish collected from marsh sites in CY2007 ranged from 10 nanograms per gram (ng/g) at site WCA-2-F1 to 206 ng/g at site WCA-2-U3 (**Table 6** and **Figure 10**). The average annual basinwide concentration in mosquitofish collected in CY2007 is 71 ng/g (**Table 6)** (for all locations see **Figure 3**), which represents a 35 percent increase from the basinwide mean concentration in 2006 (46 ng/g). The mean aliquot for tissue-mercury concentrations in mosquitofish for the period of record (1998–2007; n = 524) was 75.7 ng/g. In CY2007, THg levels in mosquitofish declined at three of 14 sites (**Table 6**). Five out of the 14 sites have increased by at least 50 percent since 2006. From 2005 to 2007, overall concentrations have increased (**Figure 10**). These observed increases should, however, be viewed with caution as they may be related to the change in primary analytical laboratory. In October 2007, all fish mercury analysis responsibilities were transferred from FDEP to the District. Both agencies use different instrumentation for mercury detection (see section *Laboratory Quality Control*). Investigation is currently under way to determine if there are analytical differences in mercury quantification for both instrumentation types. Based on visual inspection, there was an increase for all stations except CA2NF, CA315 and CA35alt. For most stations (WCA-2-U3, WCA-2-F1, CA2NF, CA33, CA3F1, ROTENF1, ROTENC, HOLEYC, L67F1) this increasing trend was statistically significant (all Spearman $\rho \approx 0.95$, $p < 0.001$).

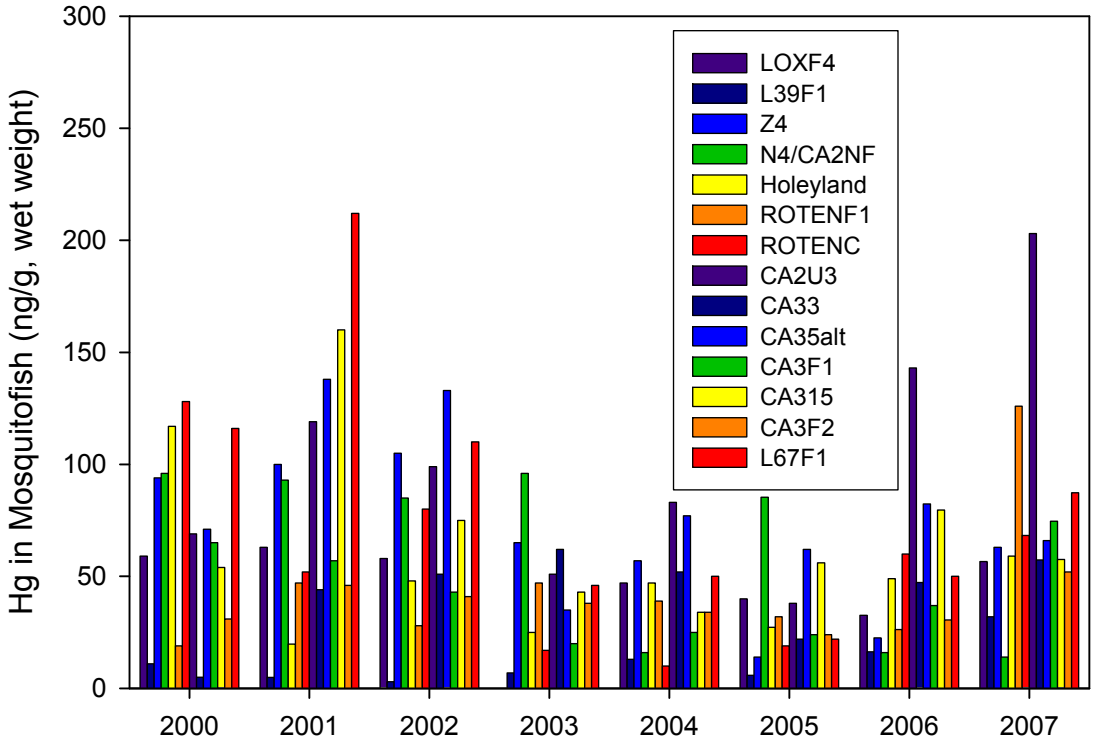

Figure 10. Mercury concentrations in mosquitofish collected at ECP and non-ECP sites for the period of record (1998–2007). Not all sites were sampled in all years (see **Table 6**).

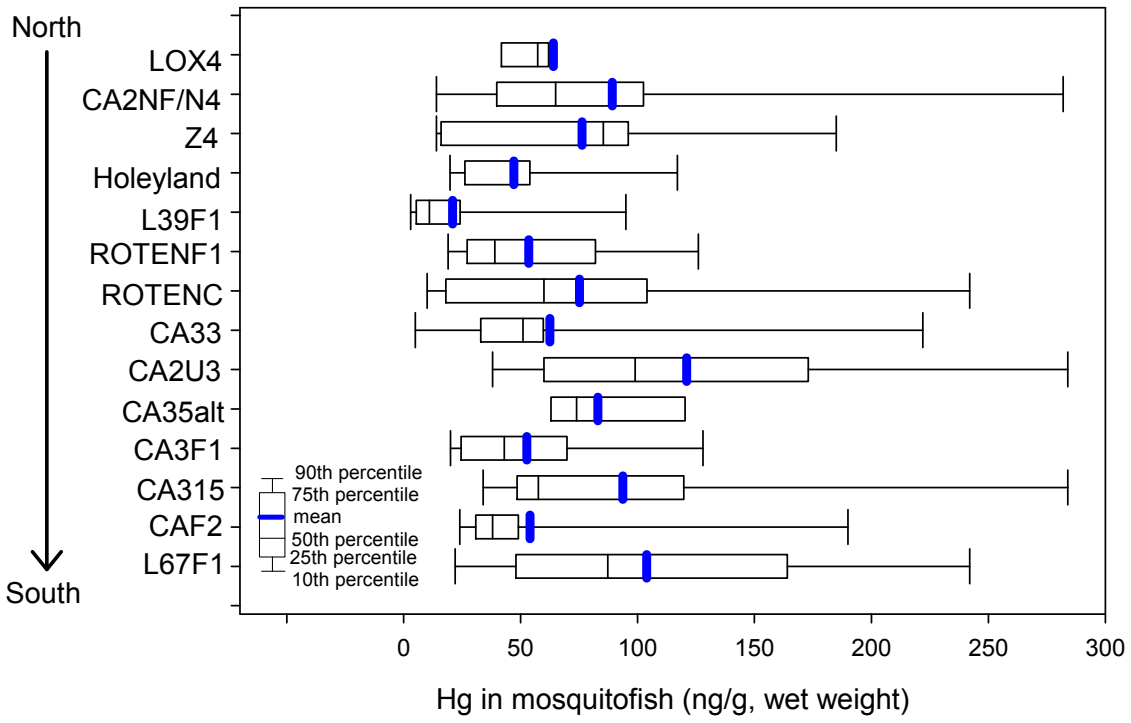

Figure 11. Hg concentration distributions in mosquitofish (*Gambusia* sp.) collected at non-ECP sites for the period of record (i.e., 1998–2007). Not all sites were sampled in all years (see **Table 6**).

Figure 11 shows that the spatial variability in mean mosquitofish THg levels is relatively high. A few stations reveal a consistent low or high level (e.g., L39F1, LOX4, and CA2U3); however, there does not appear to be a clear north-to-south concentration gradient that is more commonly observed with large-bodied fish (LMB, sunfish).

Sunfish

Mercury levels in sunfish collected from downstream sites in CY2007 (n = 195) ranged from a low of 17 ng/g in a redear sunfish from site L39F1 to a high of 945 ng/g in a bluegill from site L67F1. This pattern of minimum and maximum contrasts to 2006; as for 2007, the maximum was observed at the opposite end of the EPA at site CA33. The grandmean of all sites in 2006 was 153 ng/g. For CY2007 the grandmean is 187 ng/g, indicating a 19 percent increase.

In CY2007, sunfish continued to show significant spatial variation in Hg levels (**Table 7**; **Figure 12**; df = 11; H = 119; $p < 0.001$). Fish from sites L67F1, HOLYBC and WCA-2-U3 contained the highest median concentrations (ranging from 237–300 ng/g) and were greater than all other sites (Dunn's Method, $p < 0.05$), except HOLYBC, which was not greater than CA3F2. Because of differences in sizes and species of sunfish collected, these 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; Gabriel, 2007), 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, $p = 0.098$, $df = 11$, $f = 1.61$). After removing the impact of weight on fish THg variability, THg levels were not significantly different with location ($p < 0.084$, $df = 11$, $f = 1.66$), however only marginally.

With the above ANCOVA results showing a borderline spatial impact, an informative next step is to test the spatial variability using standardization. After filtering for bluegill and normalizing by fish length. there were significant differences between sites (Kruskal-Wallis ANOVA on Ranks; $df = 11$; $H = 52$; $p < 0.001$), therefore demonstrating the importance of spatial location on THg levels in bluegill. Overall, L67F1, CA315 and CA35ALT had the highest levels (2.144 to 2.639 ng/g/mm); however most pair-wise comparisons were not statistically different, which likely resulted from several sites have low numbers after the standardizing process. The only statistically significant differences (Dunn's Methods, $p < 0.05$) were between CA35ALT versus L39F1, CA35ALT versus CA3F2, and CA35ALT versus CA3F1 with the former being higher.

As observed over the past several years when data was pooled across sites, fish species was a significant factor in tissue mercury concentration in CY2007 (Kruskal-Wallis ANOVA on Ranks; $df = 3$; $H = 20.2$; $p < 0.001$). Mercury levels were statistically lower in redear (median = 152 ng/g) and bluegill (median = 123 ng/g) than in the other species (Dunn's method, $p < 0.05$): spotted sunfish, median = 238 ng/g; and warmouth, median = 240 ng/g. There were no statistical differences between bluegill and redear or between spotted and warmouth sunfish species. These species-specific medians are much different results than found in 2006. Despite redear showing the lowest THg concentration out of the four species, its average concentration more than doubled from 2006 to 2007.

From visual inspection of **Figure 12**, sunfish appeared to exhibit clear temporal variability in mercury burdens for most sites; however, these apparent trends may be once again confounded by temporal differences in size or species of *Lepomid* collected. For example, the marked decline in mercury levels for CY2007 in sunfish from L39F1 may be an artifact of only collecting redear and bluegill (see above) as compared to previous samples. Similarly, the decline in Hg levels in fish apparent at site CA33 may also be due to increased numbers of redear and bluegill (11 of 20). Spearman correlations were developed to evaluate if concentrations increase progressively with time, specifically only for stations that showed a visual increase since the start of the POR [LOX4, HOLEY, WCA2U3, and CA33 (See **Figure 12**)]. Spearman correlation was used instead of ANCOVA to evaluate the effect of time because the dependent variable (concentration) for each case was non-normally distributed (other ANCOVA rules apply; see Zar, 1996). To exclude this variability due to species and size, the sunfish dataset for 2007 was censored to assess only bluegill. To reduce size-related effects further, Hg levels were normalized by total fish length. Following standardization, two of the four stations showed progressive increase with time: HOLEY ($p = < 0.001$, $\rho = 0.66$) and WCA-2-U3 ($p = 0.04$, $\rho = 0.22$).

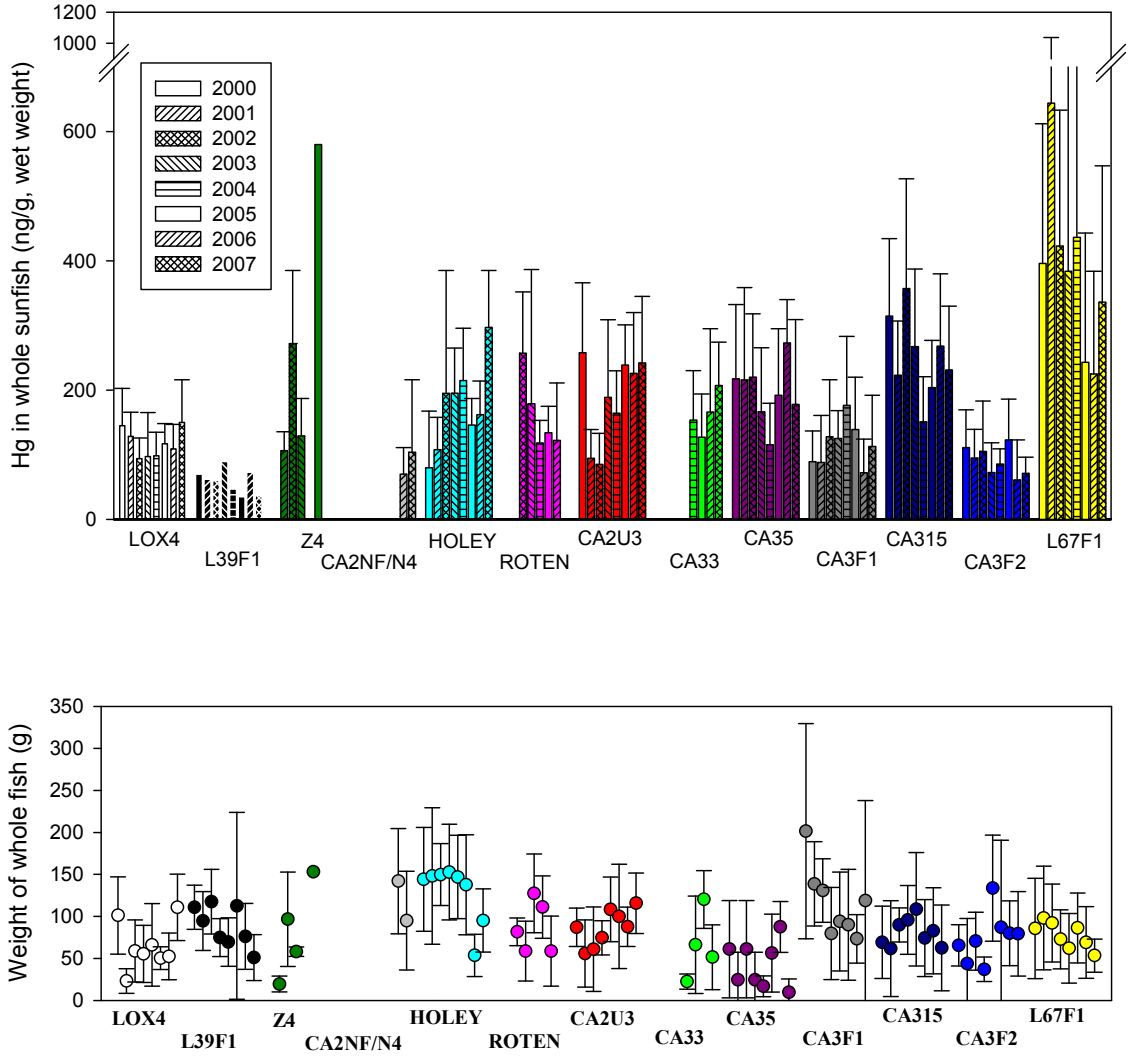

Figure 12. THg concentration (top) and weights (bottom) of whole sunfish (*Lepomis* spp.) collected at ECP and non-ECP sites for the POR. Prior to 2006, collections were made at site Z4 (CA2NF/N4 after January 1, 2006).

Largemouth Bass

A total of 161 largemouth bass (LMB) were collected at 10 downstream sites from October 2007 through November 2007. Despite best efforts of the FWC (who were contracted to electrofish at these sites), LMB could not be collected from site CA33 and CA35ALT. LMB 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 3,060 ng/g in an eight-year-old fish from site L67F1. Site specific, age-standardized concentrations (EHg3) ranged from 639 ng/g at site CA3F1 to 1890 ng/g at site L67F1 (**Table 8** and **Figure 13**). Calculation of EHg3 was not appropriate at sites ROTENC, LOX4, L39F1, CA2NF, and CA3F2 either because the tissue mercury-age relationship was not significant or because of small sample size. Based on the sites where it was appropriate to calculate site-specific EHg3, the grandmean value was 1,109 ng/g in 2007, which represents a 41 percent increase over the grandmean estimated for 2006; however, this increase should viewed with caution as this relays on only five regression calculations for 2007 and seven for 2006. In addition, as with mosquitofish and sunfish, the increase may be the product of analytical instrumentation changeover that occurred in October 2007.

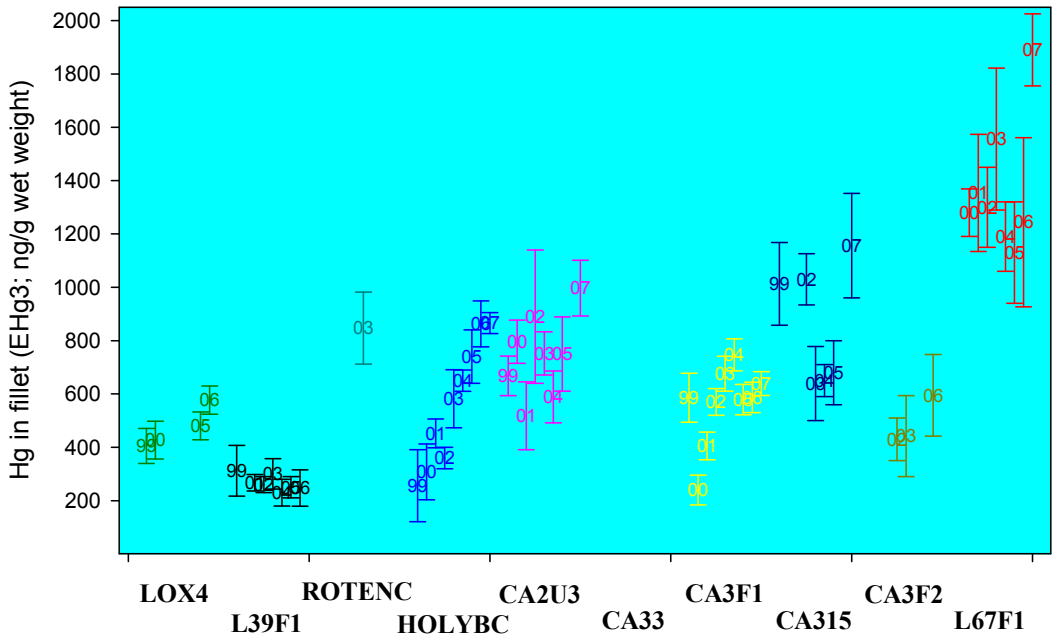

Figure 13. Age standardized (three years old) expected Hg concentration (EHg3) in largemouth bass (*Micropterus salmoides*) collected at downstream sites from 1999–2007. EHg3 was not calculated if regressions were not significant or if age distributions were narrow (see **Table 8**).

In 2007, LMB exhibited spatial patterns in tissue Hg concentrations similar to those observed in sunfish, with higher levels generally being found at the southern sites (**Table 8** and **Figure 13**). Because of a statistically significant interaction between location and age ($f = 5.1$; $df = 8$, $p < 0.001$), ANCOVA could not be used to assess differences in Hg levels among all sites.

Based on **Figure 13,** the most apparent increasing trends occur at CA3F1 and the Holey Land WMA (Rumbold, 2005; Rumbold et al., 2006; Gabriel et al;, 2007). ANCOVA was used once again to statistically validate the temporal trends in Hg levels in LMB from CA3F1 for the POR. However, due to the significant interaction between time (date) and age ($f = 10.6$, $df = 8.0$, and $p = < 0.001$), the impact of time could not be assessed. At the Holey Land WMA site (HOLYBC) the effect of time on THg levels could not be evaluated due to the insignificant interaction between age and date ($df = 8$, $f = 1.4$, $p = 0.20$). Accordingly, a clear statistically significant temporal increase in concentration is present ($df = 8$, $f = 5.0$, $p < 0.001$).

PREDATOR PROTECTION CRITERIA

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

In CY2007, 20 percent of all mosquitofish collected (considered to be at TL2 and TL3, depending on age; Loftus et al., 1998) exceeded the USEPA criterion of 77 ng/g and 13 percent exceeded the USFWS criterion of 100 ng/g. These exceedances were all from the L67F1, ROTENF1 and WCA-2-U3 stations (**Table 6**). This is large increase from 2006 where there were no exceedances of the 77 ng/g criterion for mosquitofish. Sunfish also showed an increase from 2006. For 2007, 78 percent of all sunfish, which are TL3 (*L. gulosus* at TL 4, Loftus et al., 1998), exceeded the 77 ng/g criterion, 68 percent exceeded the USFWS 100 ng/g criterion and 10 percent exceeded the EPA 346 ng/g criterion (**Table 7)**. In 2006, 73 percent of all sunfish exceeded TL3 criteria and 56 percent exceeded USFWS criteria of 100 ng/g. As discussed previously by Rumbold (2005), these findings are significant because sunfish and mosquitofish represent the preferred prey item of many fish-eating species in the Everglades. All largemouth bass collected at stations WCA-2-U3, L67F1 and HOLYBC, which accounts for 41 percent of all LMB collected, exceeded the guidance value for TL4 fish [based on the following calculation: LMB (where whole body THg concentration) = 0.695 x fillet THg; (Lange et al., 1998)]. Four percent of all bass exceeded the Florida Department of Health's human "no consumption" advisory of 1,500 ng/g, all of which were all collected from site L67F1. In 2006, 46 percent of all LMB exceeded TL4 criteria. Based on 2007 findings, certain Everglades populations of fish-eating avian and mammalian wildlife continue to be at risk of adverse effects from mercury exposure depending on where they forage.

WADING BIRD FEATHERS FROM EVERGLADES CONSTRUCTION PROJECT INTERIOR MARSHES

In early 2008 (April 30 and May 16), the District's Water Quality Monitoring Division attempted to collect chick Great Egret feather samples at the L67, Cypress City, in addition to Alley North (**Table 9**) on three separate occasions. However, collection was not successful due to lack of nesting. But, researchers at the University of Florida were able to make two collections at the Cypress City colony (**Table 9**). Despite the heterogeneity in sampling methods, locations, and sample quantities from year to year, there is an apparent decline in mercury concentrations within great egret chicks for the period of record (1994 through 2008), thus suggesting a decrease in mercury exposure (**Table 9**).

Establishing a benchmark for critical feather THg concentration has been difficult because of observed or suspected interspecies differences in mercury sensitivity, particularly between piscivores and nonpiscivores and between freshwater birds and seabirds. However, Bouton et al. (1999) and Spalding et al. (2000) reported results of a controlled dosing study that combined feather analysis with toxicological observations of great egrets. Great egret juveniles were dosed with MeHg-containing gelatin capsules at 0.5 mg Hg/kg food (n = 5) and were found to have subtle behavioral changes and statistically significant differences in blood chemistry, liver biochemistry, and weight index (Bouton et al., 1999; Spalding et al., 2000). At five weeks, chicks in this dose group had 19 μg/g THg in feathers and showed a significant decline in packed cell volume (i.e., lowest observed effects level) (Spalding et al., 2000).

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 and, as a consequence, their potential exposure to mercury (utilizing the Electronic Databases for State Employees at http://dlis.dos.state.fl.us/cgi-bin/services/index.cfm). No new reports in 2007 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 2B).

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 (two to three weeks old) at colonies within WCA-3A.

Year	JW1	L67	Cypress City	Alley North
1994[1,2]	21±6 (25±8,9)	16±4 (NA)	NS	NS
1995[2]	14 ± 3 (N/A±8)	16 ± 6 (16±6,14)	NS	NS
1999	7 ± 1 (4 ± 2,13)	NC (4±2,20)	NS	NS
2000	7 ± 1 (3±2,10)	NC (3±1,10)	NS	NS
2001	Failed to initiate nesting	NC (7±3,13)	NS	NS
2002	Colony abandoned	NC (2±0.5,6)	NS	NS
2003	Failed to initiate nesting	NC (5±2,3)	NC (6±2,15)	NS
2004	Failed to initiate nesting	4 ± 2 (1±1,10)	5 ± 2 (2±1,10)	NS
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
2008[3]	NS	NA	NC 0.2, 2	NA

[1]Concentrations standardized to a bill length of 5.6 centimeters (cm)
[2]Data from P. Frederick et al. (1997)
[3]Data from P. Frederick, 2008, University of Florida, unpublished results
NA – Data 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, 19 days old in 2007, and 28 days old in 2008

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 Everglades Forever Act 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 submitted an updated non-ECP mercury monitoring plan for approval by the FDEP.

On April 18, 2008, modification requests were approved and the updated non-ECP was issued. Specific changes to the non-ECP monitoring as a result of the modification are summarized below, as reflected in this appendix. For updates on the ECP monitoring program, refer to Appendix 5-4 of this volume.

DOWNSTREAM FISH MONITORING (PROGRAM HGFS):

- Monitoring eliminated at stations L39F1, Z4, and RotenF1
- Station CA33 was officially replaced with CA33ALT (this station has been sampled as an alternate to CA33 since 2004)

DOWNSTREAM SURFACE WATER MONITORING (PROGRAM HGLE):

- Site S12A changed to S12D (on October 1, 2007)
- Surface water mercury sampling removed from stations S5A, S9, S10C, S12D, S140, S141, S151, S38B, and S190 (on April 18, 2008)
- Site S12D changed from project code HGLE to PIN (on April 18, 2008)

ATMOSPHERIC MERCURY DEPOSITION (NADP/MDN):

- Station FL04 was last sampled on October 17, 2006, and FL97 was first sampled on November 14, 2006; FL34 was moved to the ground on March 3, 2006

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Attachment:

Statistical Analysis and Summary of HgRR8 Mercury Round Robin Data

Xu-Feng Niu[1] and Andrew Tintle[2]

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[1] Department of Statistics, Florida State University, Tallahassee, FL

[2] Florida Department of Environmental Protection, Tallahassee, FL

Statistical Analysis and Summary of the HgRR8 Mercury Round Robin Data

Xu-Feng Niu[1] and Andrew Tintle[2]

[1] *Department of Statistics*
Florida State University
Tallahassee, FL 32306

[2]*Florida Department of Environmental Protection*
2600 Blair Stone Road, MS 6511
Tallahassee, FL 32399

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1. Introduction

The Mercury Round Robin (HgRR) Inter-laboratory Comparison Program was initiated by the Florida Department of Environmental Protection (FDEP) for the purpose of assessing the comparability of total and methyl mercury data. Participating laboratories received 9 unknown samples of ambient water from the Florida Everglades for analysis of total and/or methyl mercury.

One-way layout linear models were used in mercury Round Robin data analysis (Lin and Niu, 1998). Let Y_{ij} be the testing result of the *i*th laboratory on the *j*th replicates at a given testing site. The linear model has the form:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}, \quad i = 1, \cdots, p_*; \quad j = 1, \cdots, r, \tag{1}$$

where p_* is the number of participating laboratories without any outliers, and r is the number of replicates from each laboratory. The sample size is $n = p_* \times r$. The random errors ε_{ij} are assumed to be independently and normally distributed with mean zero and variance σ^2. The parameters in model (1) are denoted as $\beta = (\mu, \alpha_1, \cdots, \alpha_{p_*-1})'$.

It is quite often that the parameter estimates in a linear model are highly influenced by testing results from few laboratories. Lin and Niu (1998) suggested assessing the influence of each laboratory by its *Cook-Weisberg distance* (Cook and Weisberg 1980). Specifically, the *Cook-Weisberg distance* for assessing the influence of the *i*th laboratory is

$$D_I = \frac{(\hat{\beta}_I - \hat{\beta})'(X'X)(\hat{\beta}_I - \hat{\beta})}{p_* s_*^2} = \frac{r(\overline{Y}_{i\bullet} - \overline{Y}_{\bullet\bullet})^2}{p_* s_*^2}, \tag{2}$$

where $\hat{\beta}$ is the vector-parameter estimate in the linear model based on testing results from the p_* laboratories, $\hat{\beta}_I$ is the vector-parameter estimate without using the testing results from the *i*th laboratory, and s_*^2 is the sample variance of the experimental error terms calculated based on the residuals from laboratories without outliers. If the *Cook-Weisberg distance* for a laboratory is large, the parameter estimates in the linear model are highly influenced by the testing results from this laboratory. Under the normality assumption for model (1), D_I can be compared to the *F*-distribution with p_* and n-p_* degrees of freedom.

When the number of testing laboratories without any outliers is over 10, the scoring system recommended by Lin and Niu (1998) claims that the results from a laboratory are *highly influential* if its *Cook-Weisberg distance is large than 3*. Niu and Tintle (2003) studied this scoring system for small samples and pointed out that when the number of participating laboratories is less than 10, the scoring system needs to be modified.

Specifically, Niu and Tintle (2003) recommend the following two scoring systems for the analysis of environmental laboratory testing data. The two systems are based on the number of testing laboratories without any outliers.

Table A1. Rating of Laboratory by Site based on the absolute *t*-value or the C-W distance when the number of testing laboratories without any outliers is 10 or more

Rating	Absolute *t*-value or C-W distance
5 (Very Good)	0.00 to 2.00
4 (Good)	2.01 to 4.00
3 (Satisfactory)	4.01 or larger
2 (Questionable)	**C-W distance between 3.00 and 10.00**
1 (Poor)	C-W distance larger than 10.00
0 (Unacceptable)	With one or more outliers

Table A2. Rating of Laboratory by Site based on the absolute *t*-value or the C-W distance when the number of testing laboratories without any outliers is less than 10

Rating	Absolute *t*-value or C-W distance
5 (Very Good)	0.00 to 2.00
4 (Good)	2.01 to 4.00
3 (Satisfactory)	4.01 or larger
2 (Questionable)	**C-W distance between 5.00 and 10.00**
1 (Poor)	C-W distance larger than 10.00
0 (Unacceptable)	With one or more outliers

In this report, we present the statistical analysis and summary of the HgRR8 Mercury Round Robin data set. Three sites, G310, G335, and S6, were selected for sampling surface water. For each of the three sites, three replicates were provided to each participating laboratory. Fifteen participating laboratories provided total mercury (ng/L) results while eight laboratories reported methyl mercury (ng/L) measurements.

Similar to the HgRR Mercury Round Robin data analyses performed by Niu and Tintle (2004a, 2004b, 2005, 2006), the first scoring system in Table A1 will be applied to the total mercury data and the second scoring system specified in Table A2 will be used for the Methyl Mercury data analysis in this study.

2. HgRR8 Mercury Round Robin Analysis and Scores (Total Mercury)

a). Results for the Three Sites

HgRR8-Table 1. ANOVA Summary Table and Laboratory Performance
For the Total Mercury Results at Site G310

ANOVA Summary Table
(Labs H, J, and N are excluded)

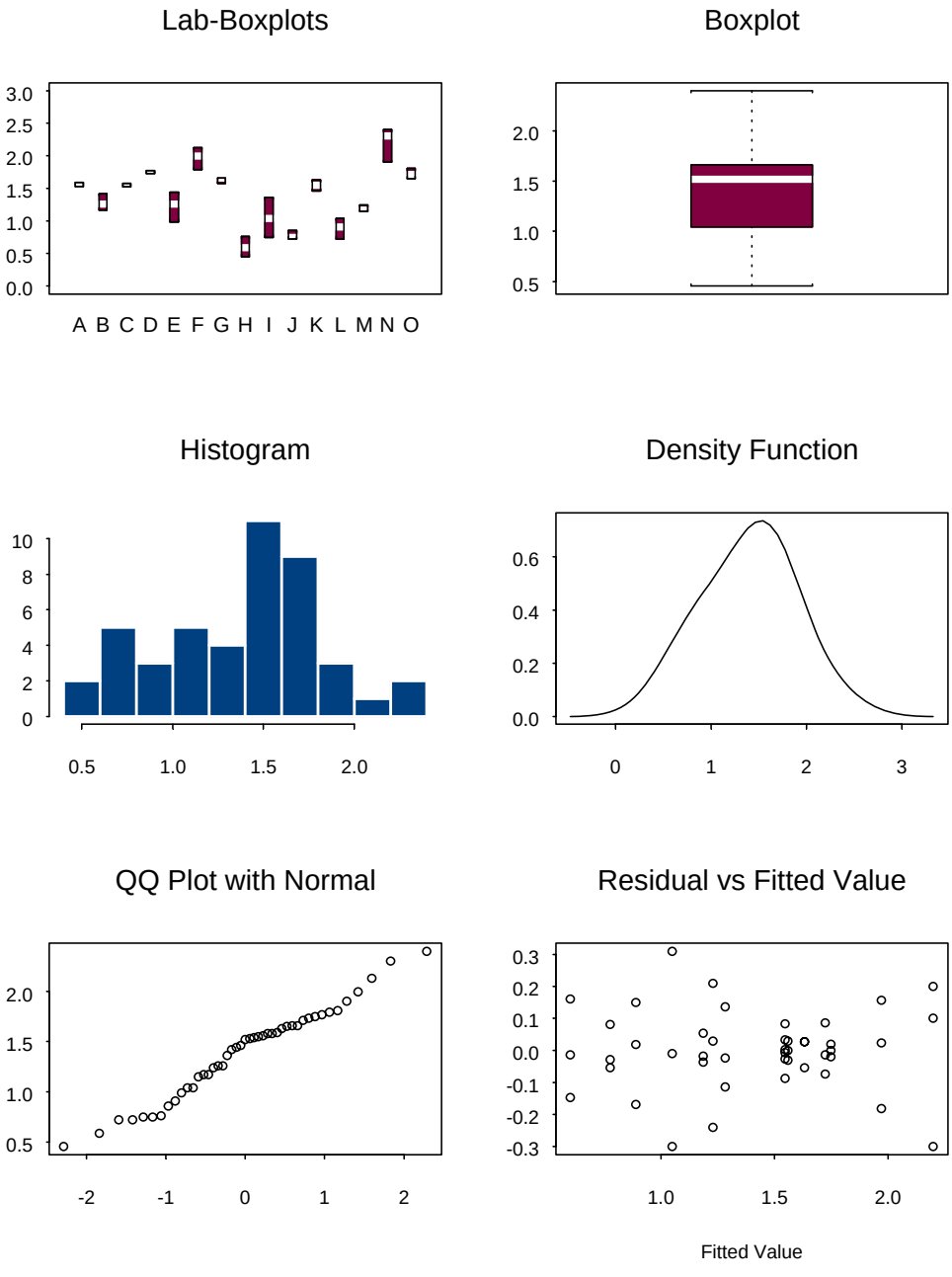
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	11	3.359	0.305	15.58	2.34×10^{-8}
Residuals	24	0.470	0.0196		

Consensus Mean 1.448

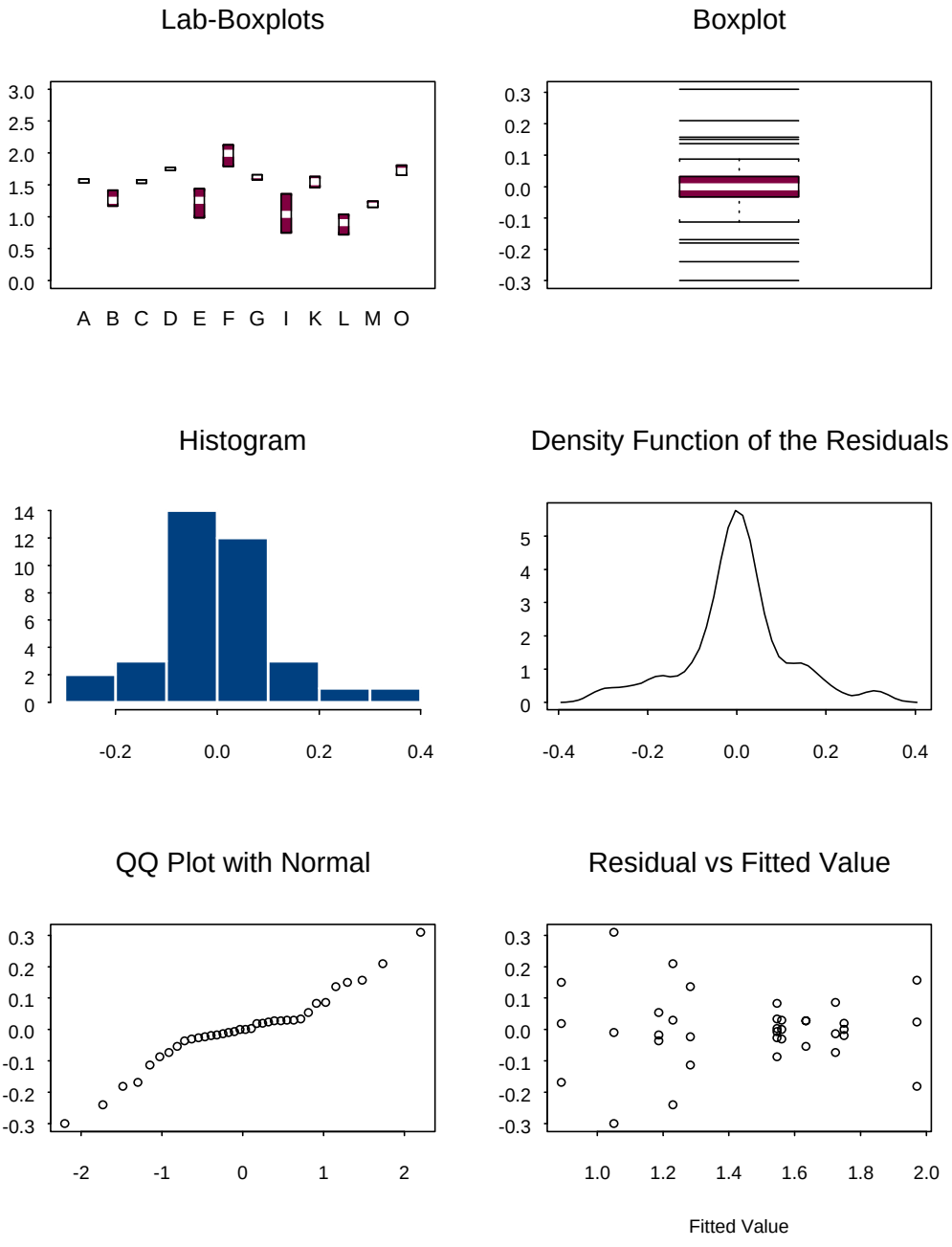
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	1.560	0.240	1.45	5
Lab-B	1.283	0.115	-2.12	4
Lab-C	1.547	0.202	1.28	5
Lab-D	1.750	1.121	3.91	4
Lab-E	1.230	0.249	-2.81	4
Lab-F	1.971	2.966	6.77	3
Lab-G	1.633	0.503	2.40	4
Lab-H	0.601	5.689	Highly Influential	2
Lab-I	1.050	1.079	-5.14	3
Lab-J	0.777	3.448	Highly Influential	2
Lab-K	1.547	0.202	1.28	5
Lab-L	0.890	2.302	-7.20	3
Lab-M	1.187	0.396	-3.37	4
Lab-N	2.200	5.796	Highly Influential	2
Lab-O	1,723	0.959	3.56	4

HG8-Figure 1a. Exploratory Analysis Plots for Site G310, (Total Mercury, Original Measurements)



HG8-Figure 1b. Residual Plots for Site G310, (Labs H, J, and N are excluded)



HgRR8-Table 2. ANOVA Summary Table and Laboratory Performance
For the Total Mercury Results at Site G335

ANOVA Summary Table
(Labs F, H, I, J, and N are excluded)

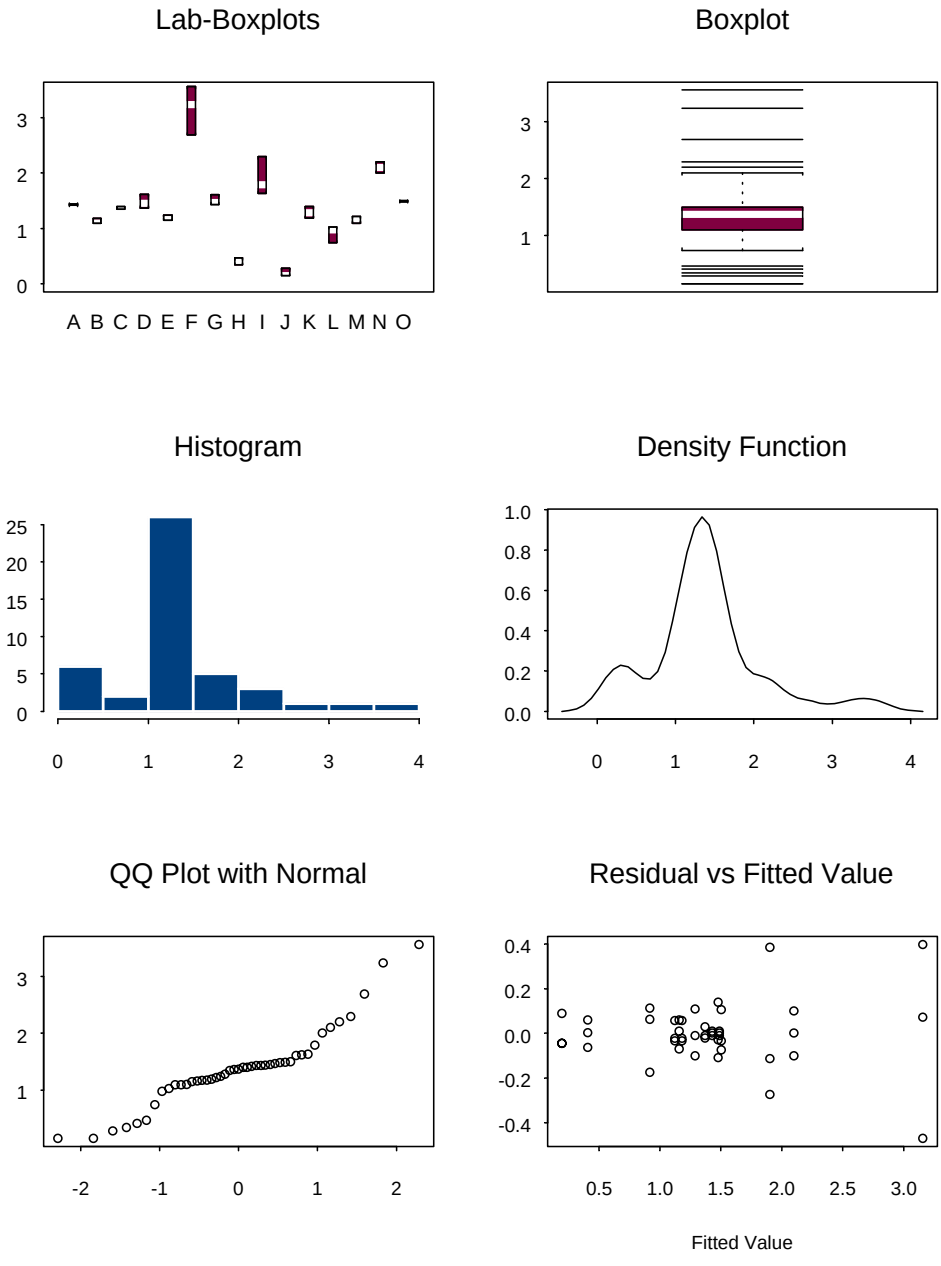
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	9	1.026	0.114	16.31	2.25×10^{-7}
Residuals	20	0.140	0.007		

Consensus Mean 1.295

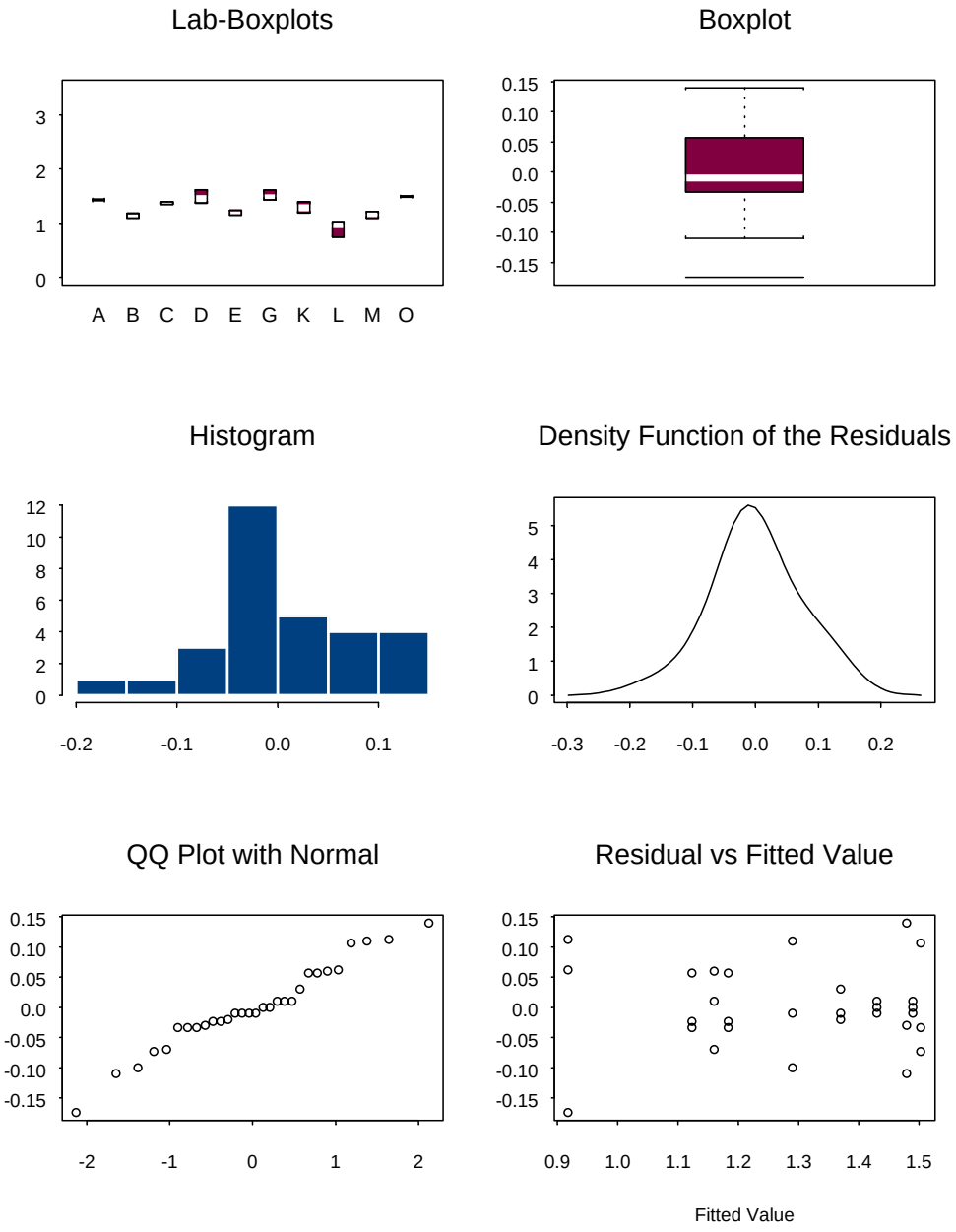
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	1.430	1.708	2.95	4
Lab-B	1.123	0.217	-3.74	4
Lab-C	1.370	0.922	1.64	5
Lab-D	1.480	2.548	4.04	3
Lab-E	1.183	0.014	-2.43	4
Lab-F	3.159	With	Outliers	0
Lab-G	1.503	2.996	4.55	3
Lab-H	0.408	21.169	Extremely Influential	1
Lab-I	1.903	With	Outliers	0
Lab-J	0.195	34.016	Extremely Influential	1
Lab-K	1.290	0.248	-0.104	5
Lab-L	0.918	2.738	-8.23	3
Lab-M	1.160	0.064	-2.94	4
Lab-N	2.100	26.835	Extremely Influential	1
Lab-O	1.490	2.736	4.26	3

HG8-Figure 2a. Exploratory Analysis Plots for Site G335, (Total Mercury, Original Measurements)



HG8-Figure 2b. Residual Plots for Site G335,
(Labs F, H, I, J, and N are excluded)



HgRR8-Table 3. ANOVA Summary Table and Laboratory Performance
For the Total Mercury Results at Site S6

ANOVA Summary Table
(Labs D, H, I, J, L, and N are excluded)

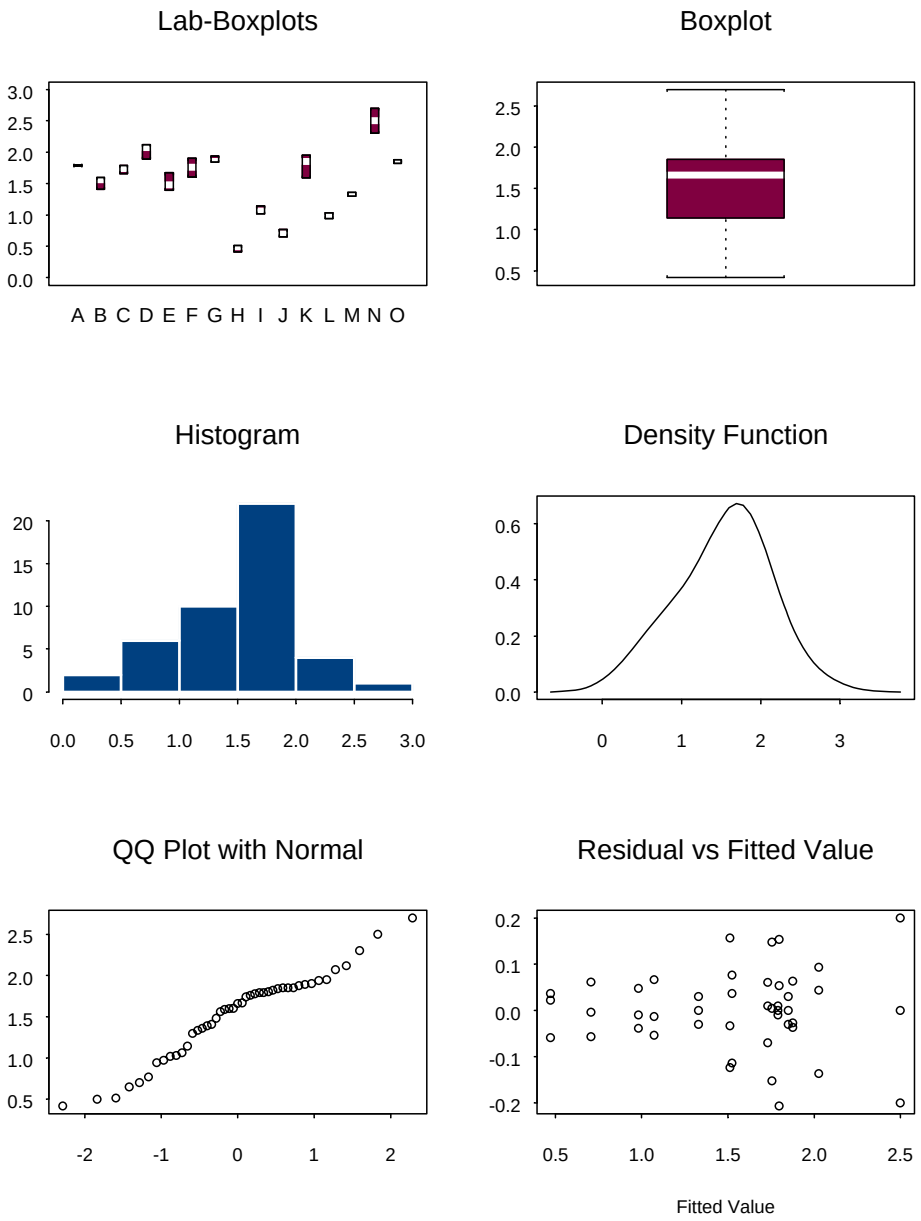
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	8	0.828	0.104	9.65	0.0000389
Residuals	18	0.193	0.011		

Consensus Mean 1.685

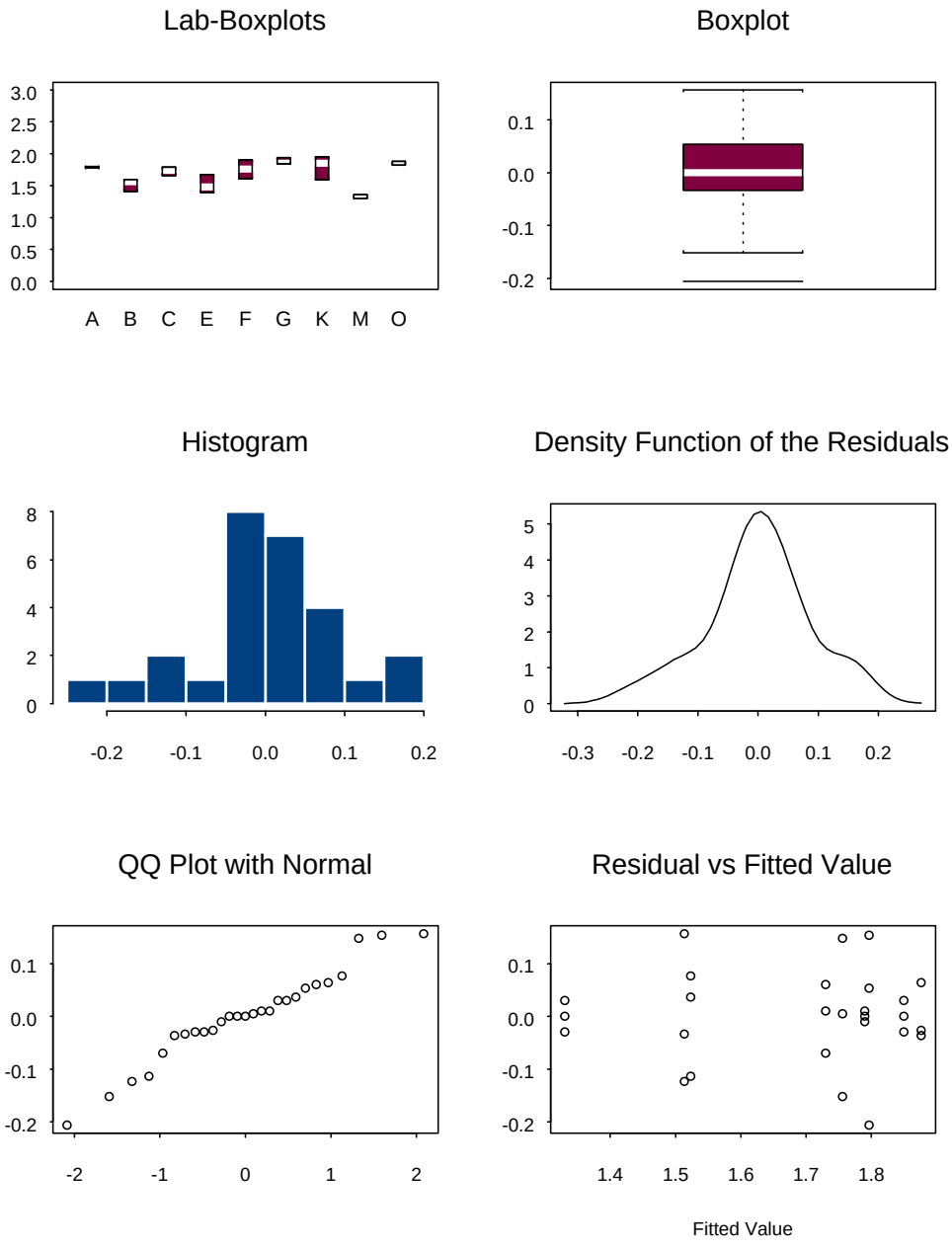
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	1.790	1.257	1.86	5
Lab-B	1.523	0.0005	-2.87	4
Lab-C	1.730	0.746	0.80	5
Lab-D	2.027	4.564	Highly Influential	2
Lab-E	1.513	0.004	-3.05	4
Lab-F	1.756	0.951	1.26	5
Lab-G	1.877	2.229	3.40	4
Lab-H	0.474	20.465	Extremely Influential	1
Lab-I	1.073	3.814	Highly Influential	2
Lab-J	0.707	12.421	Extremely Influential	1
Lab-K	1.797	1.322	1.98	5
Lab-L	0.982	5.491	Highly Influential	2
Lab-M	1.330	0.726	-6.30	3
Lab-N	2.500	17.362	Extremely Influential	1
Lab-O	1.850	0.959	2.92	4

HG8-Figure 3a. Exploratory Analysis Plots for Site S6,
(Total Mercury, Original Measurements)



HG8-Figure 3b. Residual Plots for Site S6,
(Labs D, H, I, J, L, and N are excluded)



b). Summary Results for Total Mercury

Results on total mercury data from the three sites are presented in Table 4a. The fifth and sixth columns in the table show the total scores and average scores for the fifteen participating laboratories. Five laboratories, A, B, C, E, and K, had average scores 4 or above. The codes of the participating laboratories for the HgRR8 Mercury Round Robin exercises are given in Table 4b.

For each participating laboratory, a *t*-value is calculated for each site based on the average measurement with respect to the consensus mean value of that site. A boxplot is constructed for each laboratory using its *t*-values for the three sampling sites. Figure 4a shows the *t*-values for the participating laboratories based on the total mercury results in the HgRR8 Mercury Round Robin exercise. Figure 4b plots the *t*-values within the interval (-20, 20).

The *t*-value plots are not used to evaluate the laboratories' overall performance because the rating of the laboratories is based on the presence of outliers and the Cook-Weisberg (C-W) distance in addition to the absolute *t*-values. The *t*-value plot serves the purpose of identifying systematic mean bias (high or low) with respect to the consensus mean value. For example, Figure 4a shows that the *t*-values of Laboratories B, E, H, J, L, and M (especially Labs H and J) are all below zero, which indicate that these laboratories were reporting values systematically lower than the consensus mean values at these sites. While Laboratories A, C, D, F, G, N, and O (in particular Labs F and N) tend to give systematically higher measurements than the consensus mean values.

HgRR8-Table 4a. Summary Table for Laboratory Performance
Based on Total Mercury Results

Lab	G310 [a]	G335	S6	Total-Score [b]	Average
Lab-A	5	4	5	14.0	4.67
Lab-B	4	4	4	12.0	4.00
Lab-C	5	5	5	15.0	5.00
Lab-D	4	3	2	9.0	3.00
Lab-E	4	4	4	12.0	4.00
Lab-F	3	0	5	8.0	2.67
Lab-G	4	3	4	11.0	3.67
Lab-H	2	1	1	4.0	1.33
Lab-I	3	0	2	5.0	1.67
Lab-J	2	1	1	4.00	1.33
Lab-K	5	5	5	15.0	5.00
Lab-L	3	3	2	8.0	2.67
Lab-M	4	4	3	11.0	3.67
Lab-N	2	1	1	4.0	1.33
Lab-O	4	3	4	11.0	3.67

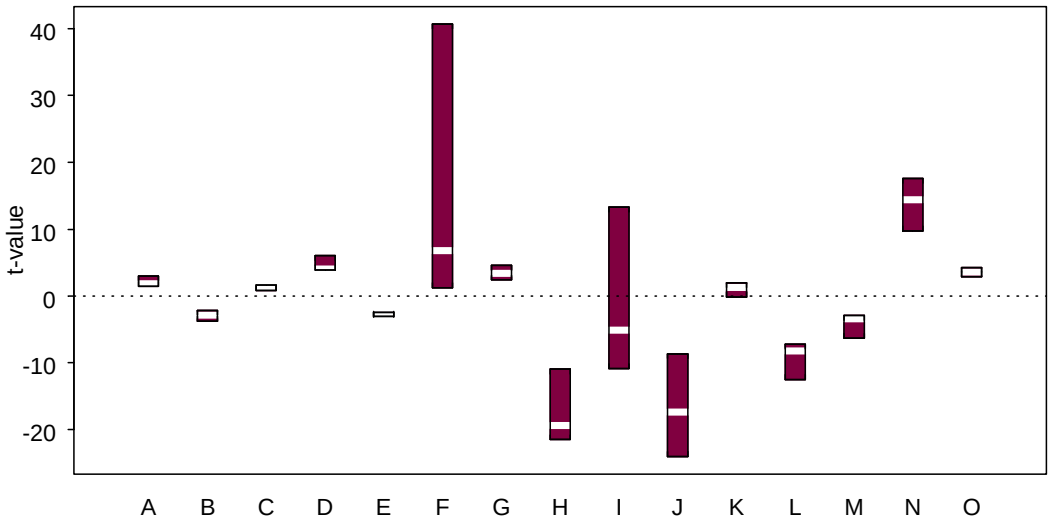
a. The 5-point scoring scale defined in Table A1 is used to assess a laboratory's performance on each site of HgRR8, with 5.0 = *the best* and 0.0 = *the worst* scores.
b. The total score for a participating laboratory over the three sites with 15.0 = *the highest* and 0.0 = *the lowest* scores.

HgRR8-Table 4b. Total Mercury Participating Laboratory Names

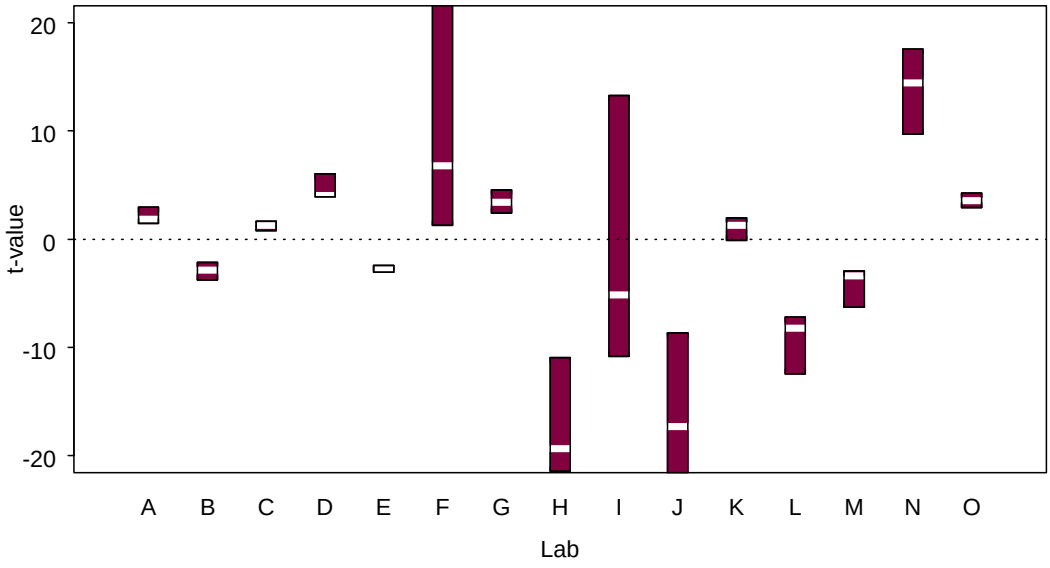
Real Name	**Name Used in the Analysis**
CEBAM Analytical	Lab-A
Flemish Institute for Technological Research VITO	Lab-B
IVL Swedish Environmental Research Institute	Lab-C
Brooks Rand LLC	Lab-D
Frontier Geosciences	Lab-E
Florida State Univ-Department of Oceanography	Lab-F
Battelle Marine Science Laboratory	Lab-G
NC Dept. of Environmental and Natural Resources	Lab-H
Florida International University	Lab-I
Jupiter Environmental Laboratories, Inc	Lab-J
USGS - Middleton	Lab-K
City of Portland	Lab-L
Institute Jozef Stefan	Lab-M
FL Dept. of Environmental Protection	Lab-N
City of San Jose	Lab-O

HgRR8-Figure 4. Boxplots of t-values at the three sites (Total Mercury)

a). All the t-values are plotted



b). Only t-values within (-20, 20) are plotted



3. HgRR8 Mercury Round Robin Analysis and Scores (Methyl Mercury)

a). Results for the Three Sites

HgRR8-Table 5. ANOVA Summary Table and Laboratory Performance
For the Methyl Mercury Results at Site G310

ANOVA Summary Table
(Labs E and G are excluded)

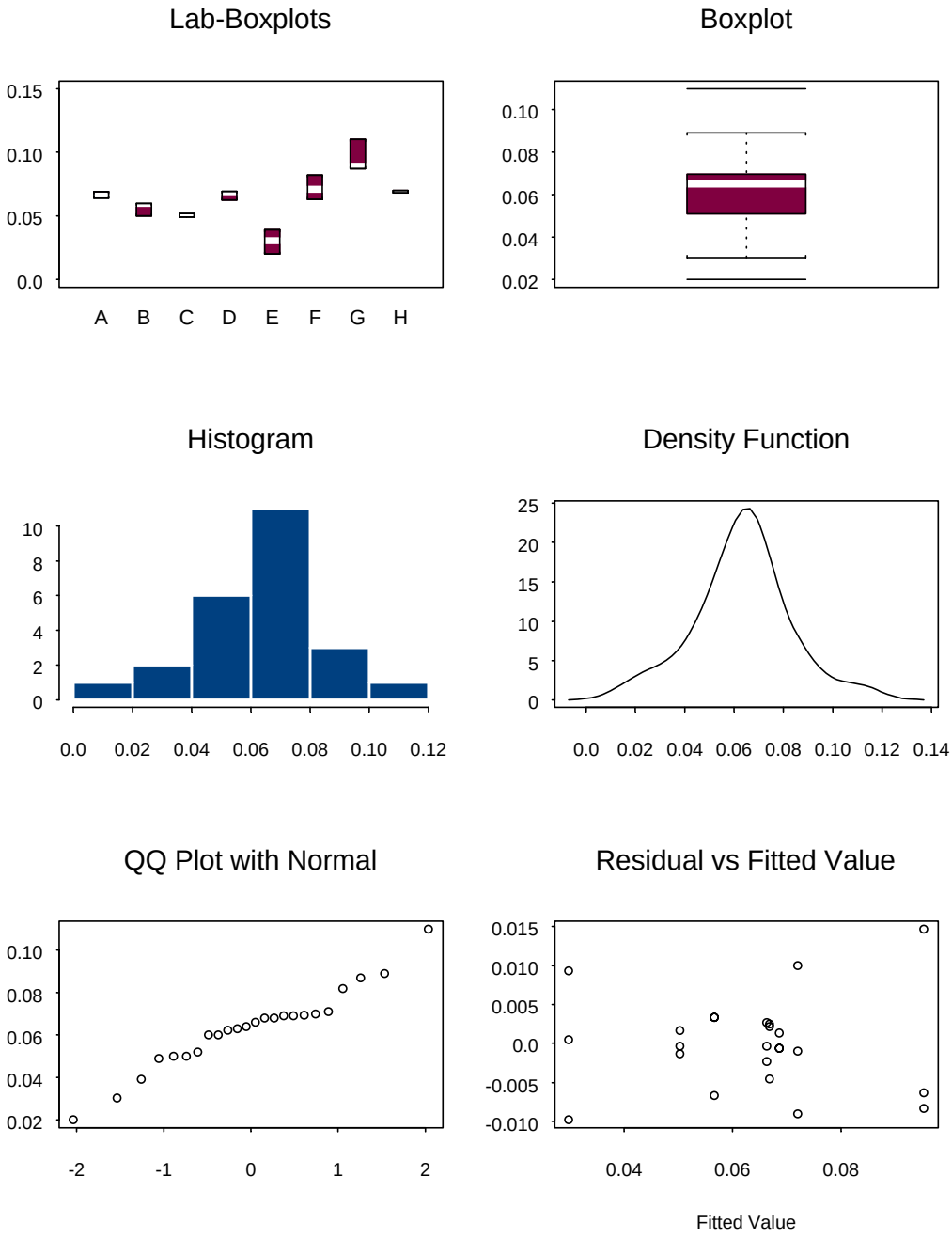
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	5	0.00102	0.000203	8.12	0.00149
Residuals	12	0.00030	0.000025		

Consensus Mean 0.0635

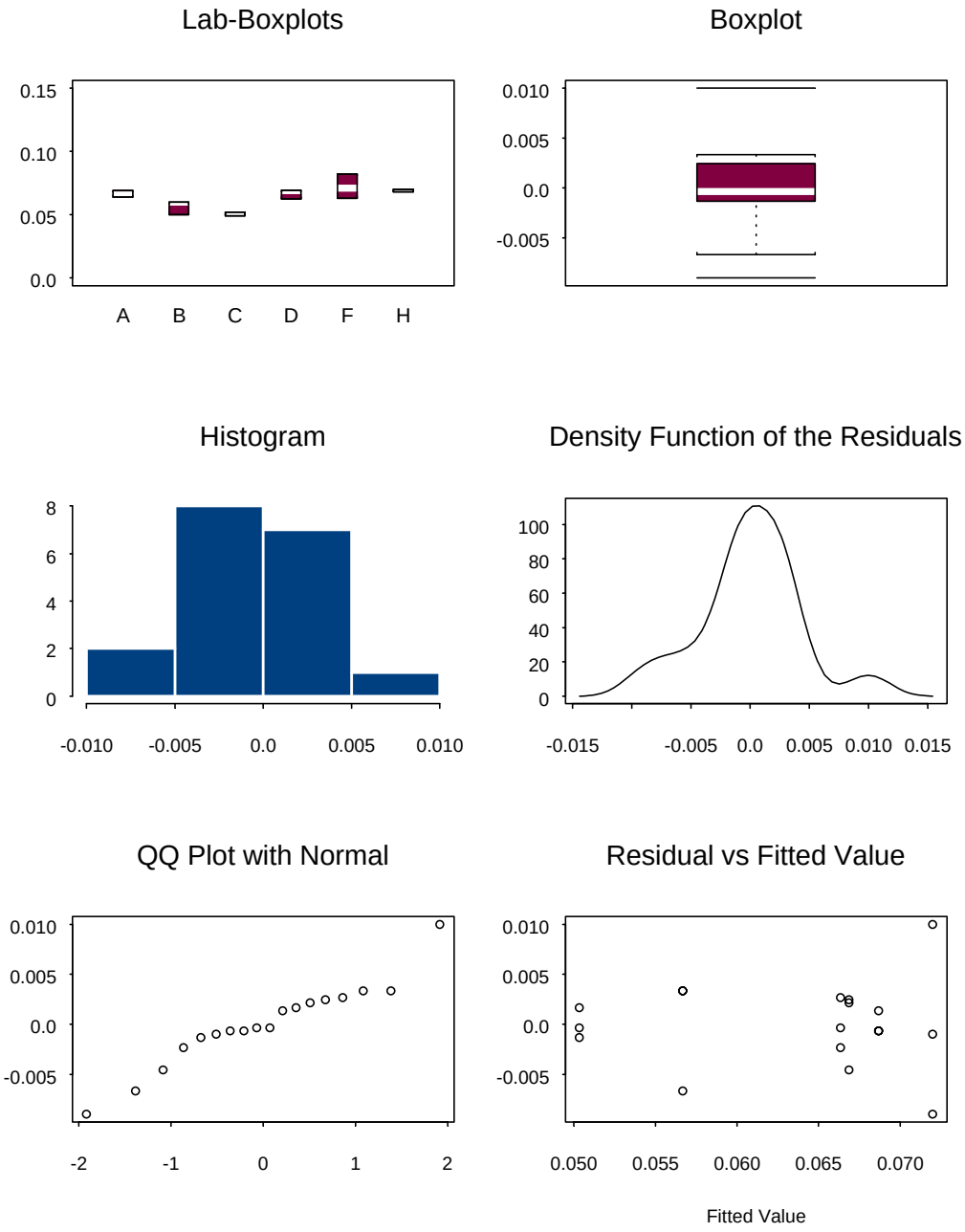
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	0.066	0.07	1.08	5
Lab-B	0.057	0.32	-2.58	4
Lab-C	0.050	1.24	-4.99	3
Lab-D	0.067	0.10	1.29	5
Lab-E	0.030	8.31	Highly Influential	2
Lab-F	0.072	0.57	3.23	4
Lab-G	0.095	7.65	Highly Influential	2
Lab-H	0.069	0.22	1.97	5

HG8-Figure 5a. Exploratory Analysis Plots for Site G310, (Methyl Mercury, Original Measurements)



HG8-Figure 5b. Residual Plots for Site G310, (Labs E and G are excluded)



HgRR8-Table 6. ANOVA Summary Table and Laboratory Performance
For the Methyl Mercury Results at Site G335

ANOVA Summary Table
(No Lab is excluded)

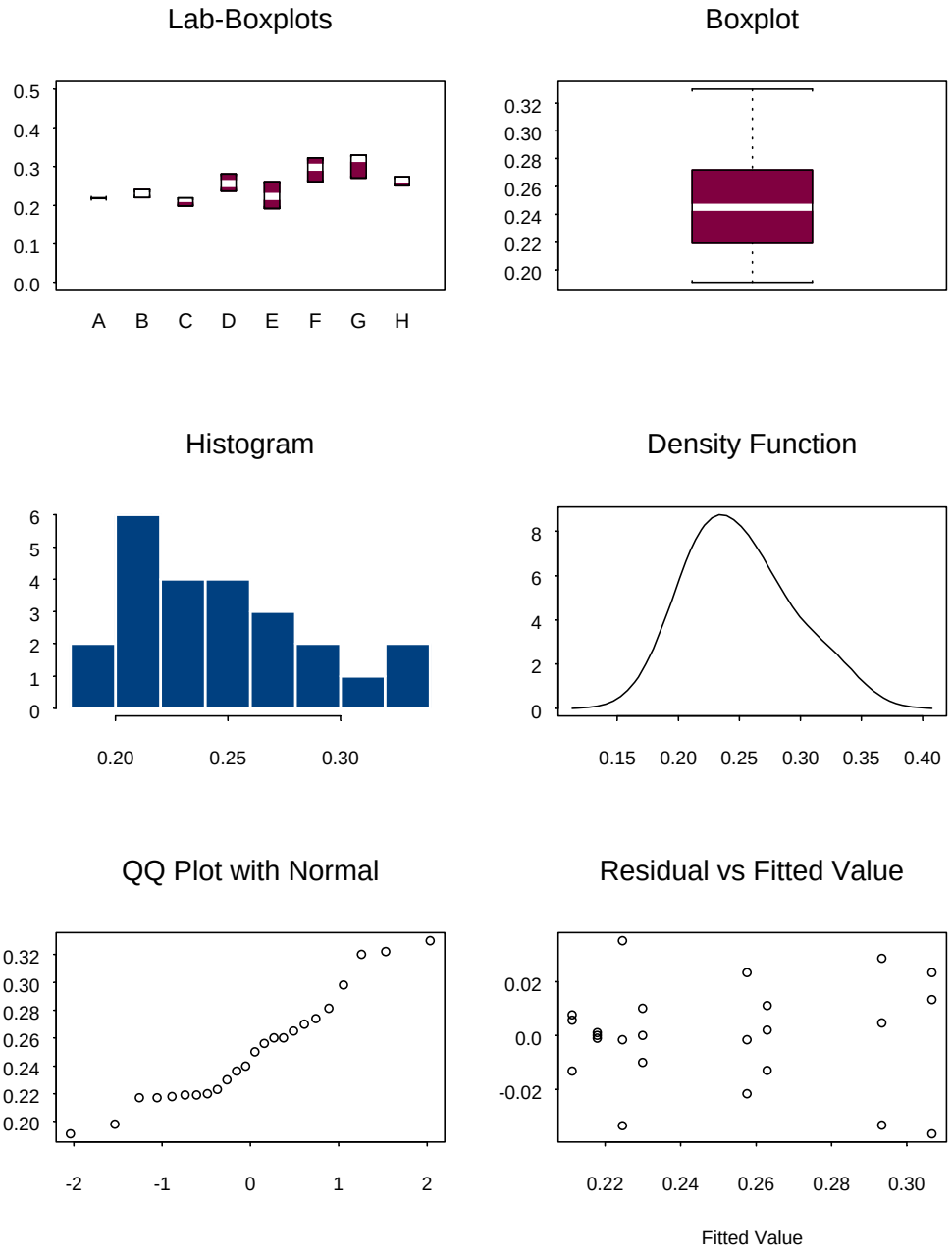
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	7	0.0266	0.0038	7.43	0.00046
Residuals	16	0.0082	0.00051		

Consensus Mean 0.251

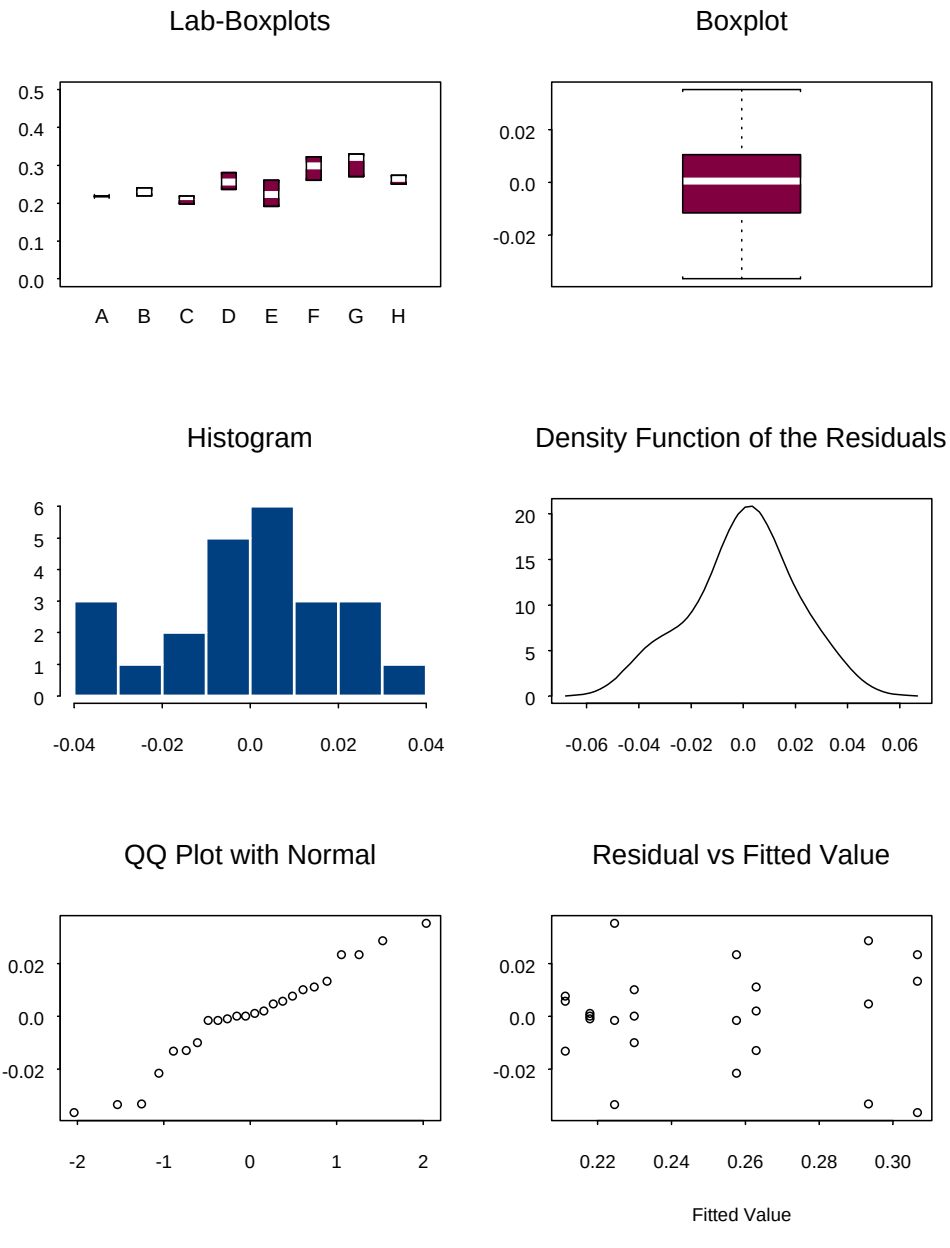
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	0.218	0.778	-2.67	4
Lab-B	0.230	0.311	-1.68	5
Lab-C	0.211	1.129	-3.21	4
Lab-D	0.258	0.037	0.58	5
Lab-E	0.225	0.492	-2.12	4
Lab-F	0.293	1.339	3.50	4
Lab-G	0.307	2.305	4.59	3
Lab-H	0.263	0.113	1.02	5

HG8-Figure 6a. Exploratory Analysis Plots for Site G335, (Methyl Mercury, Original Measurements)



HG8-Figure 6b. Residual Plots for Site G335,
(No Lab is excluded)



HgRR8-Table 7. ANOVA Summary Table and Laboratory Performance
For the Methyl Mercury Results at Site S6

ANOVA Summary Table
(Labs F and G are excluded)

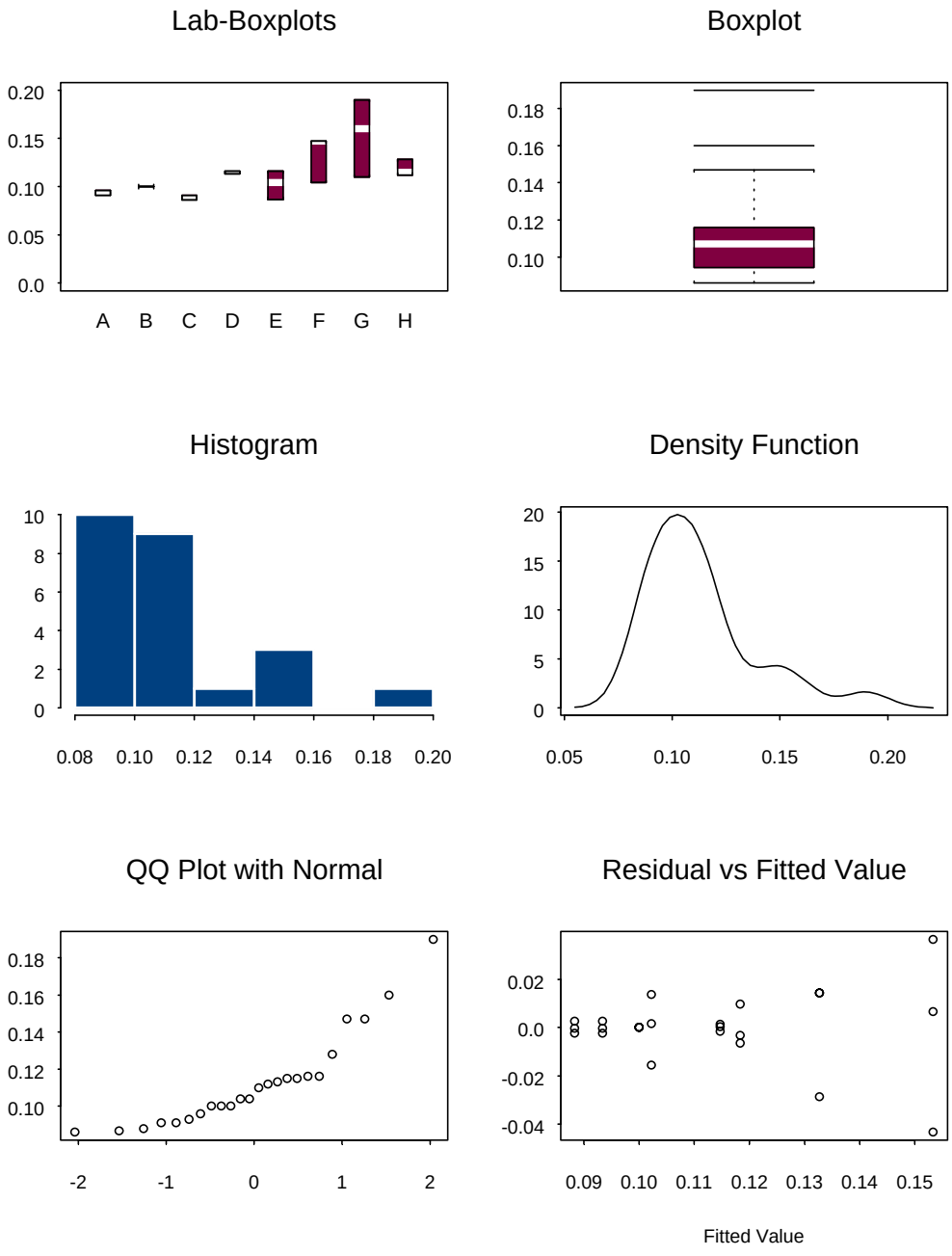
	Df	Sum of Squares	Mean Squares	F-value	p-value
Laboratory	5	0.00207	0.00041	8.15	00147
Residuals	12	0.00061	0.000051		

Consensus Mean 0. 1028

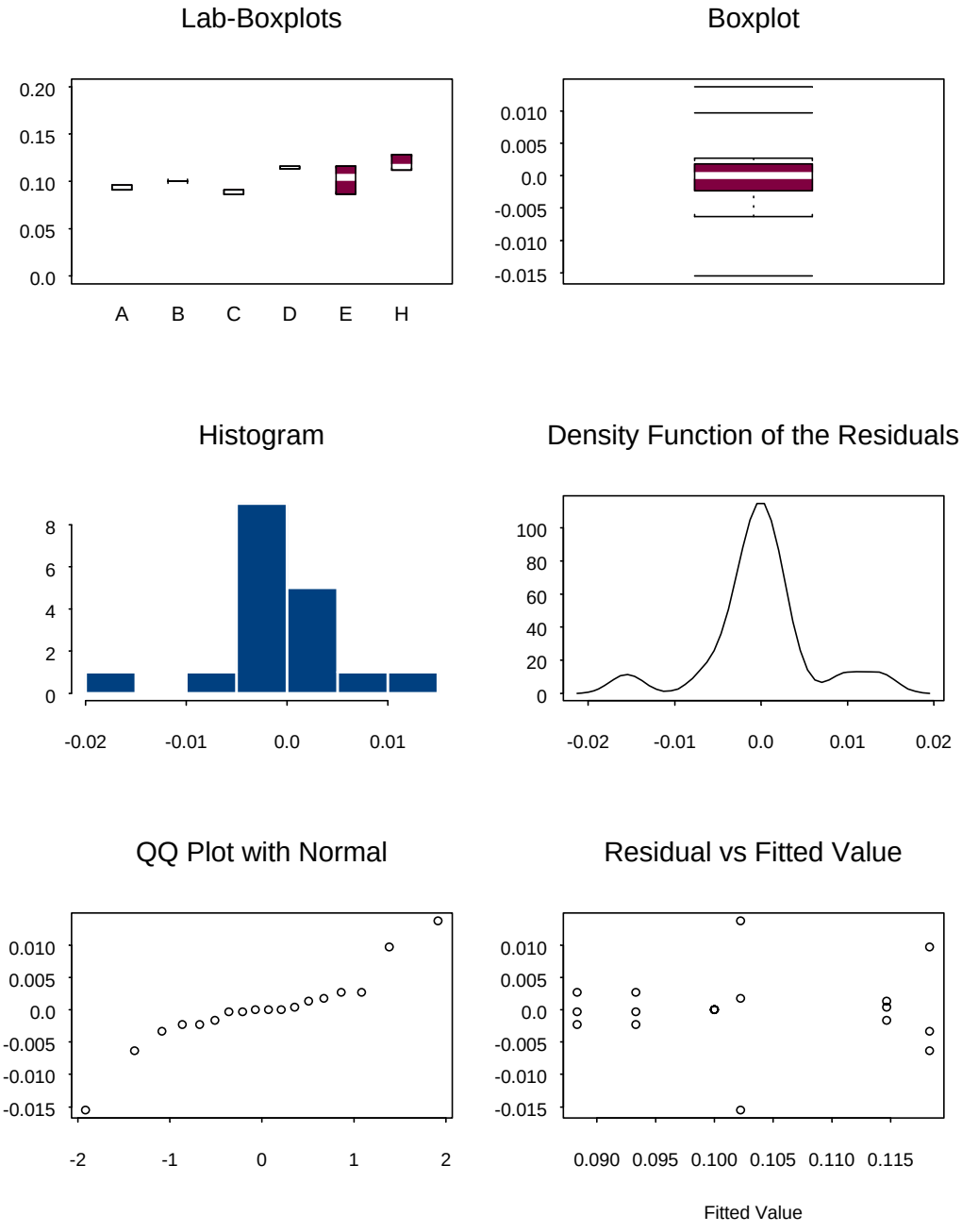
Laboratory Performance

Lab	Mean	C-W Distance	t-value	Score
Lab-A	0.093	0.887	-2.53	4
Lab-B	0.100	0.078	-0.75	5
Lab-C	0.088	2.068	-3.86	4
Lab-D	0.115	1.384	3.16	4
Lab-E	0.102	0.003	-0.16	5
Lab-F	0.133	With	Outliers	0
Lab-G	0.153	With	Outliers	0
Lab-H	0.118	2.374	4.13	3

HG8-Figure 7a. Exploratory Analysis Plots for Site S6, (Methyl Mercury, Original Measurements)



HG8-Figure 7b. Residual Plots for Site S6,
(Labs F and G are excluded)



b). Summary Results for Methyl Mercury

Results on Methyl mercury data from the three sites are presented in Table 8a. The fifth and sixth columns in the table show the total scores and average scores for the nine participating laboratories. Four laboratories A, B, D, and H, had average scores 4 or above. The codes of the participating laboratories for the HgRR8 Methyl Mercury Round Robin exercises are given in Table 8b.

For each participating laboratory, a *t*-value is calculated for each site based on the average measurement with respect to the consensus mean value of that site. A boxplot is constructed for each laboratory using its *t*-values for the three sampling sites. Figures 8a and 8b show the *t*-values for the participating laboratories based on the methyl mercury results in the HgRR8 Methyl Mercury Round Robin exercise.

The *t*-value plots are not used to evaluate the laboratories' overall performance because the rating of the laboratories is based on the presence of outliers and the Cook-Weisberg (C-W) distance in addition to the absolute *t*-values. The *t*-value plot serves the purpose of identifying systematic mean bias (high or low) with respect to the consensus mean value. For example, Figure 8a shows that the *t*-values of laboratories D, F, G, and H are all above zero, which indicates that these laboratories were reporting values systematically higher than the consensus mean values at the sites. On the other hand, laboratories B and C tend to give systematically lower measurements than the consensus mean values.

HgRR8-Table 8a. Summary Table for Laboratory Performance
Based on Methyl Mercury Results

Lab	G310[c]	G335	S6	Total-Score[d]	Average
Lab-A	5	4	4	13.0	4.33
Lab-B	4	5	5	14.0	4.67
Lab-C	3	4	4	11.0	3.67
Lab-D	5	5	4	14.0	4.67
Lab-E	2	4	5	11.0	3.67
Lab-F	4	4	0	8.0	2.67
Lab-G	2	3	0	5.0	1.67
Lab-H	5	5	3	13.0	4.33

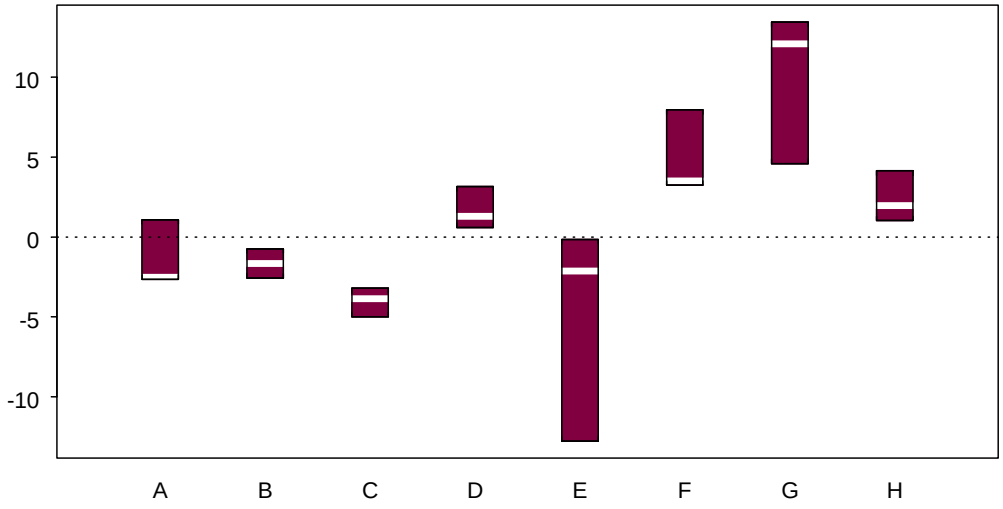
c. The 5-point scoring scale defined in Table A2 is used to assess a laboratory's performance on each site of HgRR8, with 5.0 = *the best* and 0.0 = *the worst* scores.
d. The total score for a participating laboratory over the three sites with 15.0 = *the highest* and 0.0 = *the lowest* scores.

HgRR8-Table 8b. Methyl Mercury Participating Laboratory Names

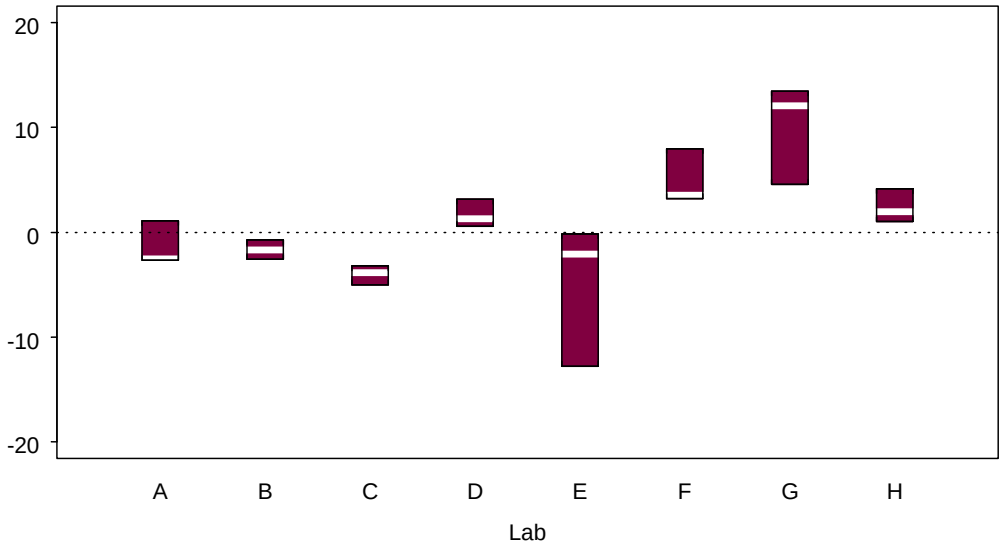
Real Name	Name Used in the Analysis
CEBAM Analytical	Lab-A
IVL Swedish Environmental Research Institute	Lab-B
Brooks Rand LLC	Lab-C
Battelle Marine Science Laboratory	Lab-D
Florida International University	Lab-E
USGS - Middleton	Lab-F
FL Dept. of Environmental Protection	Lab-G
City of San Jose	Lab-H

HgRR8-Figure 8. Boxplots of t-values at the three sites (Methyl Mercury)

a). All the t-values are plotted



b). Only t-values within (-20, 20) are plotted



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