Appendix 4-4: Annual Permit Compliance Monitoring Report for Mercury in the Stormwater Treatment Areas

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KEY FINDINGS AND OVERALL ASSESSMENT

This report summarizes data from compliance monitoring of mercury (Hg) storage, release, and biomagnification in Stormwater Treatment Areas (STAs) during Water Year 2005 (WY2005) (May 1, 2004 through April 30, 2005).

Key findings are as follows:

1. **All STAs:** There were no violations of the Florida Class III numerical water quality standard (WQS) of 12 nanograms (ng) of total mercury per liter (THg/L) during the reporting year at any of the STAs. As such, the project has met the requirements of Section 6.i of the Hg monitoring program of the referenced permits.
2. **STA-1W:** Stormwater Treatment Area 1 West (STA-1W) subsumed the Everglades Nutrient Removal (ENR) Project in April 1999; the ENR project had served as the prototype STA and had been in operation since 1994. After 10 years of operation this STA continued to have only low concentrations of both total mercury (THg) and methylmercury (MeHg) in surface water, and consistently exhibited a negative percent change in both THg and MeHg (i.e., concentrations in the outflows were consistently lower than in the inflow). Furthermore, MeHg biomagnification in resident large-bodied fishes (e.g., sunfish and largemouth bass) has remained relatively constant over the monitoring period at levels almost an order of magnitude lower than observed in fishes from the downstream Everglades. Hg levels in fish at this STA do not appear to pose a threat to fish-eating wildlife based on the U.S. Fish and Wildlife Service (USFWS) and the U.S. Environmental Protection Agency (USEPA) predator protection criteria.
3. **STA-1E**: The District began monitoring water-column concentrations of THg and MeHg on a biweekly basis at this STA in January 2005. As of April 30, 2005, the STA had not satisfied the start-up criteria that water-column concentrations in the interior marshes should not be significantly greater than the concentrations of the corresponding species in inflow samples.
4. **STA-2:** During the water year, both THg and MeHg remained at low concentration in the outflow from the STA as compared to previous years, and exhibited either a negative or very small positive percent change across the STA. Tissue-Hg levels also remained low in mosquitofish, including fish from Cell 1, as compared to other STAs and downstream marshes. Although continuing to exhibit significant among-cell differences, Hg levels in

sunfish appeared to decline in Cell 1. Hg levels also declined in largemouth bass from both the discharge canal and Cell 1 (young-of-the-year fish). Risks to fish-eating wildlife foraging at STA-2 are reported in an accompanying appendix (Appendix 4-7 of this volume).

5. **STA-3/4:** Although the newly operational STA had higher concentrations of both THg and MeHg in the outflow than the inflow (i.e., positive percent change across the STA), levels were comparable to water-column concentrations observed elsewhere. Tissue-Hg was only slightly elevated in mosquitofish as compared to fish from the other STAs, and was similar to, or lower than, levels found in mosquitofish inhabiting downstream sites. Resident sunfish and bass also contained elevated Hg levels compared to fish from other STAs, but not as compared to downstream sites. Because this STA only began flow-through operation in early 2004, Hg levels in these large-bodied fish collected in October represent baseline exposures.

6. **STA-5:** Water-column concentrations of both THg and MeHg remained low at STA-5 in WY2005 relative to spikes observed during previous years. While THg exhibited a large negative percent change across the STA, MeHg was at greater concentration in the outflow than in the inflow in two quarters and, as a result, exhibited only a slight negative percent change for the year. Mosquitofish collected near the outflows contained almost identical levels of Hg as fish collected just upstream of inflow culverts. Likewise, sunfish caught in the discharge canal of STA-5 contained Hg levels similar to fish in the supply canal. Although bass from the discharge contained greater Hg, they were also much larger than fish from the supply canal. Hg levels did not differ among years in bass from the discharge canal. Fish from all three different trophic levels (TLs) contained Hg levels lower than both USFWS and USEPA predator protection criteria.

7. **STA-6:** During the water year, the STA dried out and, as observed in previous years, upon reflooding water-column concentrations exhibited spikes in THg and MeHg concentrations in outflows. However, these spikes in water-column concentrations were not as high as previous years. More importantly, these transient spikes do not appear to be having an obvious effect on Hg levels in resident fish populations either within the treatment cells or immediately downstream in the discharge canal. Hg levels in mosquitofish captured in 2004 were comparable to fish from other STAs and lower than concentrations in mosquitofish from downstream Everglades sites. Although tissue-Hg in bluegill sunfish tended to be lower in 2003, a year in which the STA did not dry down, levels were even lower in 1999, a year in which the STA did dry down. When bluegill in the discharge canal were assessed, 2003 fish contained the highest tissue concentration. A downward trend in Hg levels in bass (least square mean Hg concentration in bass collected from the discharge canal in 2004 was significantly lower than all earlier years) would also seem to suggest that the transient spikes in water-column concentrations are not having a significant impact.

INTRODUCTION

This is the annual permit compliance monitoring report for mercury (Hg) in Stormwater Treatment Areas (STAs). This report summarizes the Hg-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 STA-1W, STA-2, STA-3/4, STA-5, and STA-6 (Nos., 503074709, 0126704, 192895, 0131842, and 2629183090, respectively). This report summarizes the results of monitoring in Water Year 2005 (WY2005) (May 1, 2004 through April 30, 2005). The results of Hg monitoring at sites downstream of the STAs in accordance with these permits, as well as non-Everglades Construction Project (non-ECP) discharge structures (see Permit No. 06,502590709) will be reported separately in Appendix 2B-1 of the *2006 South Florida Environmental Report – Volume I* (2006 SFER).

This report consists of key findings and an overall assessment, an introduction and background, a summary of the Mercury Monitoring and Reporting Program, and monitoring results. The background section briefly summarizes previously identified concerns regarding possible impact of STA operation on South Florida's Hg problem. The following section summarizes sampling and reporting requirements of the Mercury Monitoring Program within the STAs. Monitoring results are summarized and discussed in two subsections: (1) results from pre-operational monitoring, and (2) results from STA operational monitoring. Recent results from the Mercury Monitoring and Reporting Program describe significant spatial distributions and, in some instances, between-year differences in Hg concentrations.

BACKGROUND

The STAs are constructed wetlands designed to remove phosphorus from stormwater runoff originating from upstream agricultural areas and Lake Okeechobee releases. The STAs are being built as part of the Everglades Construction Project (ECP) authorized under the Everglades Forever Act [EFA; Section 373.4592, Florida Statutes (F.S.)]. When completed, the ECP will include six large treatment marshes totaling about 47,000 acres.

Even prior to passage of the EFA, concerns were being raised that, in attempting to reduce downstream eutrophication, the restoration effort could inadvertently worsen the Hg problem known to be present in the Everglades (Ware et al., 1990; Mercury Technical Committee, 1991). This stemmed from studies in other areas that showed flooded soils in new impoundments to be a source of inorganic Hg (Cox et al., 1979). Of greater concern, studies had shown wetlands to be an important site of Hg methylation; methylmercury (MeHg) is the more bioaccumulative and toxic form of Hg (St. Louis et al., 1994; for review, see Rudd, 1995). Decomposition of flooded terrestrial vegetation and soil carbon in new reservoirs had been reported to stimulate the sulfate-reducing bacteria (SRB) that methylate inorganic Hg (Kelly et al., 1997; Paterson et al., 1998). Environments that favor methylation drive bioaccumulation. For example, Paterson et al., (1998) found that annual fluxes of MeHg increased 10 to 100 times through a zooplankton community after impoundment. Newly created reservoirs have also been found to contain fish with elevated Hg burdens (Abernathy and Cumbie, 1977; Bodaly et al., 1984; Bodaly and Fudge, 1999). This so-called "reservoir effect" can occasionally persist for several decades after initial flooding (Bodaly et al., 1984; Verdon et al., 1991; for review, see Fink et al., 1999). For instance, Verdon et al. (1991) reported that Hg levels in northern pike (*Esox lucius*) increased from 0.61 to 2.99 parts per million (ppm) and were still increasing nine years after initial flooding. Given these observations, Kelly et al. (1997) recently recommended that in siting a new reservoir (1) total land area flooded should be minimized and (2) flooding the wetlands, which contain larger quantities of organic carbon than the uplands, should be avoided.

However, applying these observations directly to the Everglades was problematic because most of these observations were made in deepwater lakes or reservoirs in temperate regions. In a report to the SFWMD, Watras (1993) stated that "the boreal and temperate watersheds, wetlands and reservoirs studied to date are very different geologically, hydrologically, meteorologically and ecologically from the subtropical systems in the Everglades." He recommended monitoring and integrating mass balance and process-oriented studies to improve our understanding of how the system would behave. Such studies were initiated in 1994 with the start-up of the prototype STA, the Everglades Nutrient Removal (ENR) Project. Baseline collections at the ENR Project (funded by the SFWMD and others) found no evidence of MeHg spikes in either surface water (PTI, 1994 attributed to KBN, 1994a; Watras, 1993; 1994) or resident fishes (mosquitofish and largemouth bass; PTI, 1994 attributed to KBN, 1994b). During the first two years of operation, median concentrations of THg and MeHg in unfiltered surface water were reported to be 0.81 and 0.074 nanograms per liter (ng/L), respectively (Miles and Fink, 1998). These low levels persisted in later years; from January 1998–April 1999, median water-column concentrations in the interior marsh (i.e., excluding inflows and outflows) were 0.81 ng THg/L and 0.04 ng MeHg/L (Rumbold and Fink, 2002b). Resident fishes also continued to have only low Hg levels: 8 to 75 nanograms per gram (ng/g) in mosquitofish, and 100 to 172 ng/g in three-year-old bass (Miles and Fink, 1998; SFWMD, 1999b; Lange et al., 1999). Finally, a mass balance assessment found the ENR Project to be a net sink for both THg and MeHg, removing approximately 70 percent of the inflow mass (Miles and Fink, 1998). Nonetheless, to provide continuing assurance that the ECP does not exacerbate the Hg problem, construction and operating permits for the STAs, issued by the FDEP, require the SFWMD to monitor levels of THg and MeHg in various abiotic (e.g., water and sediment) and biotic (e.g., fish and bird tissues) media, both within the STA and the downstream receiving waters.

Results from monitoring programs at STAs constructed and operated after the ENR Project have revealed transitory spikes in MeHg production (for details, see previous annual reports including Rumbold and Fink, 2002b). These monitoring results combined with the results of a 1999 field study on the effect that drought and muck fires had on mercury cycling in the Everglades (Krabbenhoft and Fink, 2001) have demonstrated that spikes can sometimes occur following drydowns and rewetting. Accumulating evidence suggests that oxidation of sulfide pools in the sediments (e.g., organic sulfide, disulfides, and acid volatile sulfides) during the drydown can lead to increased methylation upon rewetting of the marsh either by providing free sulfate, which stimulates the sulfate-reducing bacteria or, in highly sulfidic areas, by reducing porewater sulfide, which can inhibit methylation (for discussion of sulfide inhibition see, Benoit et al., 1999a and b).

SUMMARY OF THE MERCURY MONITORING AND REPORTING PROGRAM

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

EVERGLADES MERCURY BASELINE MONITORING AND REPORTING REQUIREMENTS

Levels of THg and MeHg in the pre-operational soils of each of the STAs and various compartments (media) of the downstream receiving waters define the baseline condition from which to evaluate Hg-related changes, if any, brought about by the operation of the STAs. The pre-ECP Hg baseline conditions are defined in the Everglades Mercury Background Report, which summarizes all of the relevant Hg studies conducted in the Everglades through July 1997. This was during the construction, but prior to the operation of the first STA. Originally prepared for submittal in February 1998, the report was revised to include the most recent data released by the USEPA and the U. S. Geological Survey (USGS) and was submitted in February 1999 (FTN Associates, 1999).

PRE-OPERATIONAL MONITORING AND REPORTING REQUIREMENTS

Prior to completion of construction and flooding of the soils of each STA, the District is required to collect 10-cm core samples of soil at six representative interior sites for THg and MeHg analyses. Prior to initiation of discharge, the District is also required to collect biweekly samples of supply canal and interior unfiltered water for THg and MeHg analyses. When concentrations at the interior sites are found not to be significantly greater than that of the supply canal, this information is reported to the permit-issuing authority, and the biweekly sampling can be discontinued. Discharge begins after all the start-up criteria are met.

OPERATIONAL MONITORING

Following approval for initiation of routine operation of an STA and thereafter, the permits require that the following samples be collected at the specified frequencies and analyzed for specified analytes:

Water

On a quarterly basis, 500-ml unfiltered grab samples of water are collected in pre-cleaned bottles using the ultraclean technique at the supply canals and outflows of each STA. They are analyzed for MeHg and THg (this includes the sum of all Hg species in sample, e.g., Hg^0, Hg^I, and Hg^{II}, as well as organic Hg). THg results are compared with the Florida Class III water quality standard of 12 ng/L to ensure compliance. Outflow concentrations of both THg and MeHg are compared to concentrations at the supply canal.

Sediment

Triennially, sediment cores are collected at depth from 0 to 10 cm at six representative interior sites. Each depth-homogenized core is then analyzed for THg and MeHg.

Prey Fish

Semiannually, grab samples of between 100 and 250 mosquitofish (*Gambusia* sp.) are collected using a dip net at the supply canal sites, interior sites, and outflow sites of each STA. Individuals are composited from each size, and the homogenate is subsampled in quintuplicate. Each subsample is then analyzed for THg. 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).

Top Predator Fish

Annually, 20 largemouth bass are collected primarily via electroshocking methods at representative supply canal and discharge canal sites and representative interior sites in each STA. The fish muscle (fillet) samples are analyzed for THg as an indicator of potential human exposure to Hg.

In 2000, the District began routine collection of sunfish at the same frequency, intensity (i.e., n = 20), and locations as largemouth bass. This permit revision fulfilled a USFWS recommendation (USFWS recommendation 9b in USACE Permit No. 199404532; for details, see correspondence to Bob Barron, USACE, dated July 13, 2000). Sunfish, analyzed as whole fish, also serve as a surrogate for attempts to monitor Hg in wading birds that do not nest in the STAs. (For details on the monitoring program tracking Hg in wading birds in downstream areas, see

Appendix 2B-1 of this volume.) The addition of sunfish to the compliance monitoring program was approved by the FDEP on March 5, 2002 (correspondence from F. Nearhoof, FDEP).

It should be recognized that tissue concentrations in each of the three monitored fishes will reflect ambient MeHg levels, i.e., integrate exposure, as a function of a combination of factors including body size, age, rate of population turnover, and trophic position. Mosquitofish should respond rapidly to changing ambient MeHg concentrations due to their small size, lower trophic status, short life span, and rapid population turnover. 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). Alternatively, owing to their specific life history characteristics, sunfish (sunfish are thought to have an average life span of four to seven years in the wild) and bass should take a greater amount of time to respond, in terms of tissue concentrations, to changes in ambient MeHg availability. Most importantly, they represent exposure at higher trophic levels with a requisite time lag for trophic exchange. Furthermore, this focus on a three-year old bass, while appropriate to assess exposure to fishermen, complicates interpretation because its tissue concentration will reflect integration over a three-year period. The key is to use these species-related differences to better assess MeHg availability within the system.

It is important to also recognize that virtually all (> 85 percent) of the Hg in fish muscle tissues 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 for MeHg, can be interpreted as being equivalent to the analysis of MeHg. Further details regarding rationales for sampling scheme, procedures, and data reporting requirements are set forth in the Everglades Mercury Monitoring Plan revised in March 1999 (Appendix 1 of QAPP, June 7, 1999).

QUALITY ASSURANCE MEASURES

For a quality assurance/quality control (QA/QC) assessment of the District's Mercury Monitoring Program during WY2005, see Appendix 2B-1 of this volume.

STATISTICAL METHODS

The proper interpretation of residue levels in tissues can sometimes prove problematic due to the confounding influences of age or species of collected animals. For comparison, special procedures are used to normalize the data (Wren and MacCrimmon, 1986; Hakanson, 1980). To be consistent with the reporting protocol used by the FWC (Lange et al., 1998 and 1999), Hg concentrations in largemouth bass were standardized to an expected mean concentration in three-year-old fish at a given site by regressing Hg against age (hereafter symbolized as EHg3). To adjust for the month of collection, otolith ages were first converted to decimal ages using protocols developed by Lange et al. (1999). Sunfish were not aged, so age normalization was not available. Instead, arithmetic means were reported. However, efforts were made to estimate a least square mean (LSM) Hg concentration based on the weight of the fish. Additionally, the distribution of the different species of *Lepomis* (warmouth, *L. gulosus*; spotted sunfish, *L. punctatus*; bluegill, *L. macrochirus*; and redear sunfish, *L. microlophus*) that were collected during electroshocking was also considered (qualitatively) as a potential confounding influence on Hg concentrations prior to each comparison.

Where appropriate, analysis of covariance (ANCOVA), using the SAS General Linear Model (GLM) procedure, was used to evaluate spatial and temporal differences in Hg concentrations, with age (largemouth bass) or weight (sunfish) as a covariate. However, use of ANCOVA is predicated on several critical assumptions (for review, see ZAR, 1996). These assumptions are that (1) regressions are simple linear functions; (2) regressions are statistically significant (i.e., nonzero slopes); (3) covariate is a random, fixed variable; (4) both the dependent variable and residuals are independent and normally distributed; and (5) slopes of regressions are homogeneous (parallel) (i.e., no interactions).

Regressions also require that collected samples exhibit a relatively wide range of covariate, that is, that fish from a given site are not all the same age or weight. Where these assumptions were not met, ANCOVA was inappropriate. Instead, standard analysis of variance (ANOVA) or student's "t" tests (SigmaStat, Jandel Corporation, San Rafael, CA) were used. Possible covariates were considered separately and often qualitatively. 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 they were used in ANOVA. If they did not meet the assumptions, then raw datasets were evaluated using nonparametric tests such as the Kruskal-Wallis ANOVA on ranks or the Mann-Whitney Rank sum test. If the multigroup null hypothesis was rejected, then groups were compared using either Tukey HSD (honestly significant difference) or Dunn's Method.

SITE DESCRIPTIONS

Site descriptions and operational plans for STA-1W, 2, 3/4, 5, and 6 are published elsewhere (SFWMD, 1997; 1998a; 1998b; 1999a; 2004); similar information on STA-1E was not available as of the date of this report. For maps of monitoring locations, see **Figures 1** through **6**.

STA1W MERCURY SAMPLING LOCATIONS

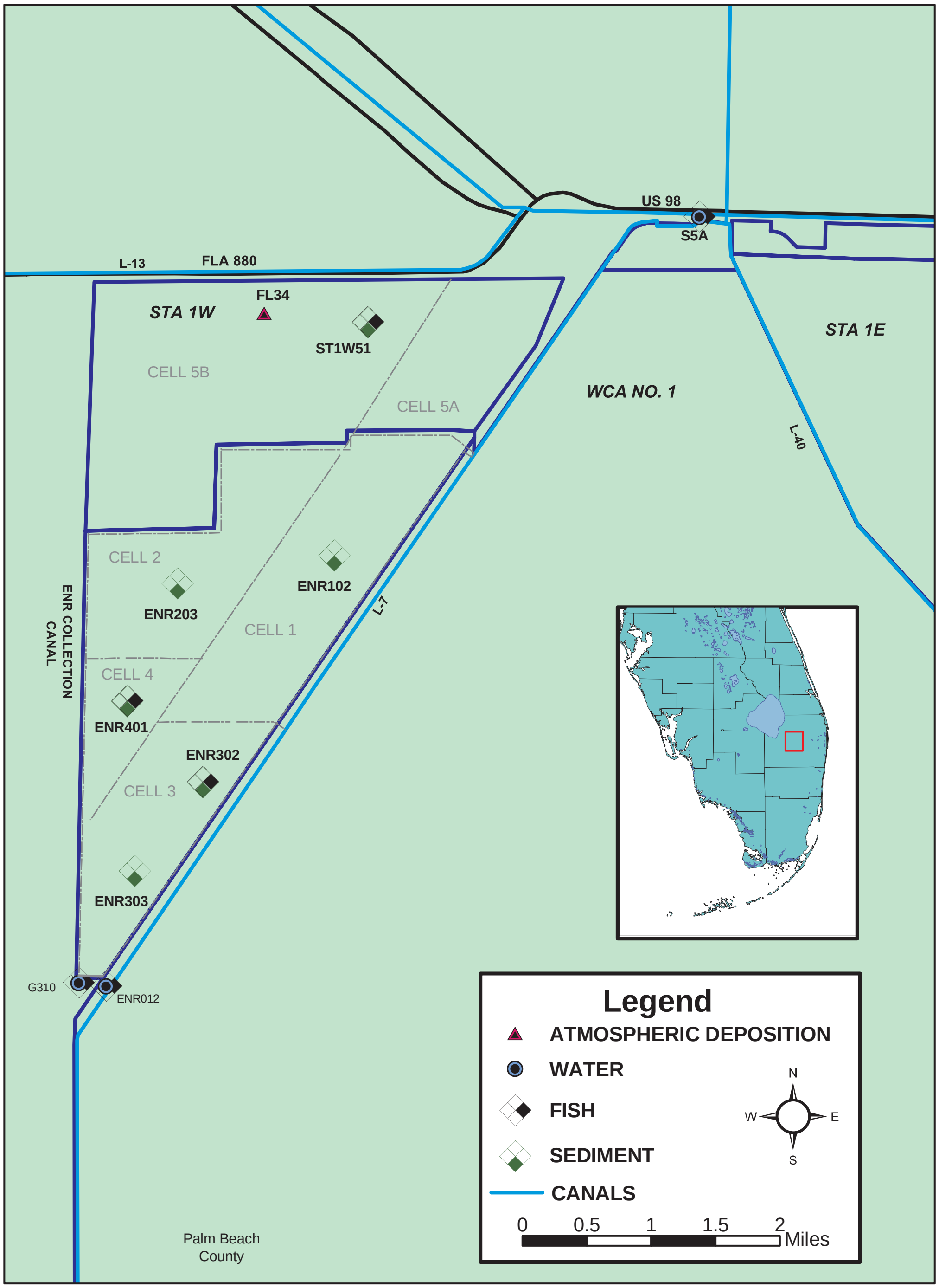


Figure 1. Stormwater Treatment Area 1 West (STA-1W) showing mercury monitoring sites.

STA1E MERCURY SAMPLING LOCATIONS

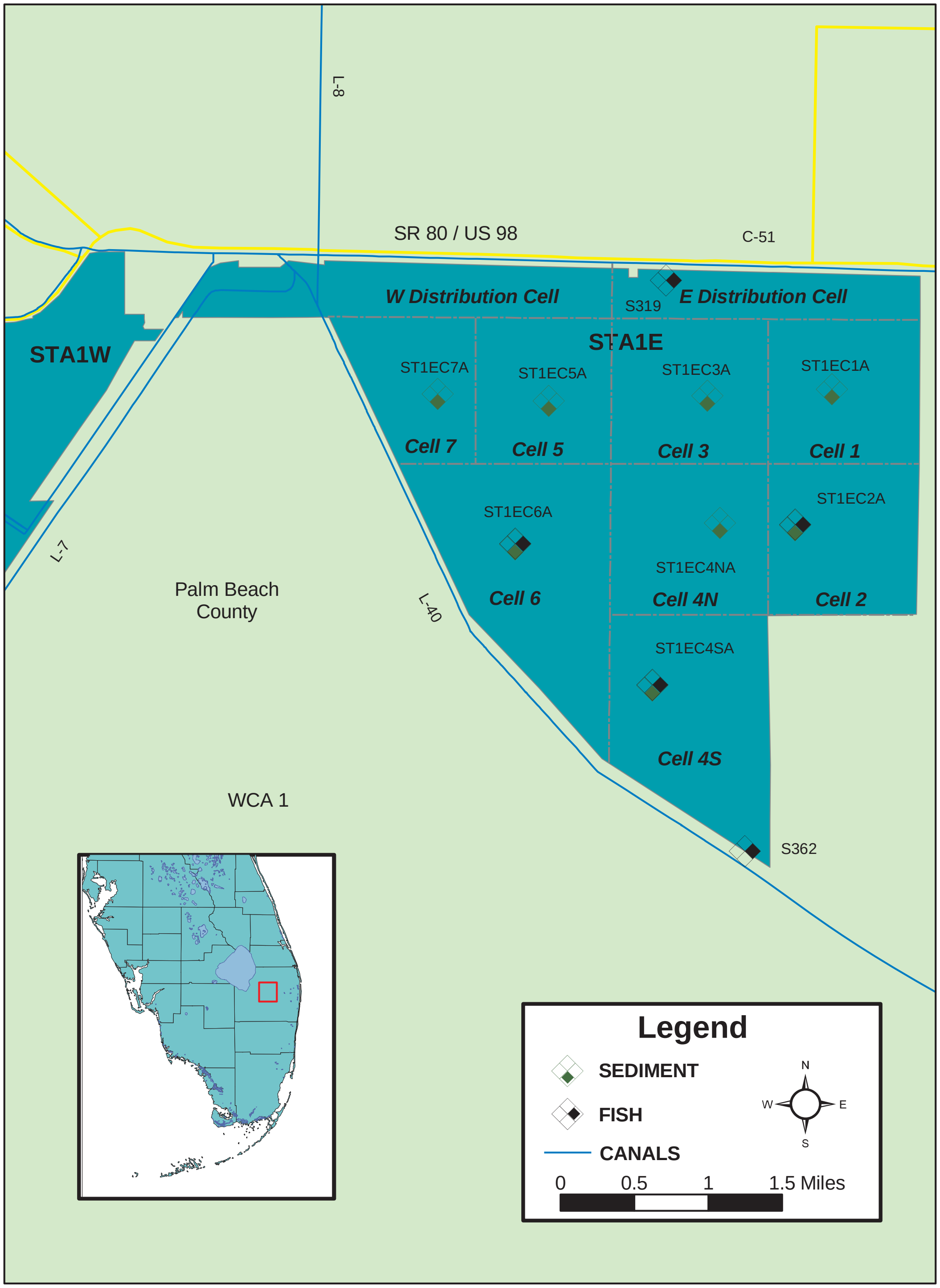


Figure 2. Map of Stormwater Treatment Area 1 East (STA-1E) showing sediment collection sites and future fish collection sites.

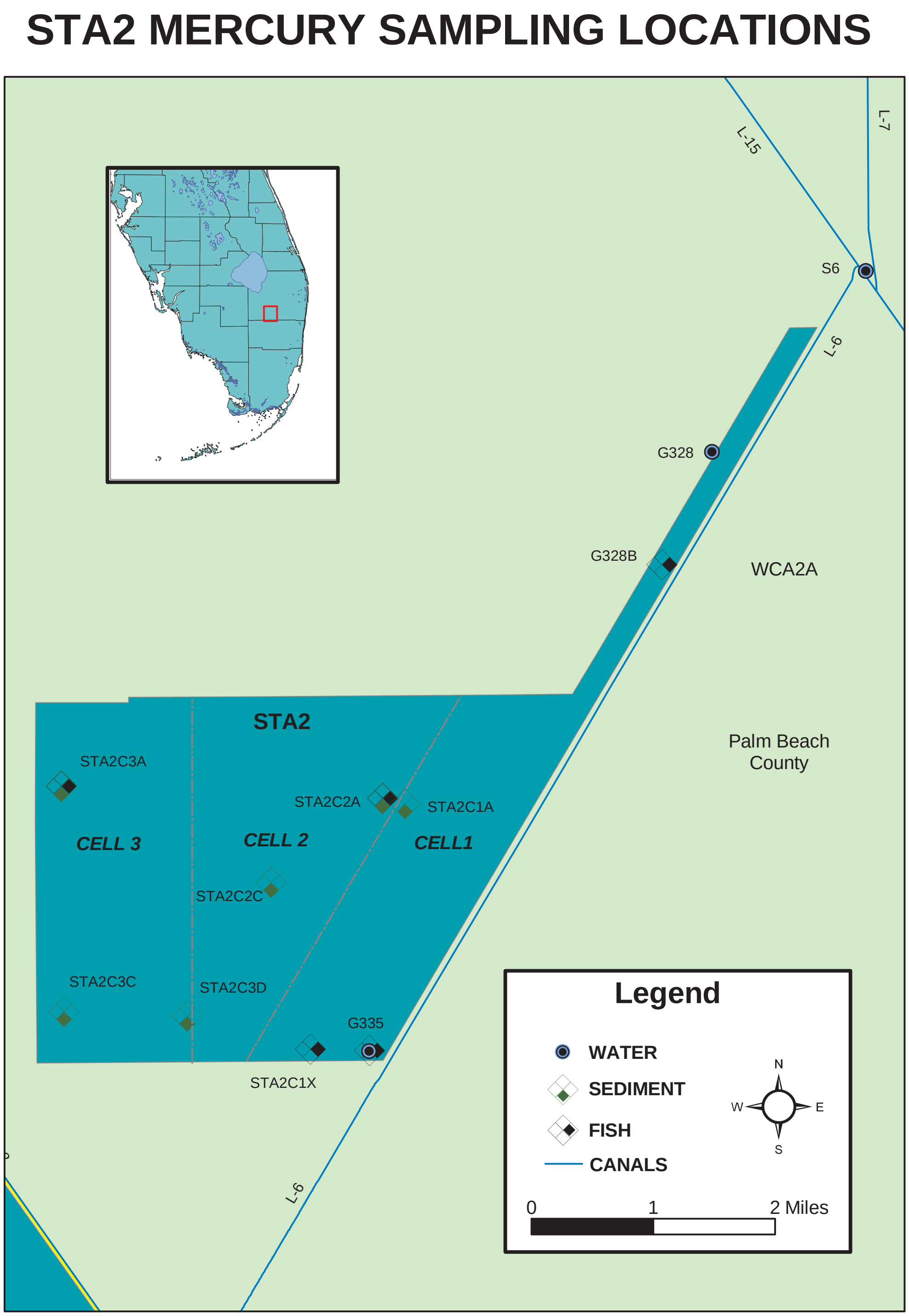


Figure 3. Map of STA-2 showing mercury monitoring sites.

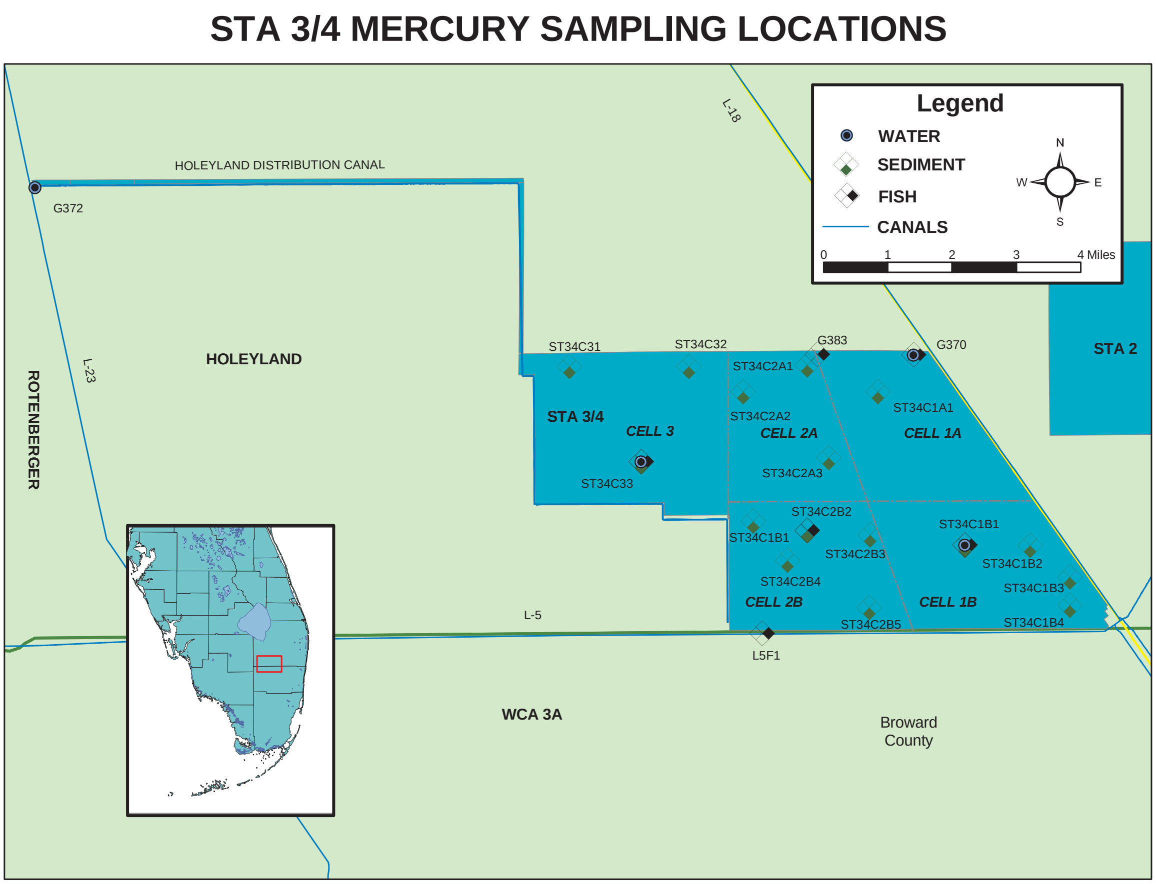


Figure 4. Map of STA-3/4 showing mercury monitoring sites.

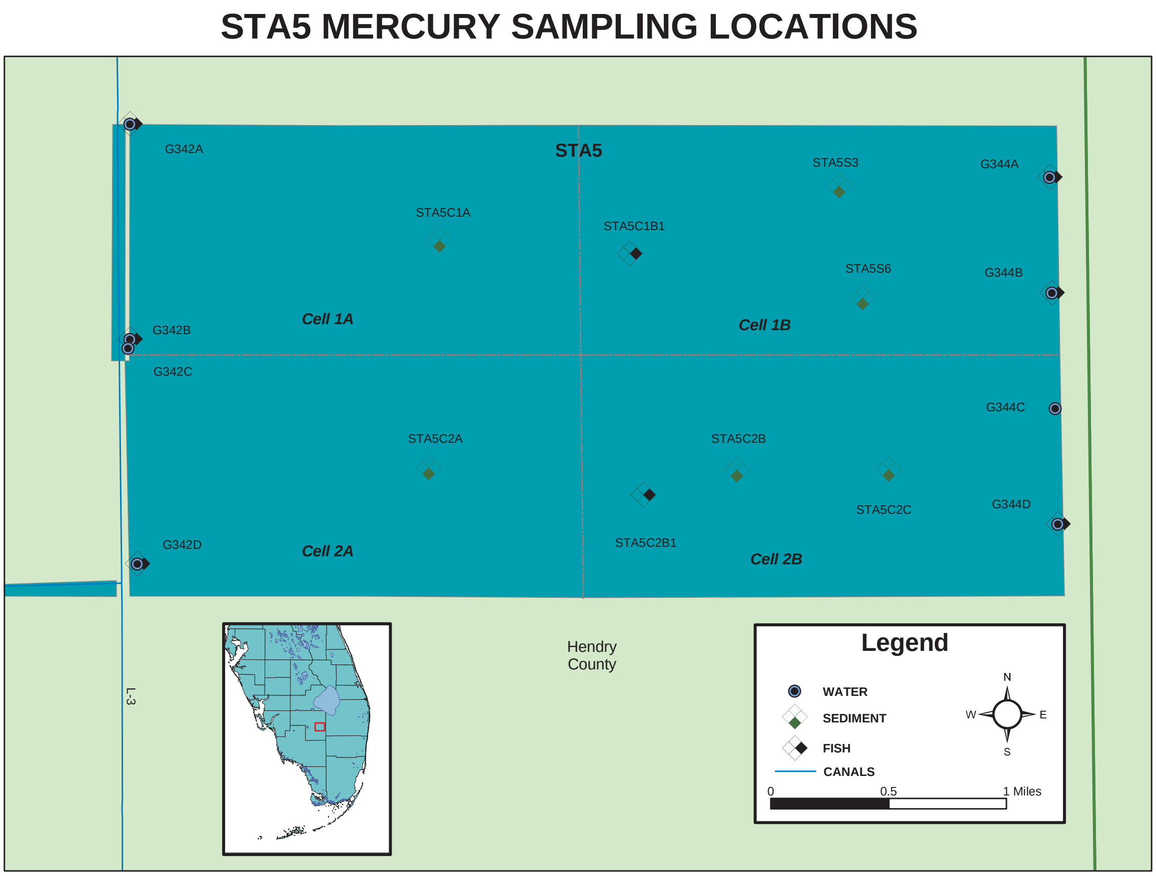


Figure 5. Map of STA-5 showing mercury monitoring sites.

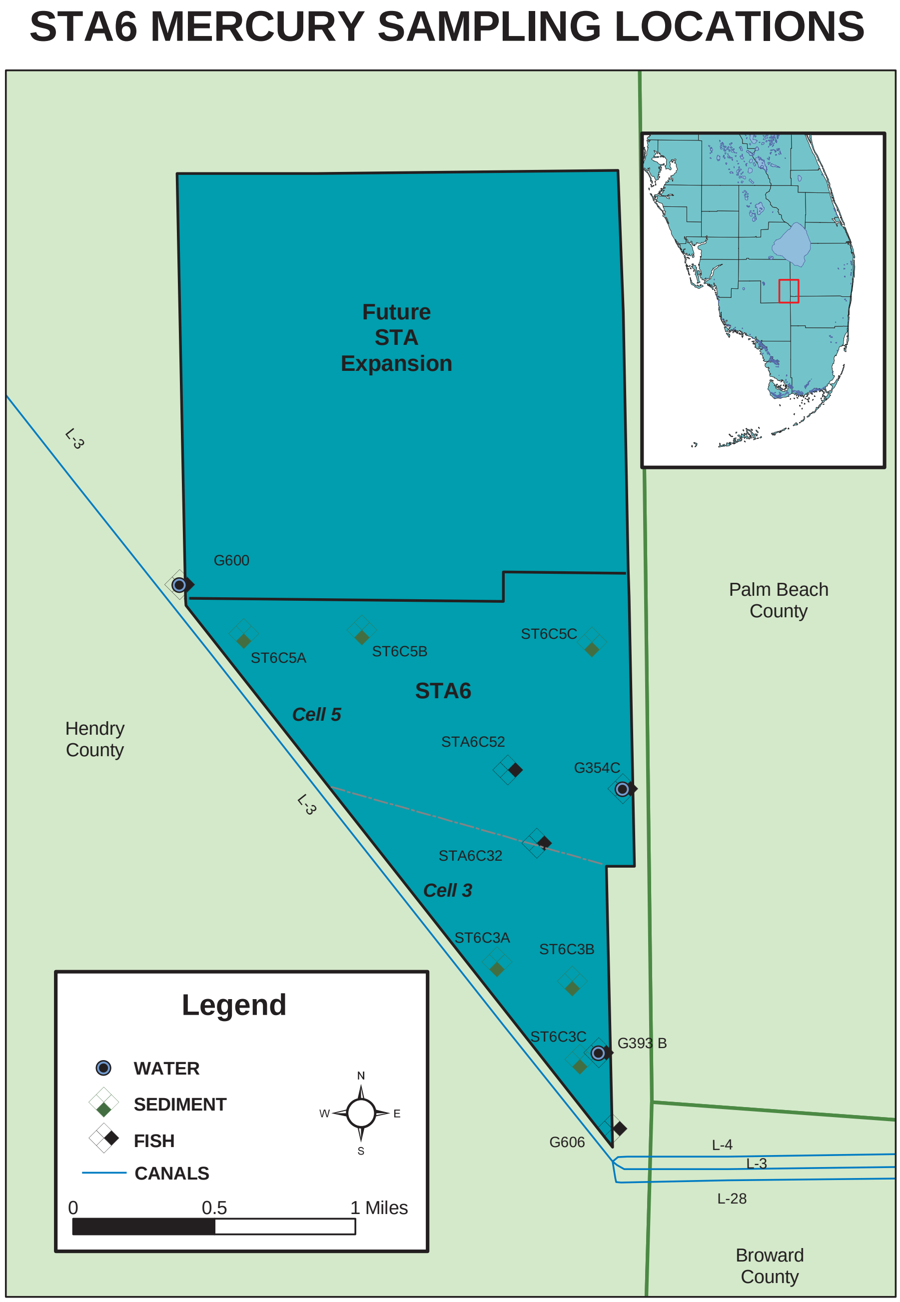


Figure 6. Map of STA-6 mercury monitoring sites.

MONITORING RESULTS

PRE-OPERATIONAL MONITORING

Results from pre-operational monitoring of STA-1W, 1E (i.e., baseline sediment), 2, 3/4, 5, and 6 have been reported previously (SFWMD, 1998c and 1999c; Rumbold and Rawlik, 2000; Rumbold et al., 2001a; Rumbold and Fink, 2002a; Rumbold and Fink, 2003a; Rumbold 2004a, Rumbold 2005).

STA-1E

The District began monitoring water-column concentrations of total mercury (THg) and methylmercury (MeHg) on a biweekly basis at STA-1E in January 2005. As of April 30, 2005, the STA had not satisfied the start-up criteria that water-column concentrations in the interior marshes should not be significantly greater than the concentrations of the corresponding species in inflow samples; during the four months of monitoring, concentrations from the interior marshes ranged from 0.77 to 2.2 ng THg/ L and from 0.21 to 0.95 ng MeHg/L.

OPERATIONAL MONITORING

STA-1W

In 2000, STA-1W subsumed the ENR Project (Treatment Cells 1 through 4, **Figure 1**), which had been in operation since 1994. STA-1W surface water passed start-up criteria during the week of January 17, 2000; flow-through operations began in early February 2000. Formal monitoring of Hg levels in STA-1W surface water began on February 16, 2000 (for discussion of results observed prior to WY2005, see Rumbold and Rawlik, 2000; Rumbold et al., 2001a; Rumbold and Fink, 2002a; Rumbold and Fink, 2003a; Rumbold, 2004a; Rumbold, 2005).

The triennial collection of sediments cores (10 cm depth) from STA-1W occurred on January 26, 2005. THg levels (**Table 1**) were within the range of concentrations observed in 10 cm sediment cores previous collected from the STAs; median concentration in cores collected from STAs pooled (and 95th percentile) was 81 ng THg/g (140 ng/g, n = 121). MeHg levels in sediments from STA-1W were relatively low compared to other STAs; median concentration for STAs pooled (and 95th percentile) was 0.45 ng MeHg/g (3.2 ng/g; n = 115). The sediment core from Cell 5 appeared to differ from sediments in other cells being close to the median MeHg concentration. These low levels of sediment MeHg likely reflect a minimal net methylation rate that is the primary factor as to why this STA has such low MeHg concentrations in the water column and fish.

When sediment data were pooled across cells within STA-1W, no significant among-year difference (e.g., 1999, 2002, 2005) was found in THg concentrations (ANOVA, df = 2, 15; F = 3.3, p = 0.06). Although this p value hints at a possible trend, it should be noted that mean concentrations were highest in 1999 (105 ng/g) and lowest in 2002 (69 ng/g). Thus, any between-year difference would not likely have involved sediments collected in 2005. There was also no significant difference in MeHg levels between sediments collected in 2002 and 2005 (Kruskal-Wallis One Way ANOVA on Ranks H = 0.315, df = 1, p = 0.589). This analysis of MeHg levels did not include cores taken in 1999, because five out of six cores collected that year were analyzed by the FDEP laboratory which, at the time, had a higher MDL than the primary laboratory for sediments and consequently results were reported as below detection.

As shown in **Table 2** and **Figure 7**, concentrations of both THg and MeHg in surface water at the outflows of STA-1W remained low as compared to the inflow and as compared to (inflows and outflows of) other STAs. It is notable that both constituents were at nearly identical concentration in the two outflows over the last three quarters (**Figure 7**). As observed in previous water years, THg concentration spiked at the inflow during the water year (28 ng THg/l; **Table 2**); however, as before, this was a result of suspended solids in the unfiltered samples (the analytical laboratory noted that the sample had a significant amount of suspended particulate matter) and likely had no environmental impact. As evident from **Figure 8**, after an extended period of inundation (going back to 1997; Rumbold and Fink, 2002b), Cells 2, 4, and 5B were taken off-line during the water year and allowed to draw down to allow for construction activities (for details, see Chapter 4 of this volume). These drawdowns appeared to have no marked effect on water-column concentrations at the outflows during this water year (**Table 2**, **Figure 7**). STA-1W continued to exhibit a large negative percent change across the STA in both water-column THg and MeHg concentrations (**Table 3**). This long-term trend is also reflected in smaller loads of THg and MeHg in the two outflows (e.g., G251 and G310) as compared to the combined inflows to the cells (especially given the spikes that occurred in the inflow in 2004, **Figures 9** and **10**).

Concentrations of THg in mosquitofish are summarized in **Table 4** and are graphically presented in **Figure 11**. Levels of Hg in mosquitofish from STA-1W were similar to, or have declined slightly when compared to concentrations observed in fish collected previously from this area when it was operated as the ENR Project (see review, see Rumbold and Fink, 2002b). Furthermore, Hg levels in STA-1W mosquitofish continue to be low compared to levels currently observed in fish from other areas of the Everglades (see Appendix 2B-1 of this volume). Here again it is important to note that Hg levels in mosquitofish from STA-1W did not increase markedly (in the first semi-annual event in 2005) following the drawdowns and reflooding during the water year. Similar to water-column concentrations, mosquitofish consistently exhibited a negative percent change in tissue-Hg across STA-1W (**Table 4**). As discussed below, this pattern was also observed in sunfish and largemouth bass.

As evident from **Table 5** and **Figures 11** and **12**, STA-1W sunfish continued to have Hg levels much lower than those observed in sunfish at the other STAs and locations within the Everglades (see Appendix 2B-1 of this volume). When only bluegill from interior marshes were assessed (i.e., assessment was limited to this species to remove the variation associated with species-related difference in biomagnfication and the year-to-year and site-to-site differences in species of lepomid collected), Hg levels in fish from Cell 5 (pooled over time, median 38 ng/g in $n = 29$ fish) differed from levels in fish from both Cell 3 (median was 17 ng/g for $n = 41$) and Cell 4 (median was 13.5 ng/g for $n = 38$)($H = 23.3$, $df = 2$; $p < 0.001$, Dunn's post-hoc test $p < 0.05$); fish from the latter two cells did not differ ($p > 0.05$). When bluegill from Cell 5 were assessed for temporal differences, fish collected in 2003 and 2004 contained much lower Hg levels (median was 13 ng/g in 2003 and 14 ng/g in 2004) than fish collected in earlier years (median concentration was 45 ng/g in 1999, 64 ng/g in 2000, 38 ng/g in 2002); these between-year differences were shown to be significant when levels in 2000 were compared to 2003 or 2004 (Dunn's method, $p < 0.05$). When data on bluegill from Cells 3 and 4 were pooled, Hg levels varied over time, with highest concentrations occurring in 2000; however, no pairwise comparison between years was statistically significant ($H = 11.4$, $df = 5$, $p = 0.04$; Dunn's pairwise comparisons $p > 0.05$). When only bluegill were assessed (and then normalized further based on size), it became even more apparent that Hg levels have remained relatively low at STA-1W compared to other STAs (**Figure 12**) and downstream sites (see Figure 13 in Appendix 2B-1 of this volume).

Similar to sunfish, largemouth bass from STA-1W contained lower levels of Hg than bass from the other STAs (**Table 6**, **Figure 12)**. Moreover, STA-1W bass contained much lower Hg than fish from downstream sites in the WCAs (Figure 13, Appendix 2B-1 of this volume). As with mosquitofish and sunfish, the bass exhibited a negative percent change in Hg levels across the STA (**Table 6**); as evident from **Figure 11**, bass from the supply canal (upstream of S5A) contained substantially greater Hg levels than fish both from interior marshes and from the discharge canals. The Hg level estimated in a fillet from a three-year-old bass (EHg3) was only slightly higher in Cell 5 (61 ± 4 ng/g) as compared to Cell 3 (58 ± 13 ng/g). The regression of Hg on age was not significant for fish caught in Cell 4; however, average levels were 29 ng/g in six bass of age 2.8 years. These levels are almost an order of magnitude lower than observed in downstream sites. Visual inspection of **Figure 11** suggests no temporal patterns and, in fact, very little variation in Hg levels in these fish.

Hg levels in fish tissues can also be put into perspective and evaluated for the exposure to fish-eating wildlife. The USFWS has proposed a predator protection criterion of 100 ng/g THg in prey species (Eisler, 1987). More recently, in its Mercury Study Report to the U.S. Congress, the USEPA proposed 77 and 346 ng/g for TL 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997). STA-1W mosquitofish, sunfish, and largemouth bass continue to have tissue-Hg levels well below both the USEPA and USFWS guidance level for predator protection. Therefore, unlike most other areas of the Everglades, fish-eating wildlife foraging preferentially at STA-1W would not appear to be at risk from Hg exposure.

Table 1. THg and MeHg concentrations (ng/g, dry weight) in STA soils (i.e., 10-cm depth composited).

STA	Year	Station	n	THg	Qualifier*	MeHg	Qualifier	%MeHg
STA-1W	2005	Cell1	1	94		0.047		0.05
	2005	Cell3	1	70		0.037		0.05
	2005	Cell3	1	95		0.019		0.02
	2005	Cell4	1	82		0.067		0.08
	2005	Cell2	1	87		0.152		0.17
	2005	Cell5	1	169		0.535		0.32
	Mean			99	± 35	0.14	± 0.19	0.12%
STA-5	2004	Cell1A	1	55		0.36		0.65%
	2004	Cell1B	1	95		0.58		0.61%
	2004	Cell1B	1	125		0.69		0.55%
	2004	Cell2B	1	84		0.26		0.31%
	2004	Cell2B	1	98		0.53		0.54%
	2004	Cell2A	1	31		0.29		0.94%
	Mean			81	± 34	0.45	± 0.17	0.60%

Table 2. Concentrations of THg and MeHg (ng/L) in surface water collected quarterly (based on calander year) from the STAs.

STA	THg (ng/L)						MeHg (ng/L)			
	Yr-Quart	Inflow	qualifier*	Outflow	qualifier	THg WQS[a]	Inflow	qualifier	Outflow	qualifier
STA-1W[b]	04-2	7.8	J3	0.48	J3	<WQS	0.22		0.06	
	04-3	28.0		1.15		<WQS	0.09		0.04	
	04-4	1.9		0.38		<WQS	0.06		0.07	
	05-1	2.1		0.33		<WQS	0.24		0.08	
STA-2[c]	04-2	1.14	J3	1.60	J3	<WQS	0.08		0.21	
	04-3	1.80		1.60		<WQS	0.16		0.11	
	04-4	0.48		0.89		<WQS	0.08		0.10	
	05-1	0.71		0.48		<WQS	0.10		0.09	
STA-3/4[d]	04-2	0.77		1.35		<WQS	0.03		0.32	
	04-3	1.50		2.90		<WQS	0.06		0.59	
	04-4	1.15		1.65		<WQS	0.20		0.39	
	05-1	1.69		0.41		<WQS	0.13		0.18	
STA-5[e]	04-2	1.82		0.96		<WQS	0.11		0.12	
	04-3	1.17		1.06		<WQS	0.18		0.32	
	04-4	1.85		1.12		<WQS	0.21		0.17	
	05-1	1.45		0.31		<WQS	0.10		0.06	
STA-6[f]	04-2	1.60		5.2		<WQS	0.37		1.85	
	04-3	2.90		2.9		<WQS	1.10		0.92	
	04-4	0.72		1.04		<WQS	0.15	V	0.26	
	05-1	0.45		0.34		<WQS	0.22		0.09	

* For qualifier definitions, see FDEP Rule 62-160: J3" - estimated value, poor precision, "V" - analyte detected in both the sample and the associated method blank.

[a.] Class III water quality standard of 12 ng THg/L.

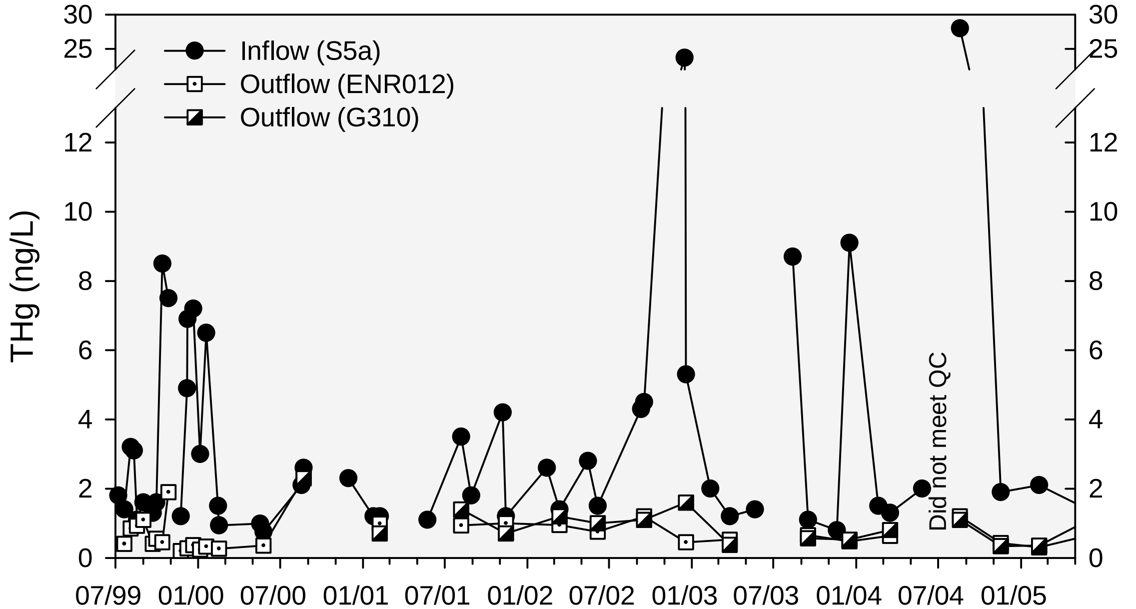
[b.] STA-1W has a single inflow and two outflows; the reported value for the latter represents mean of valid data (unqualified).

[c.] STA-2 has two inflows and a single outflow; the reported value for the former represents mean of valid data (unqualified).

[d.] STA-3/4 has two inflows; reported value is mean. Outflows are monitored at two culverts from each of the three trains; reported value is mean.

[e.] STA-5 has four inflows and four outflows; reported value represents mean of valid data (unqualified).

[f.] STA-6 has one inflow and two outflows; reported value is mean.



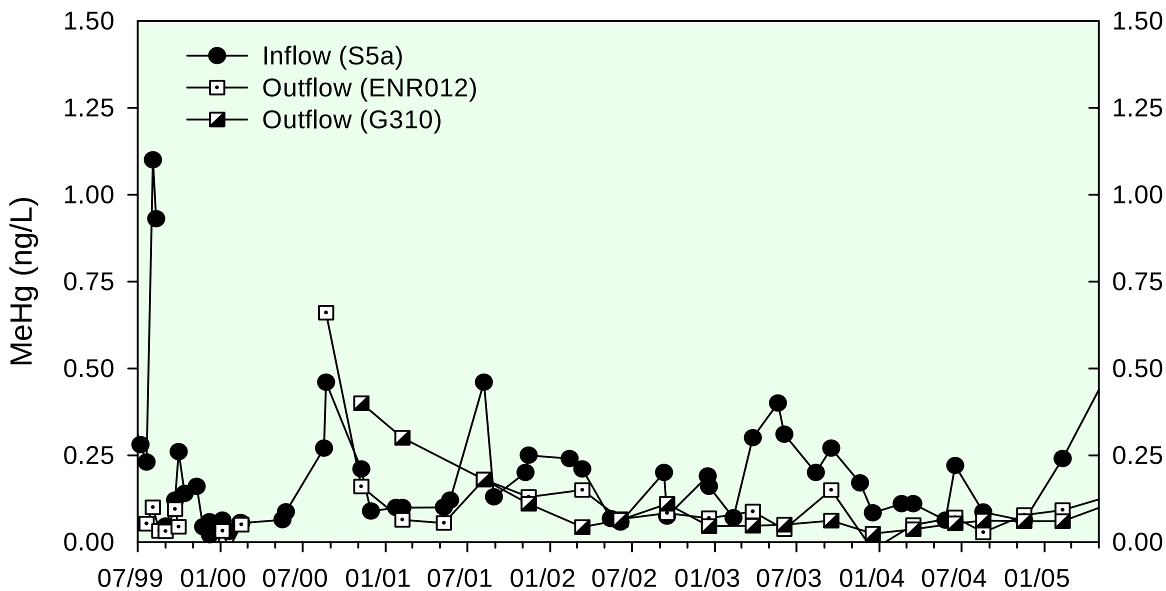


Figure 7. Concentrations of (a) THg and (b) MeHg (ng/L) in unfiltered surface water collected at STA-1W.

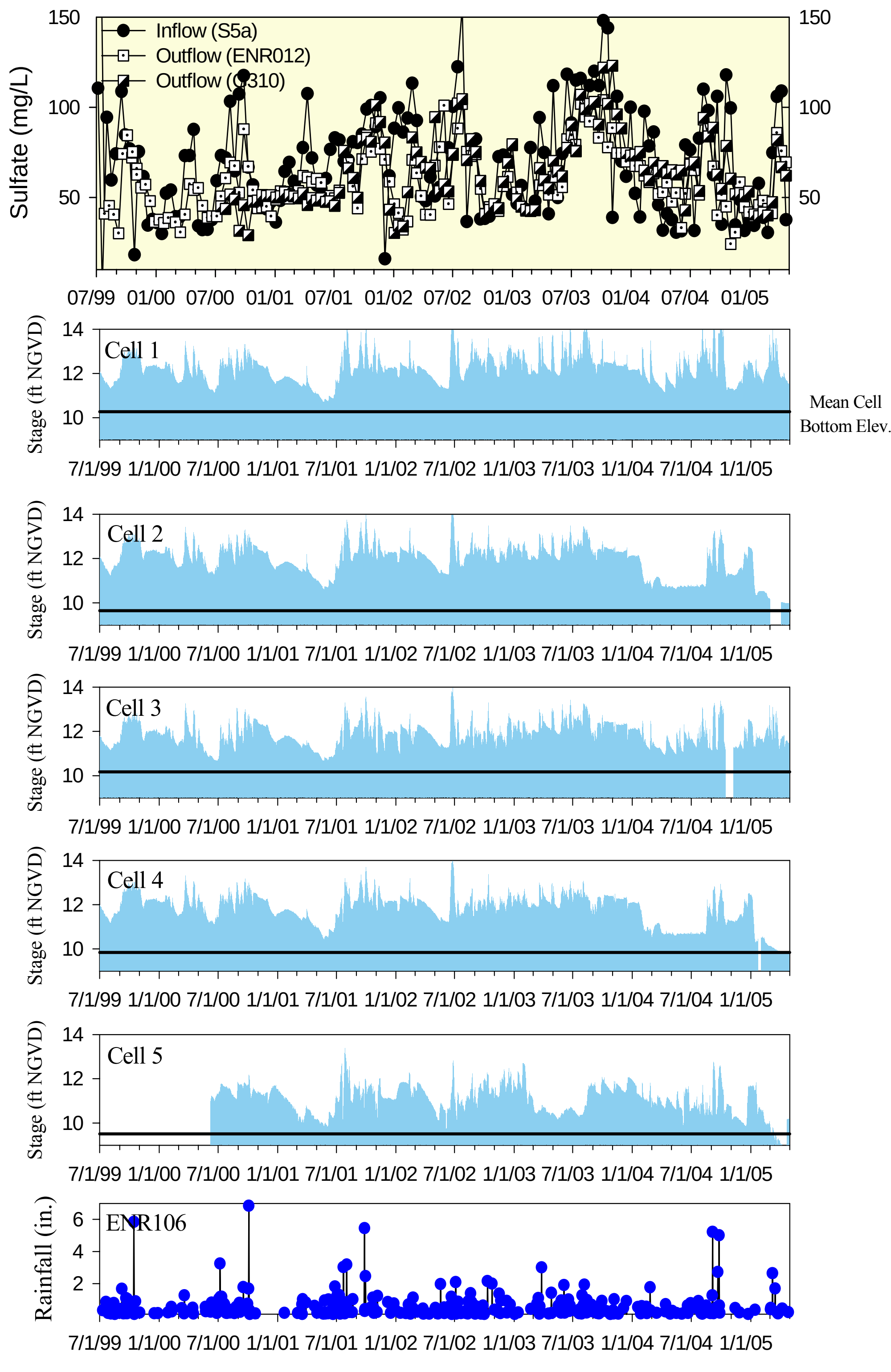


Figure 8. Water-column sulfate, stage (recorded immediately upstream of outflow culvert of cell) and rainfall at STA-1W.

Table 3. Percent change in concentration of THg and MeHg in surface water across STAs (i.e., outflow-inflow/inflow).

STA	Quarter	THg	MeHg
STA-1W	04-2	**	-72%
	04-3	-96%	-48%
	04-4	-80%	11%
	05-1	-84%	-68%
Annual median		-84%	-58%
Cumulative median		-56%	-52%
STA-2	04-2	**	164%
	04-3	-11%	-29%
	04-4	87%	19%
	05-1	-32%	-13%
Annual median		-11%	3%
Cumulative median		33%	164%
STA-3/4	04-2	74%	968%
	04-3	93%	815%
	04-4	43%	97%
	05-1	-76%	35%
Annual median		59%	456%
Cumulative median		74%	815%
STA-5	04-2	-47%	12%
	04-3	-10%	76%
	04-4	-39%	-17%
	05-1	-79%	-43%
Annual median		-43%	-3%
Cumulative median		-27%	7%
STA-6	04-2	225%	400%
	04-3	0%	-17%
	04-4	44%	**
	05-1	-24%	-58%
Annual median		22%	-17%
Cumulative median		-11%	-15%

** Only valid (unqualified) data used in calculations; see **Table 2** for raw data and qualifiers.

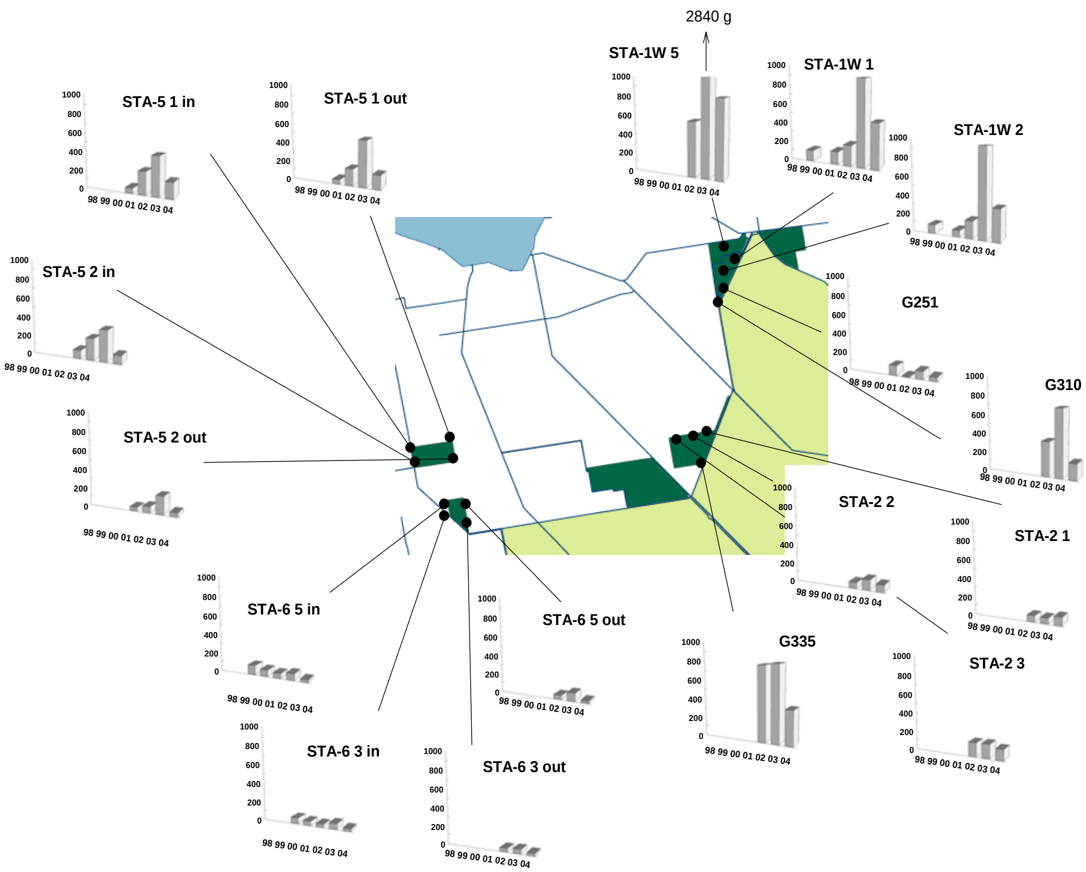


Figure 9. THg loading at STAs from WY 1998-2004. Note, y-axis scale is 1,000 grams.

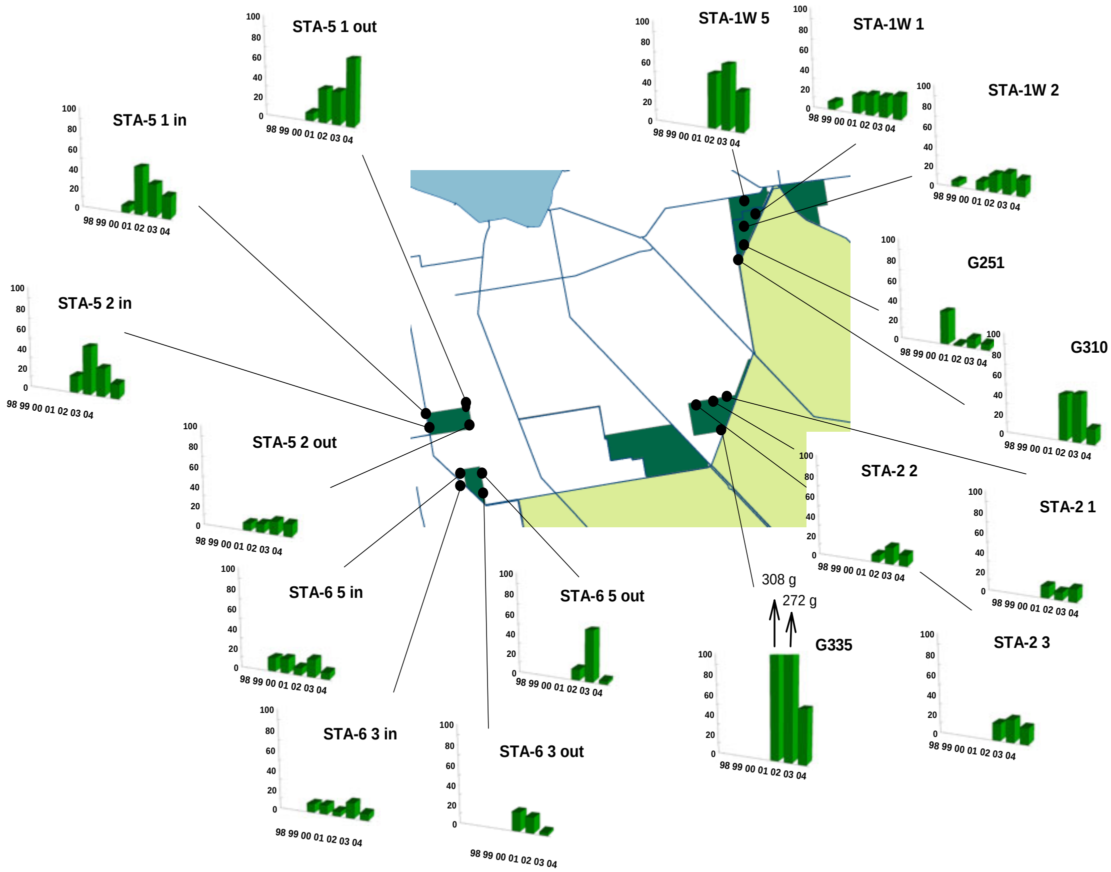


Figure 10. MeHg loading at STAs from WY1998–2004. Note, y-axis scale is to 100 grams.

Table 4. Concentration of THg in mosquitofish composites collected semiannually from STAs (units ng/g wet weight).

STA	Half-year	Inflow Fish	Interior Fish	Outflow Fish	Percent Change[1]
STA 1W	2004-2	17	8	11	-35%
	2005-1	8	4	4	--50%
Annual mean		13	7	7	-46%
Cumulative mean		28	14	13	-54%
STA 2	2004-2	10	13	17	70%
	2005-1	NA	15	14	
Annual mean		10	14	16	60%
Cumulative mean		10	74	101	910%
STA 3/4	2004-2	17	15	43	153%
	2005-1	16	31	31	94%
Annual mean		16	22	38	138%
Cumulative mean		16	22	38	138%
STA 5	2004-2	6	2	7	17%
	2005-1	13	10	13	0%
Annual mean		10	5	10	0%
Cumulative mean		29	24	32	10%
STA 6	2004-2	13	6	17	31%
	2005-1	23	11	41	78%
Annual mean		18	9	29	61%
Cumulative mean		26	14	32	23%

* Mosquitofish are collected semiannually at inflow, interior, and outflow sites.

1 - Percent change = outflow-inflow/inflow

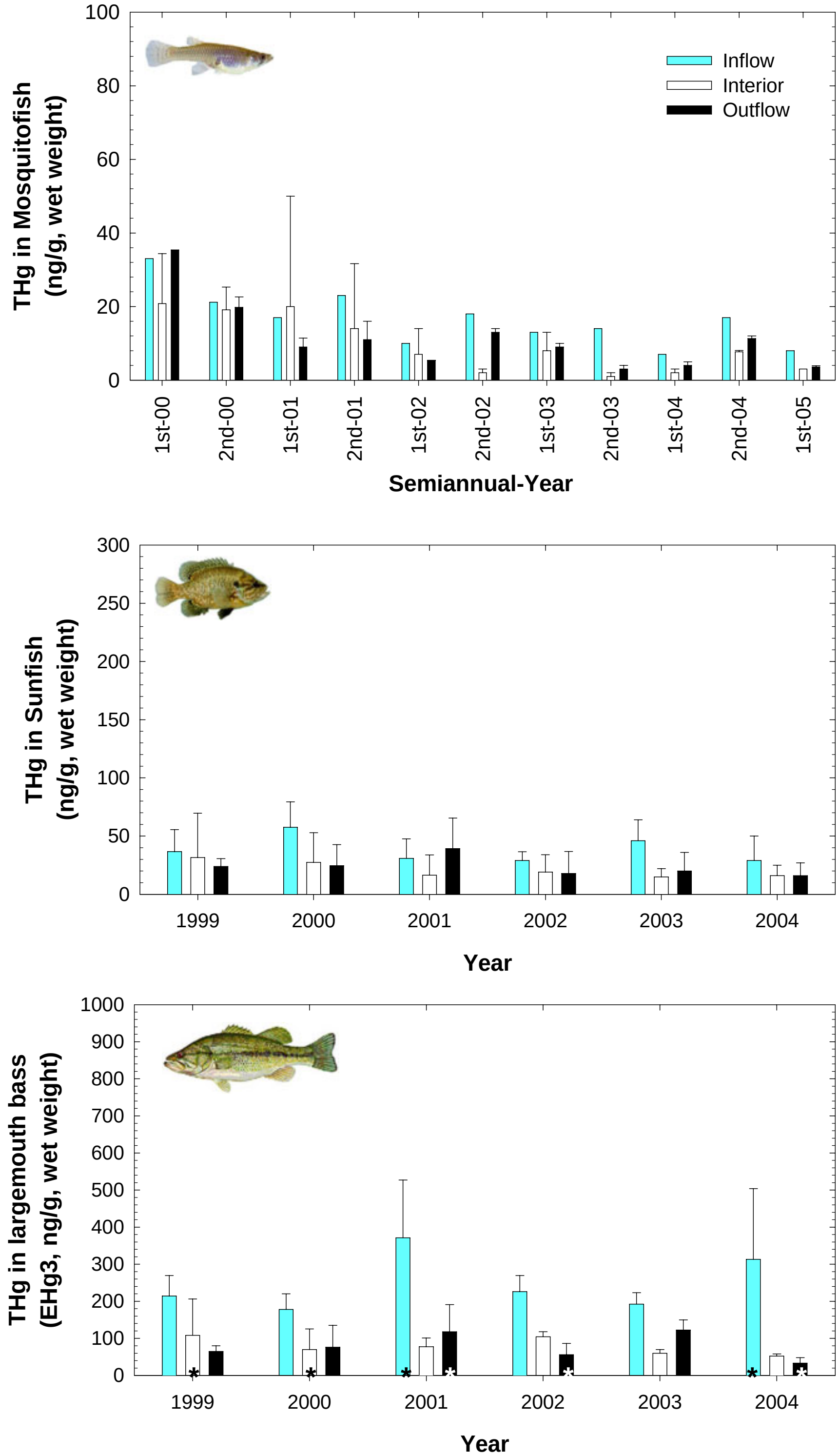


Figure 11. Mercury concentrations (ng/g, wet weight) in (*top*) mosquitofish composites (+ range), (*middle*) whole sunfish (± SD), and (*bottom*) fillets of largemouth bass (± 95% CI or, if arithmetic, SD) collected at STA-1W.

Table 5. Concentration of THg (ng/g, wet weight) in sunfish (*Lepomis* spp.) collected from STAs in 2004 (sample size in parentheses).

STA	Inflow Fish	Interior Fish	Outflow Fish	Percent Change[a]
STA-1W	29 ±21 (20)	16 ±9 (51[b])	16±11 (40)	-45%
Cum. mean[C]	38	19	23	-39%
STA-2	38 ±38(20)	87 ±101 (60)	61 ±66(20)	61%
Cum. mean	72	134	123	71%
STA-3/4	49 ±55 (40)	114 ±62 (60 [b])	101 ±53 (40 [b])	106%
Cum. mean	49	114	101	106%
STA-5	45 ±20 (20)	NA	48 ±21 (8)	7%
Cum. mean	70	96	85	21%
STA-6	42 ±14 (20)	37 ±22 (23[b])	62 ±23(20)	48%
Cum. mean	61	53	104	70%

a. Percent change = outflow-inflow/inflow

b. Where n > 20; multiple sites were sampled and pooled, i.e., multiple interior or outflows.

c. Grand mean of annual means; sunfish collected in 1999, prior to permit revision or STA operation (in the case of STA-5 and STA-1W) were included in the cumulative average.

N/A – no sunfish were collected after 2 hours of electrofishing.

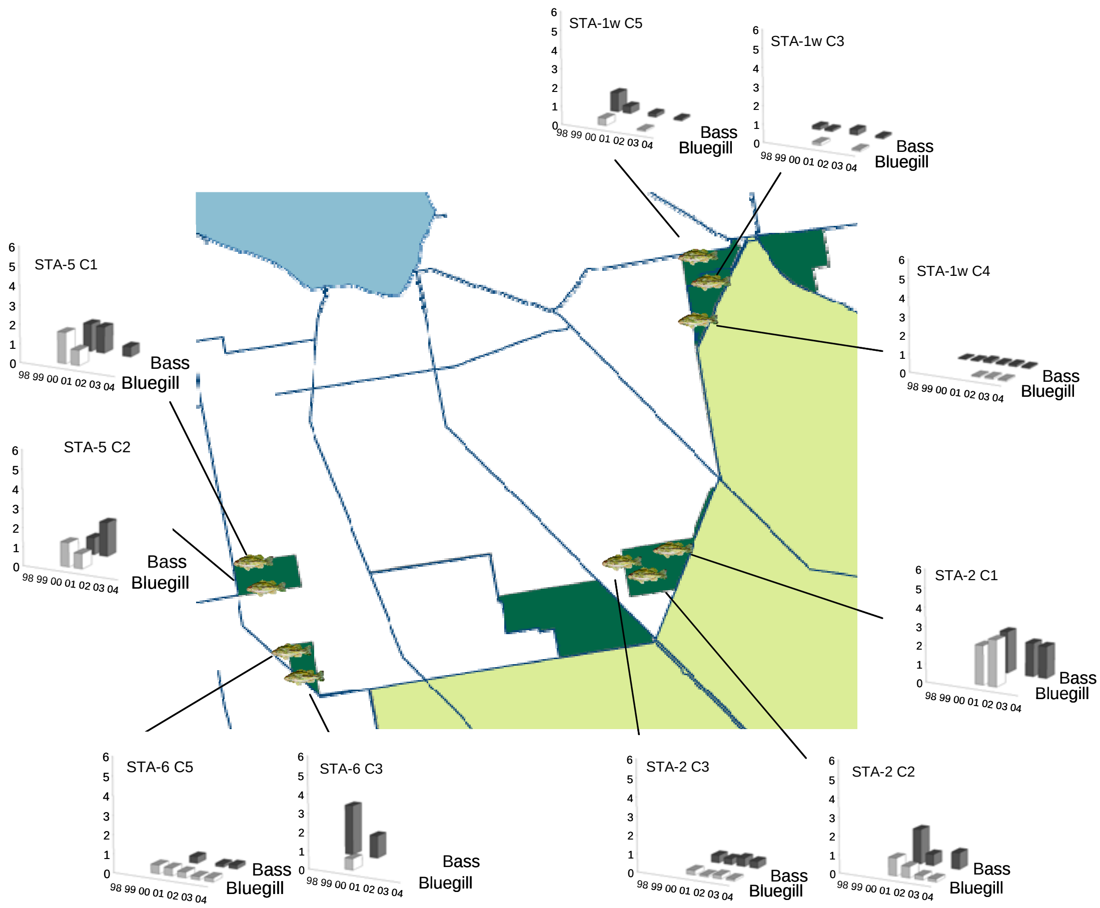


Figure 12. Spatial and temporal patterns in Hg levels in young largemouth bass (i.e., less than 1.8 years old) and bluegill sunfish (4–7 inches in length). Hg levels in fish were further normalized by dividing concentration in a given fish by its total length. Hence, the units of the y-axis are mg/kg/m (c.f. Brumbaugh et al., 2001).

Table 6. Standardized, EHg3 ± 95%, and arithmetic mean concentration (mean ± 1 SD, n; in parentheses) of THg (ng/g, wet weight) in fillets from largemouth bass collected at STAs in 2004.

STA	Inflow Fish	Interior Fish	Outflow Fish	Percent Change[a]
STA-1W	NC (1) (313 ± 191, 20)	52 ± 6 (36 ± 20, 60[b])	NC (1) (33 ± 15, 24)	-848%
Cumulative mean[c]	266	67	69	-286%
STA-2	NC (1) (149 ± 123, 20)	NC (1) (253 ± 240, 59)	218 ± 130 (284 ± 246, 20)	47%
Cumulative mean	242	308	753	67%
STA-3/4	314 ±81 (205 ± 104, 40)	NC (2) (279 ± 137, 60)	712 ± 77 (605 ± 255, 40)	66%
Cumulative mean	205	279	604	66%
STA-5	213 ±56 (115 ± 49, 20)	NA NA	NC (1) (418 ± 219, 19)	72%
Cumulative mean[d]	182	315	435	58%
STA-6	257 ±27 (203 ± 99, 20)	167 ± 64 (155 ± 160, 11)	318 ± 45 (356 ± 145, 20)	19%
Cumulative mean	266	327	495	46%

a - Percent change = outflow-inflow/inflow.

b - Where n > 20; multiple sites were sampled and pooled, i.e., multiple interior or outflows.

c – Arithmetic grandmean of annual means; bass collected in 1999 prior to operation of STA-5 and STA-1W were included.

NC = not calculated, where: (1) regression slope was not significantly different from 0, or (2) poor age distribution of collected fish.

NA = not available; no bass in sample.

STA-2

STA-2 Cells 2 and 3 met Hg start-up criteria in September 2000 and November 2000, respectively. In August 2001, flow-though operation of Cell 1 was approved under a permit modification; Cell 1 finally met start-up criteria in November 26, 2002. Operational monitoring of Hg at STA-2 began during the third quarter of 2001, following the completion of the S6 connection in May 2001 (for discussion of results observed prior to WY2005, see Rumbold and Fink, 2002b, 2003b; Rumbold 2004 and 2005).

Results from monitoring Hg concentrations in surface water at STA-2 are shown in **Tables 2** and **3**, and graphically presented in **Figure 13**. During the reporting year, THg concentration in the outflow of STA-2 did not exceed the Class III WQS of 12 ng/L. More importantly, both MeHg, which has no numerical WQS, and THg have remained at low concentration in the outflow of STA-2 as compared to previous monitoring results (**Figure 13**); annual outflow load has declined since 2003 (**Figure 9**). During the water year, both constituents were found in very similar concentrations in the inflow and outflow (i.e., very small or negative percent change across the STA, **Table 3**). It is noteworthy that Cell 1 has remained inundated since 2002 (**Figure 14**), when the weir boxes in front of the outflow culverts of the cell were reconfigured to increase control elevation. This was done to reduce the influence of outflow pump operation and prevent recurrence of the steep gradients in stage within the cell.

Results from operational monitoring of Hg concentrations in STA-2 mosquitofish are summarized in **Table 4** and **Figure 15** (note that the results from different interior sites are graphed separately for this STA because of the degree of spatial variability observed here in the past). As evident from **Figure 15**, Hg levels have declined dramatically in mosquitofish from Cell 1 and the discharge canal since 2001 and 2002 (in some cases, by an order of magnitude) and have remained low..

Sunfish from STA-2 continued to show substantial among-cell differences in Hg levels with relatively higher levels occurring in resident fish from the Cell 1 (**Figure 15**). Temporal trends in Hg levels in sunfish were difficult to accurately assess due to common confounding factors: (1) that multiple lepomid species were collected and Hg levels have been found to vary among species, and (2) sunfish size varied (i.e., fish size is a surrogate for age and Hg levels in fishes tend to increase with age). Although bluegill were caught in Cells 2 and 3 in 2004, no bluegill were collected from Cell 1. When warmouth from Cell 1 (55 percent of sunfish caught in 2004) were singled out and normalized to total length, Hg levels at site C1X differed among years ($df = 2, 20$; $F = 27.9$, $P < 0.001$) with 5.45 mg/kg/cm in 2002 fish, 2.57 mg/kg/cm in 2003 fish, and 1.72 mg/kg/cm in 2004 fish (Tukey Post-hoc test found levels in both 2004 fish and 2003 fish differed significantly from 2002 fish, but not between 2004 and 2003). When a similar analysis was carried out on redear sunfish from site C1X, fish from both 2004 and 2003 again contained significantly lower Hg levels than fish collected in 2002 (Kruskal-Wallis test; $H = 15.1$, $df = 3$, $p = 0.002$; Dunn's Test $p < 0.05$). Similar to the warmouth, 2004 fish did not differ from 2003 fish. Thus, there is an indication that Hg levels are declining in sunfish from Cell 1, though it is not readily apparent from visual inspection of **Figure 15**. Interestingly, resident bluegill from the discharge canal (most numerous species collected from the canal over the monitoring period) did not exhibit among-year differences in Hg levels (for the period from 2001–2004; ANOVA, $df = 3, 32$; $F = 1.41$; $p = 0.26$). This is interesting because, as will be discussed below, bass from the discharge canal showed a dramatic decline in Hg levels.

Concentrations of THg in fillets of resident largemouth bass from STA-2 are summarized in **Table 6** and are graphically presented in **Figure 15**. As mentioned above, bass from the discharge canal exhibited significant among-year differences in Hg levels (ANCOVA, $df = 3,75$; $F = 29.06$, $p < 0.001$); though pairwise comparisons found no difference in LSM between 2001 and 2002 (Tukey-Kramer post-hoc test, $p = 0.83$), levels in both 2004 and 2003 differed from levels in

2001 and 2002 ($p < 0.0001$), and 2004 differed from 2003 ($p < 0.0001$). This relatively rapid change in Hg levels in large-bodied fish, likely owing to changes in population structure, is not surprising given the dynamic nature (harsh environment) of this small canal during extreme pump operations (stage in canal can be drawn down 6 feet). Hg levels also declined in bass collected from site C1X in 2004, though the decline appears much more gradual than in the canal population. The decline was most evident in the young bass from Cell 1, suggesting recent declines: levels of Hg in one-year-old bass varied among years (ANOVA, df = 2, 22; $F = 4.2$, $P = 0.03$) with mean concentrations of 0.56 mg/kg in 2001, no fish from C1X in 2002, 0.49 mg/kg in 2003, and 0.34 mg/kg in 2004 (Tukey Post-hoc test found 2004 fish differed significantly from 2001 but not 2003 fish).

Risks to fish-eating wildlife foraging at STA-2 are assessed in an accompanying appendix (Appendix 4-7 of this volume), which represents an update to the probabilistic ecological risk assessment that was submitted to the FDEP in 2004 (Rumbold, 2004b; transmittal letter from R. Bearzotti, SFWMD, dated March 30, 2004).

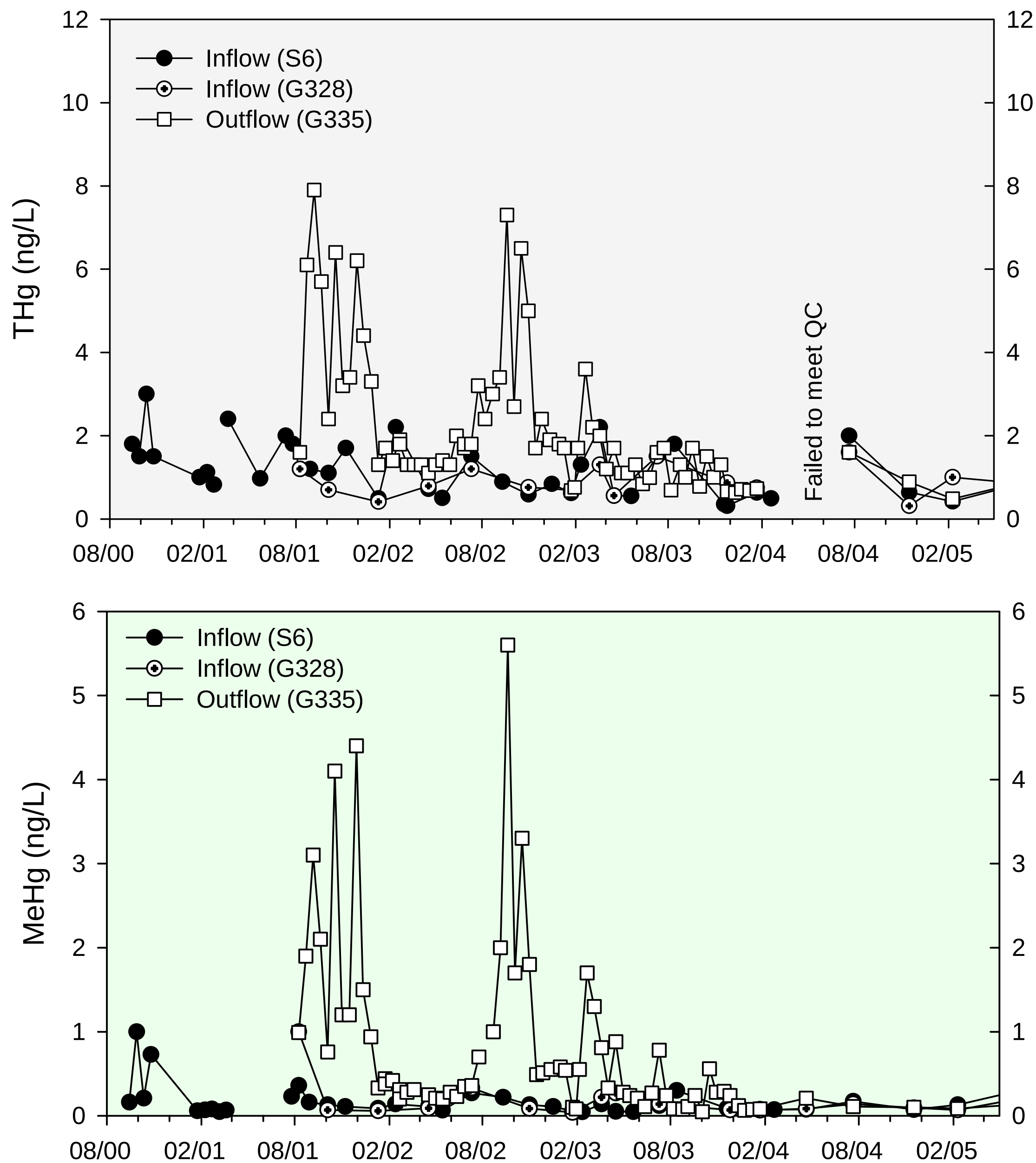


Figure 13. Concentrations of (a) THg and (b) MeHg (ng/L) in unfiltered surface water collected at STA-2, including routine and expanded sampling.

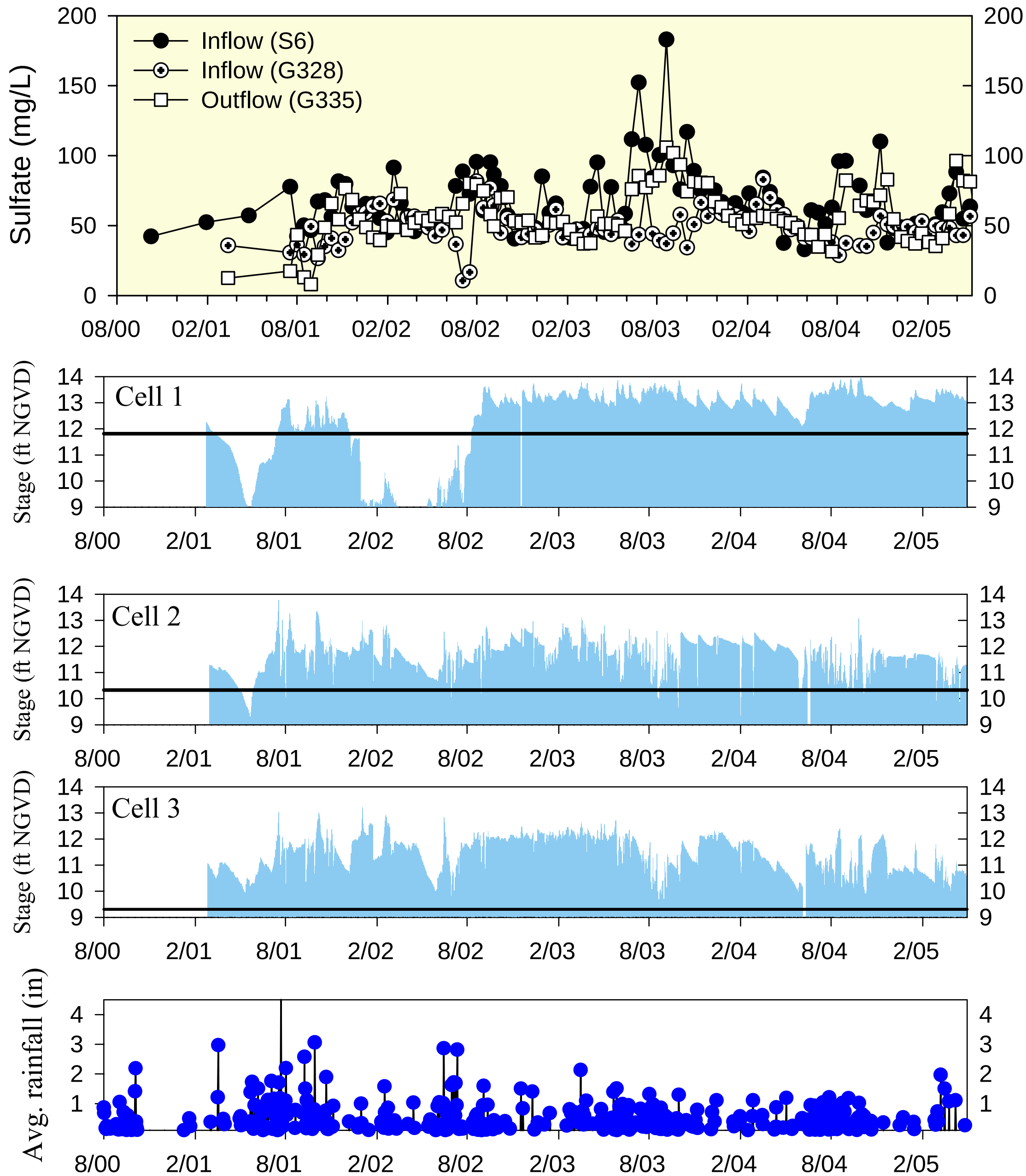


Figure 14. Water-column sulfate, stage (recorded immediately upstream of outflow culvert of cell) and rainfall totals at STA-2.

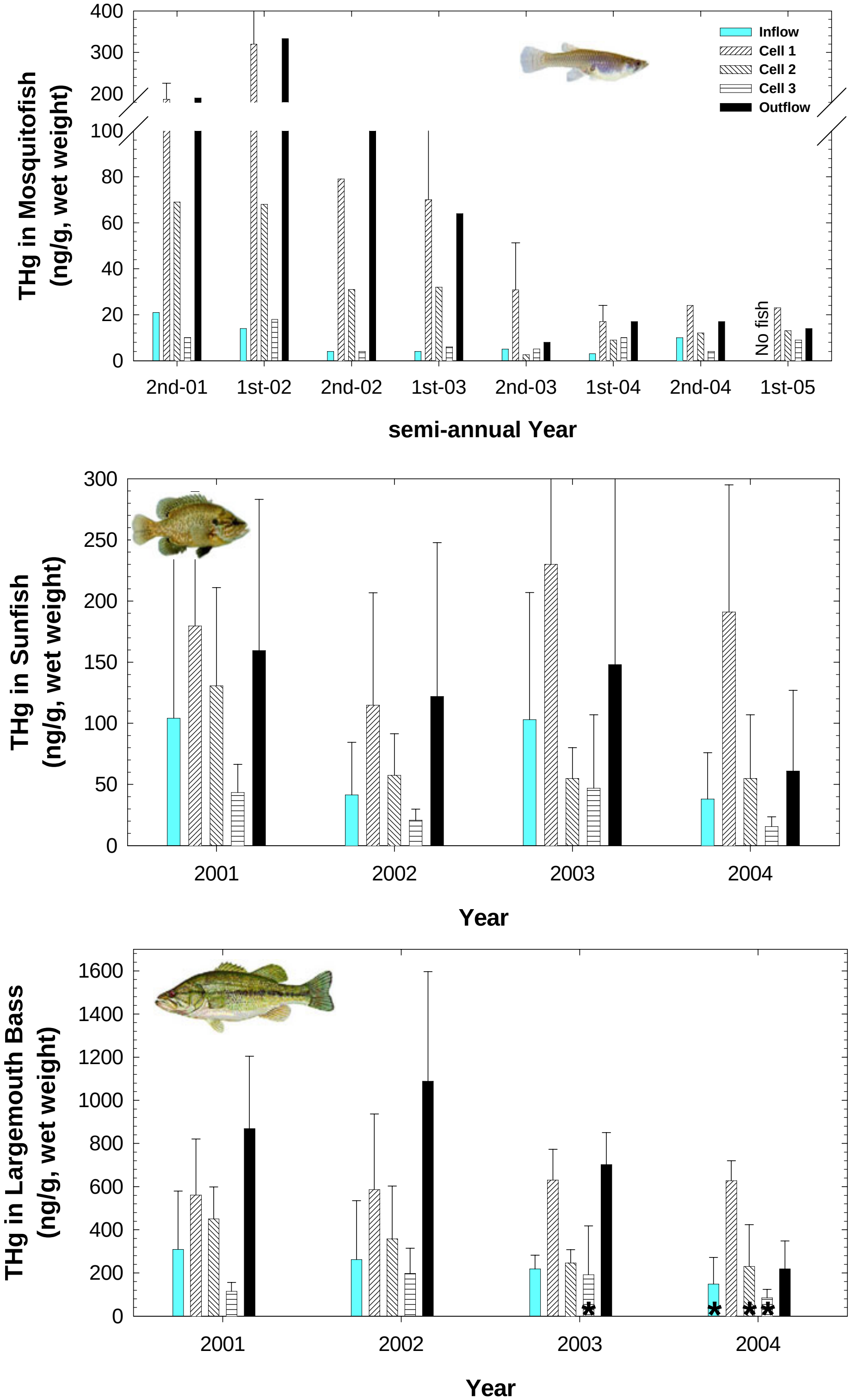


Figure 15. Mercury concentrations (ng/g, wet weight) in (*top*) mosquitofish composites (+ range), (*middle*) whole sunfish (± SD), and (*bottom*) fillets of largemouth bass [± 95-percent confidence interval (95% C.I.) or, if arithmetic, SD] collected at STA-2.

STA-3/4

As reported above, STA-3/4 Cell 1 satisfied start-up criteria for Hg in January 2004; the first discharges of treated water from this STA in February 2004. Routine operational monitoring of this flow-way began during the first quarter of 2004. STA-3/4 Cell 3 satisfied start-up criteria for Hg in June 2004; Cell 2 in August 2004, with consensus from FDEP in September 2004, at which time discharges began.

Results from monitoring Hg concentrations in surface water at STA-3/4 are shown in **Tables 2** and **3**, and graphically presented in **Figure 16**. Routine quarterly monitoring at all inflows and outflows began in the fourth quarter of 2004. As evident from **Figure 16**, although MeHg concentrations were somewhat dissimilar in the two outflows from Cell 1 during its initial operation, since the fourth quarter both THg and MeHg appear to be at very similar concentration in the outflows of each of the cells, and more importantly, as compared to the inflows. The most obvious exception to this was the relatively higher concentration of THg observed at the two inflows in the first and second quarter of 2005 (**Figure 16**), which resulted in a large negative percent change across the STA for that quarter (**Table 3**). The percent change across the STA declined over the water year for both THg (ended on a negative percent change) and MeHg. MeHg was only slightly elevated in the outflow of Cell 3 during the first and second calendar quarter of 2005. It should be noted that Cell 3 was almost dry just a few weeks earlier, at the time of the first quarterly sample (**Figure 17**) and this drawdown (for construction activities; for details, see Chapter 4 this volume) and oxidation of sediments may have played a role in the slight elevation. At this point it should be made clear that the second-quarter sampling in 2005 was carried out in April, just a few weeks following the first-quarter sampling, at the request of the FDEP laboratory, to enable them to accept a large number of samples associated with the USEPA REMAP sampling effort.

Results from operational monitoring of Hg concentrations in resident fish from STA-3/4 are summarized in **Tables 4**, **5,** and **6** and **Figure 18**. Tissue-Hg was only slightly elevated in mosquitofish from the newly operated STA as compared to mosquitofish recently collected from the other STAs (**Table 4**). The Hg levels in these mosquitofish were similar to or lower than concentrations found in mosquitofish inhabiting downstream sites (see Appendix 2B-1 of this volume).

Similar to the mosquitofish, resident sunfish in the newly operating STA contained elevated Hg levels compared to fish from other STAs (**Table 5**), but not as compared to downstream sites (see Appendix 2B-1 of this volume). The average tissue concentration in sunfish from the three cells was 114 ± 62 (n = 60; **Table 5**). Among-cell differences could not be assessed due to marked differences in lepomid species collected: Cell 1 sample consisted of 1 bluegill, 7 redear, and 12 warmouth; Cell 2 sample consisted of 15 bluegill, 1 spotted, and 4 warmouth; Cell 3 consisted of 5 bluegill, 14 redear, and 1 warmouth. Two sites were sampled to serve as outflow monitoring stations for the newly operational STA (i.e., ST34DCW and ST34DCE; two sites were sampled rather than one, to assess whether a plug in the canal for construction purposes may have created two different outflows with differing fish populations). Data on these fish were compared to results of previous sampling at the L5F1 site (located in the same canal, between ST34DCW and ST34DCE). Site L5F1 was being sampled as a default canal site because of problems in accessing the downstream marsh monitoring site CA33 (note, access problems were not an issue in 2004 and, accordingly, CA33 was sampled; see Appendix 2B-1 of this volume). When data from these sites were compared to data from samples collected from site L5F1 during the previous year, statistical differences were evident (df = 3, H = 36.9, $p < 0.001$); fish from site CA33 contained significantly higher Hg levels than the other three sites (median values were 130 ng/g at CA33, 109 ng/g at ST34DCW, 72 ng/g at ST34DCE, and 120 ng/g at L5F1). However, there were also significant among-site differences in size of fish and species of lepomid collected.

Although site CA33 had the smallest fish (median weight was 20 g), the sample of 20 sunfish included 6 warmouth and 2 spotted sunfish (the two species that typically contain significantly greater Hg) but no redear (i.e., the remainder were bluegill); none of the other sites included warmouth. Further, although ST34DCE contained the largest fish on average (median weight was 67 g), 16 of the 20 fish sampled were redear sunfish (the species that typically contains significantly lower Hg). The sample from site ST34DCW contained only 8 redear. Based on the among-species differences in Hg levels reported in the Appendix 2B-1, it is likely that the differences in composition of species of lepomids collected from the various sites confounded any among-site differences in ambient Hg conditions (especially between sites ST34DCW and ST34DCE). This conclusion was strengthened when an assessment of the bluegill sunfish collected from these sites revealed no significant among-site differences in Hg levels (df = 3, H = 0.45, p = 0.93).

Largemouth bass were also collected at sites ST34DCW and ST34DCE and were found to vary in levels of Hg; EHg3 was estimated to be 639 ± 71 ng/g at ST34DCW and 892 ± 140 ng/g ST34DCE. At this point it is difficult to speculate as to why these fish differed in Hg content when a very short time ago they were likely the same population (i.e., prior to the plug being placed in the canal). However, it should be emphasized that, owing to the fact that the STA only began flow-through operation in early 2004, Hg levels in the large-bodied fish in this water year (**Table 6**, **Figure 18**) represent baseline exposures. Fish collected from this same canal (i.e., site L5F1) in 2003 contained an average concentration of 842 ± 315 ng/g (n = 11; although an EHg3 could not be calculated – mean age of those fish was 2.98 years). An EHg3 could not be estimated for bass collected from Cell 1 or 2, because sampled populations consisted solely of young-of-the-year fish (i.e., < 1 year old); mean concentration in these young bass was 210 ± 74 ng/g in Cell 1 and 248 ± 98 ng/g in Cell 2. The age distribution was wider for bass sampled from Cell 3 allowing for an estimation of an EHg3 of 649 ± 182 ng/g; for comparative purposes, bass < 1 year old from Cell 3 contained, on average, 184 ± 79 (n = 5).

In terms of the risk to fish-eating wildlife, mosquitofish from STA-3/4 contained Hg at concentrations lower than either the USFWS (100 ng/g), or USEPA criteria (77 ng/g). However, sunfish from STA-3/4 contained levels just exceeding the USFWS criteria. This finding is significant because sunfish represent the preferred prey item of many fish-eating species in the Everglades. After adjusting the arithmetic mean Hg concentrations in fillets to whole-body concentrations (whole-body THg concentration = 0.69 x fillet THg; Lange et al., 1998) Hg levels in largemouth bass from interior marshes of STA-3/4 (mean was 192 ng/g) were less than the USEPA predator protection criteria based on TL 4 fish (i.e., 346 ng/g). Therefore, fish-eating wildlife foraging preferentially at STA-3/4 would appear to have a moderate (due to the levels in the sunfish) to low risk from Hg exposure.

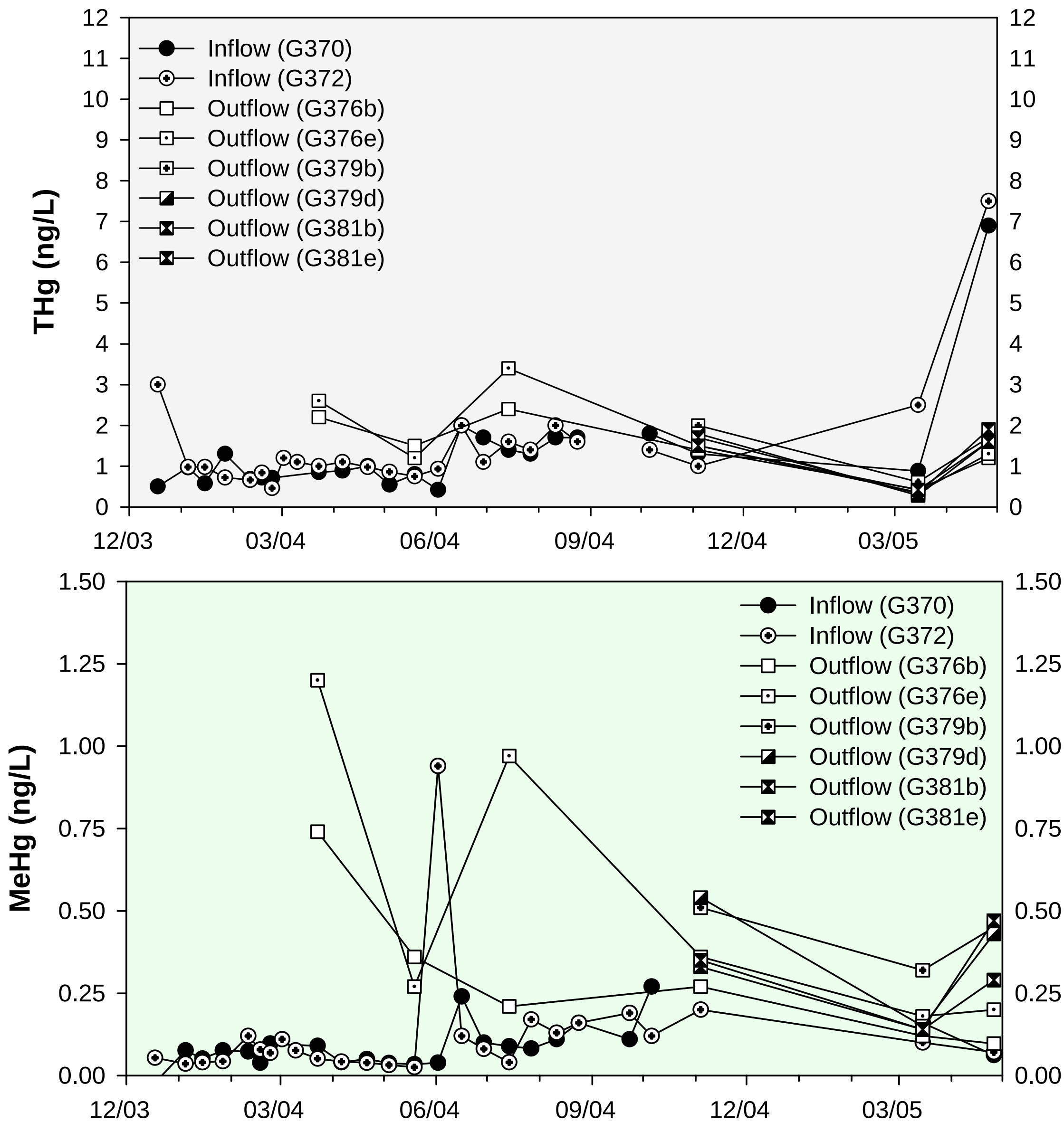


Figure 16. Concentrations of (a) THg and (b) MeHg (ng/L) in unfiltered surface water collected at STA-3/4, including results of start-up monitoring at inflows (i.e., prior to flow-through operation of all cells).

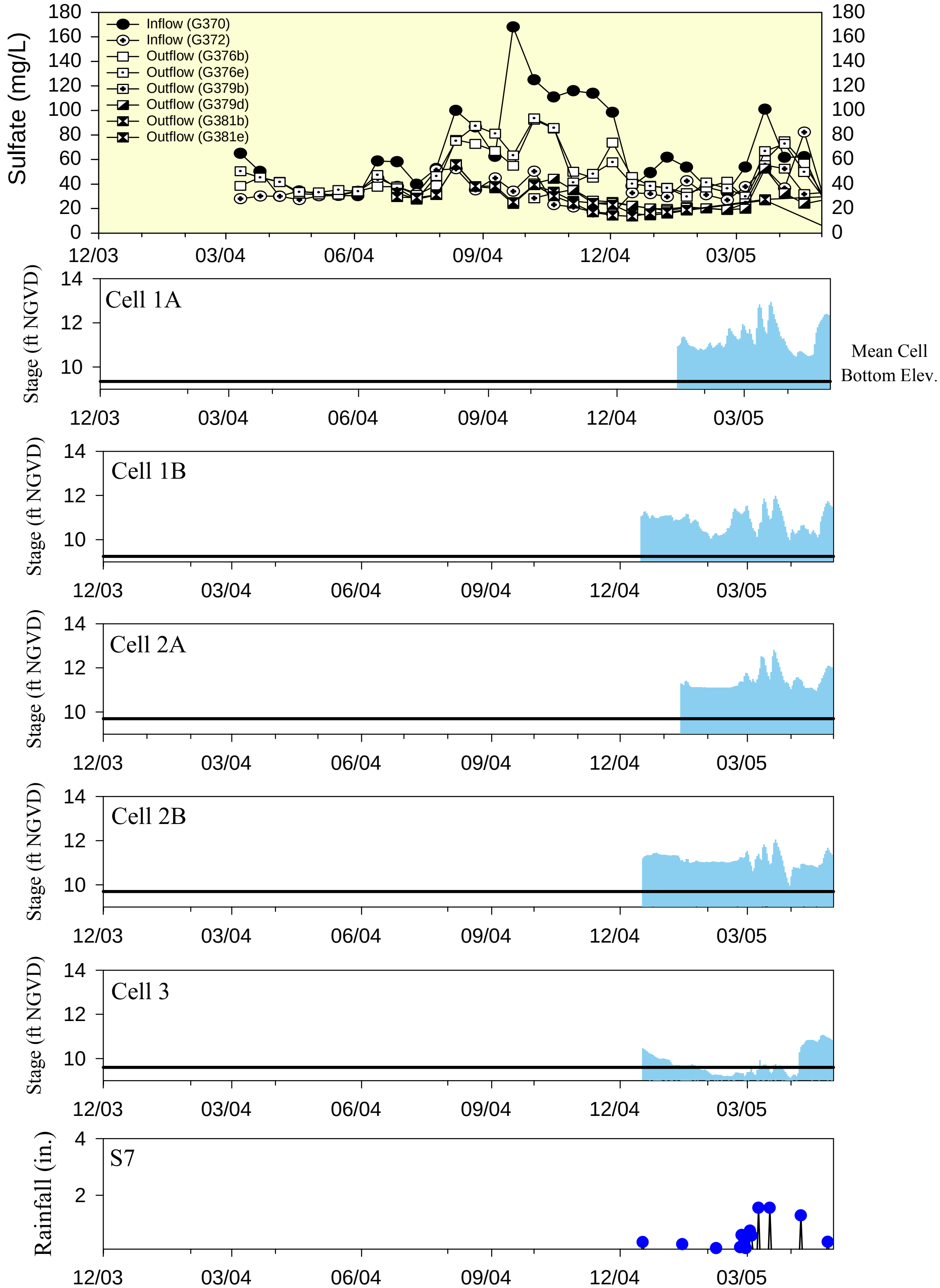


Figure 17. Water-column sulfate, stage (recorded immediately upstream of outflow culvert of cell) and rainfall at STA-3/4.

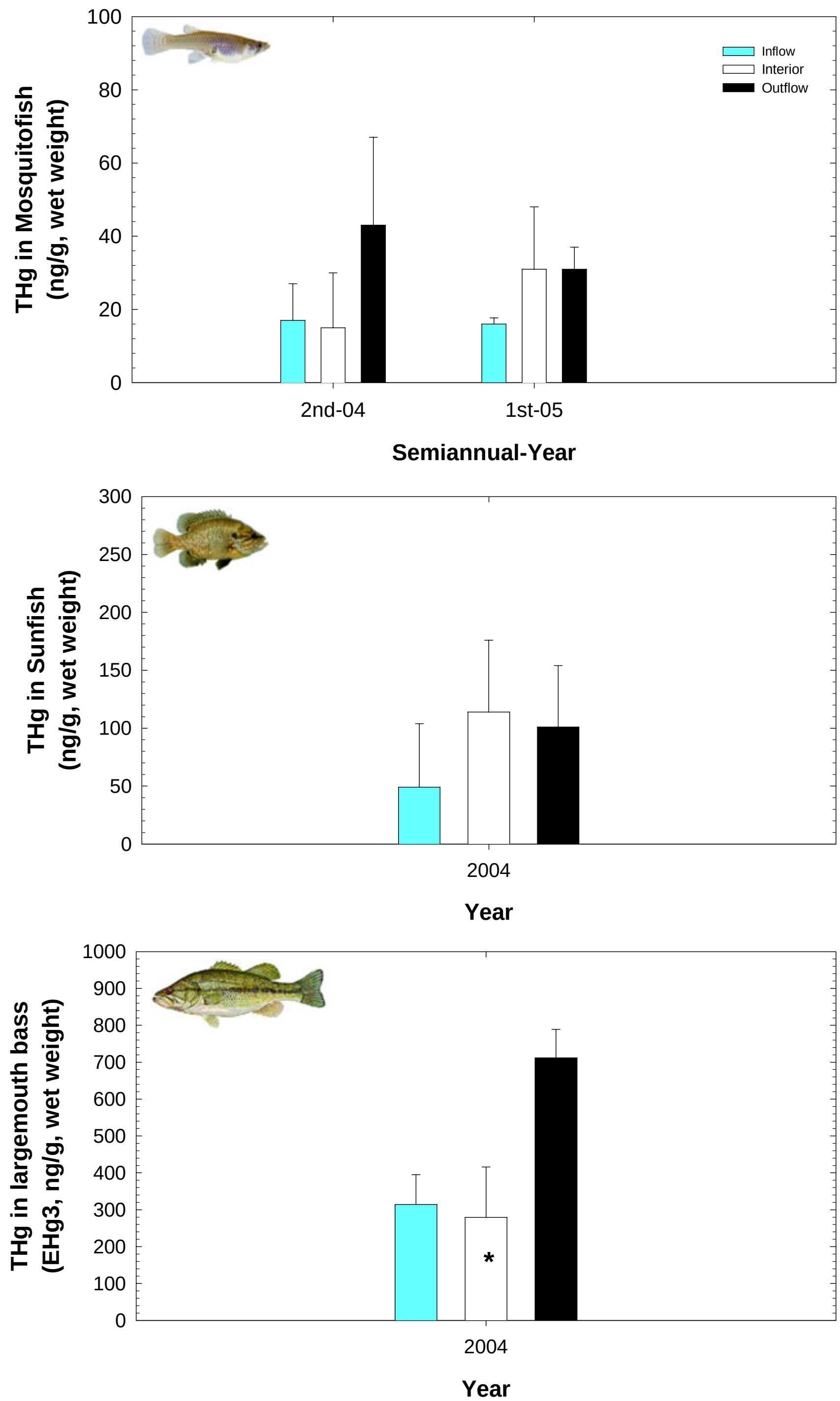


Figure 18. Mercury concentrations (ng/g, wet weight) in (*top*) mosquitofish composites (+ range), (*middle*) whole sunfish (± SD), and (*bottom*) fillets of largemouth bass (± 95% C.I. or, if arithmetic, SD) collected at STA-3/4.

STA-5

STA-5 met start-up criteria for Hg in September 1999; however, because of drought conditions and the detection of high phosphorus concentrations at the outflows, STA-5 did not begin flow-through operation until July 2000 (for discussion of results observed prior to WY2005, see Rumbold and Rawlik, 2000; Rumbold et al., 2001a; Rumbold and Fink, 2002a; Rumbold and Fink, 2003a; Rumbold, 2004a and 2005).

The triennial collection of sediments cores (10-cm depth) from STA-5 occurred on November 30, 2004. Both THg and MeHg levels in these cores (**Table 1**) were within the range of concentrations observed in 10-cm sediment cores previously collected from all the STAs pooled: median concentration (and 95th percentile) was 81 ng THg/g (140 ng/g, n = 121) and 0.45 ng MeHg /g (3.2 ng/g; n = 115). Further, there was no significant among-year (e.g., 1998, 2001, 2004) difference in either THg levels (ANOVA, df = 2,15; F = 0.26, p = 0.77) or MeHg levels (df = 2, 15; F = 0.54, p = 0.6) in the sediments from STA-5.

As shown in **Table 2** and **Figure 19**, water-column concentrations of both THg and MeHg remained low at STA-5 during the water year relative to previously observed spikes. At no time during the reporting year did THg concentrations exceed the Class III WQS of 12 ng/L. Equally important, THg exhibited a large negative percent change across the STA (**Table 3**). Alternatively, MeHg, which has no numerical WQS, was at higher concentration in the outflow as compared to the inflow during two quarters; however, the STA exhibited only slight negative percent change for the year (**Table 3**).

During the water year, stage did fluctuate in the Cells 1B and 2B (drawdowns in late August, early September, **Figure 20**); however, the fluctuations were not as severe as in previous years. It is noteworthy that these drawdowns and re-inundations were not followed by any significant spikes in water column THg or MeHg (i.e., in October 2004 or January 2005) as observed following similar events in the past. In January 2005, Cell 1B was taken off-line in order to improve the water control structures (G-343A–D) and degrade a high berm located by the outflow culverts, as part of a Long-Term Plan enhancement (for details, see Chapter 4 this volume).

Mosquitofish collected near the outflow and inflow culverts of STA-5 contained almost identical levels of Hg during the water year (**Table 4, Figure 21**). More importantly, mosquitofish from the interior marshes contained low Hg levels as compared to fish at the inflows and outflows, other STAs (**Table 4**), and as compared to levels currently found in mosquitofish inhabiting many downstream marshes or canals (see Appendix 2B-1 of this volume).

As in previous years, the Florida Fish and Wildlife Conservation Commission (FWC), which is under contract to the District to electroshock and collect large-bodied fishes for Hg monitoring, encountered difficulty in filling sample quotas at STA-5. None of the targeted species (sunfish or bass) were found in the interior marshes of Cell 1B or 2B. Sunfish caught in the supply and discharge canals of STA-5 contained similar levels of Hg (H = 0.04, df = 1, p = 0.8). These levels were also comparable to fish from other STAs (**Table 5**), but less than levels observed in downstream sunfish (see Appendix 2B-1 of this volume). In terms of temporal trends, sunfish caught from the discharge canal in 2004 contained the lowest median concentration of Hg (42.5 ng/g, n = 8) since monitoring began in 1999; among-year differences were significant (H = 15.7, df = 4, p = 0.003), with 2004 levels significantly lower than 2001 levels (Dunn's Test, $p < 0.05$; no other pairwise comparisons were significant).

Concentrations of THg in bass caught in the supply and discharge canals of STA-5 are summarized in **Table 6** and are graphically presented in **Figure 21**. As reported in previous years, bass from the discharge canal contained significantly greater concentrations of Hg than fish from the supply canal (Mann-Whitney Rank Sum Test, $T = 555.5$, $n[small] = 19$, $n[big] = 20$, $p = < 0.001$). However, this spatial difference must be interpreted cautiously, because a significant age-concentration (regression) relationship was not demonstrated in fish from the discharge canal and, consequently, ANCOVA could not used to assess covariance. In this regard, bass from the discharge canal were found to be significantly larger ($T = 487$, $p = 0.003$) and older ($T = 519.5$, $p = < 0.001$) than fish from the supply canal, and this may have skewed the results confounding any spatial difference in exposure. Temporal trends must also be assessed cautiously, because fish caught in the discharge canal were smaller (i.e., in terms of weight) than fish in previous years ($H = 18.380$, $df = 3$, $p = < 0.001$; Dunn's post hoc test showed 2004 fish were significantly smaller than 2001 fish), although an ANOVA on ranks found no difference in age among years ($H = 2.2$, $p = 0.529$). Given these possible confounding factors, which might mask true temporal differences, Hg levels in the discharge bass did not differ among years ($H = 5.3$, $df = 3$, $p = 0.15$).

In terms of the risk to fish-eating wildlife, resident fish at STA-5, representing three different trophic levels, all contained Hg levels lower than both USFWS (100 ng/g) and USEPA predator protection criteria (77 ng/g, 346 ng/g for TL 4 fish). Therefore, fish-eating wildlife foraging preferentially at STA-5 would not appear to be at elevated risk from Hg exposure.

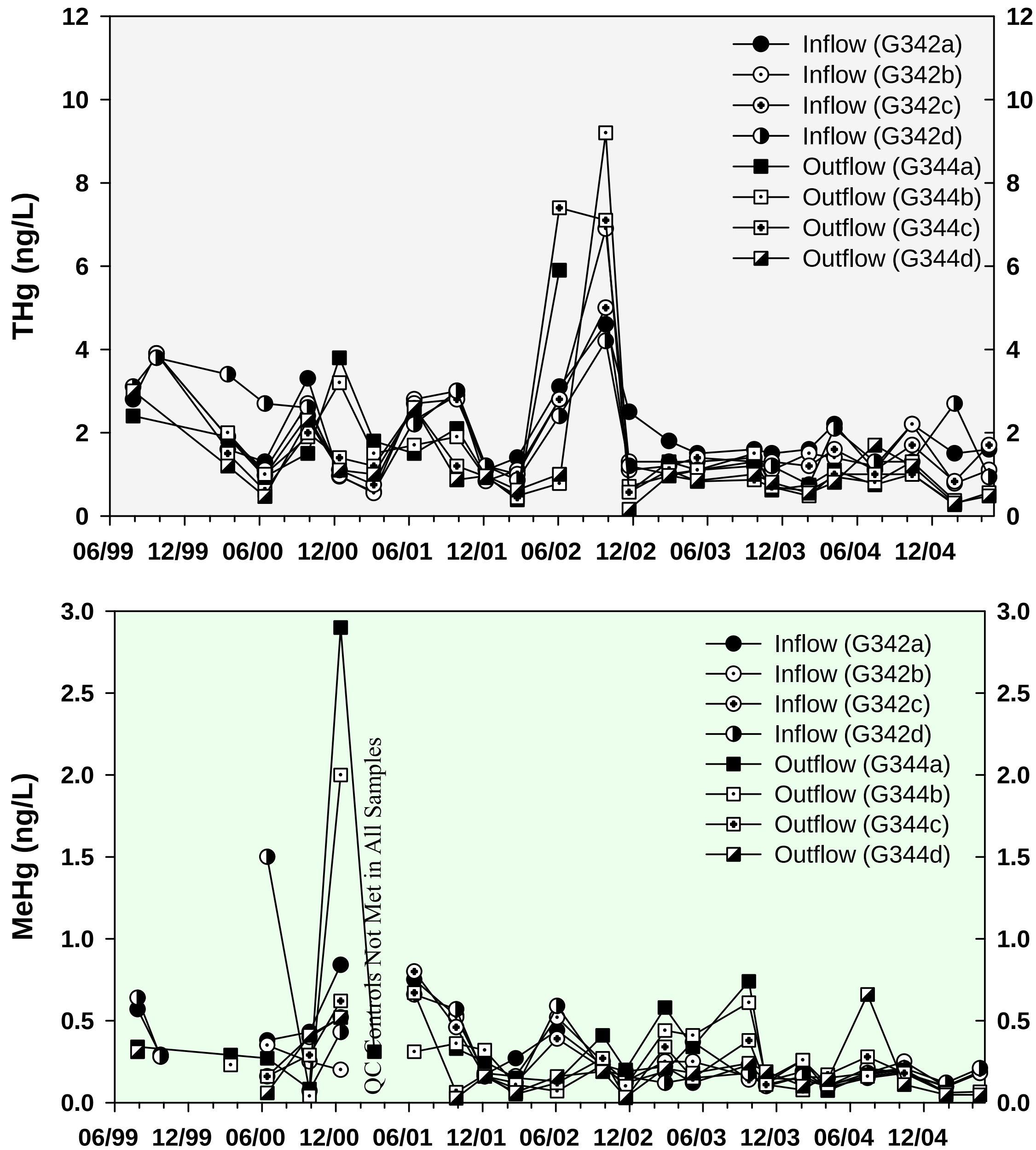


Figure 19. Concentrations of (a) THg and (b) MeHg (ng/L) in unfiltered surface water collected at STA-5.

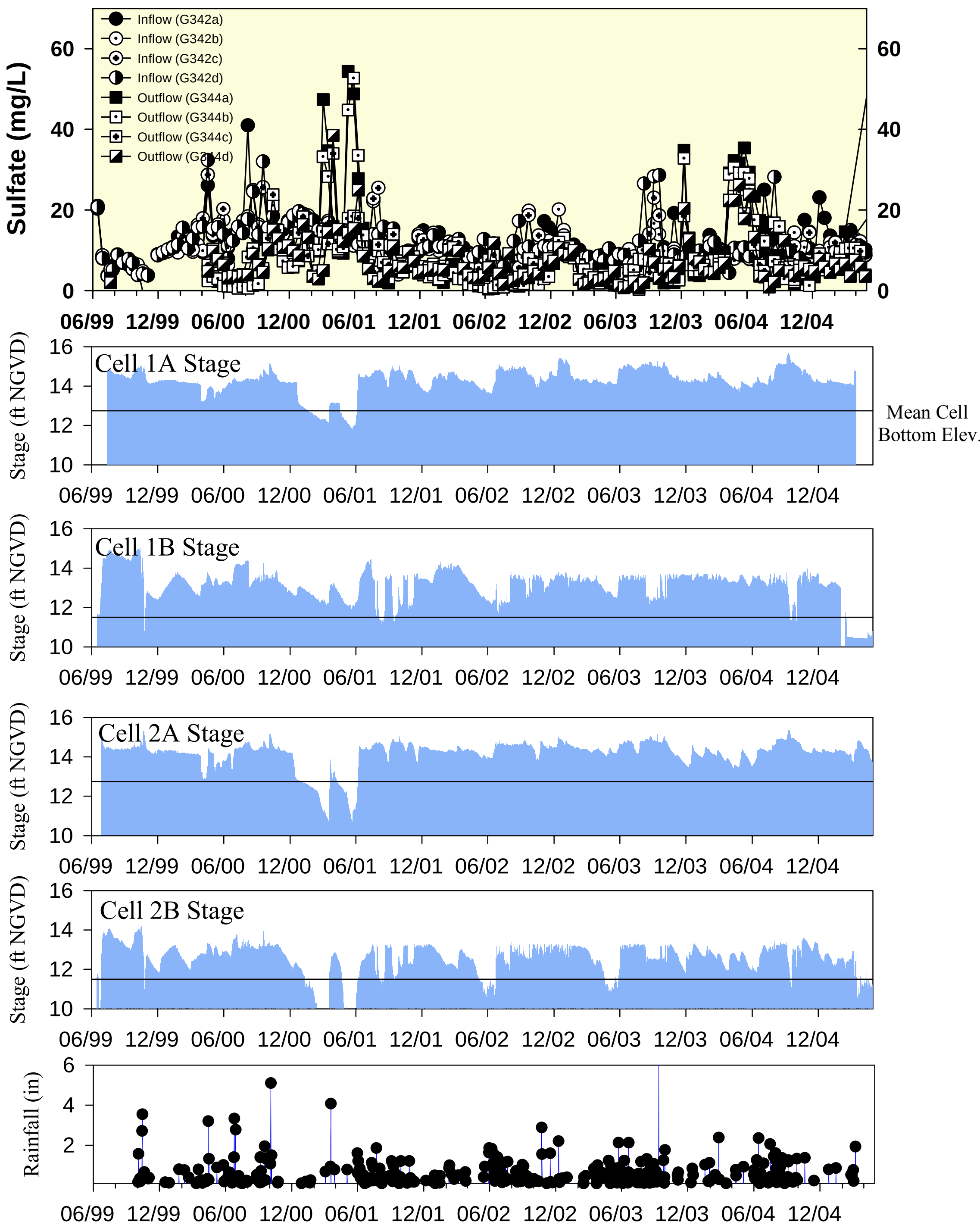


Figure 20. Water-column sulfate, stage (recorded immediately upstream of outflow culvert of cell) and rainfall at STA-5.

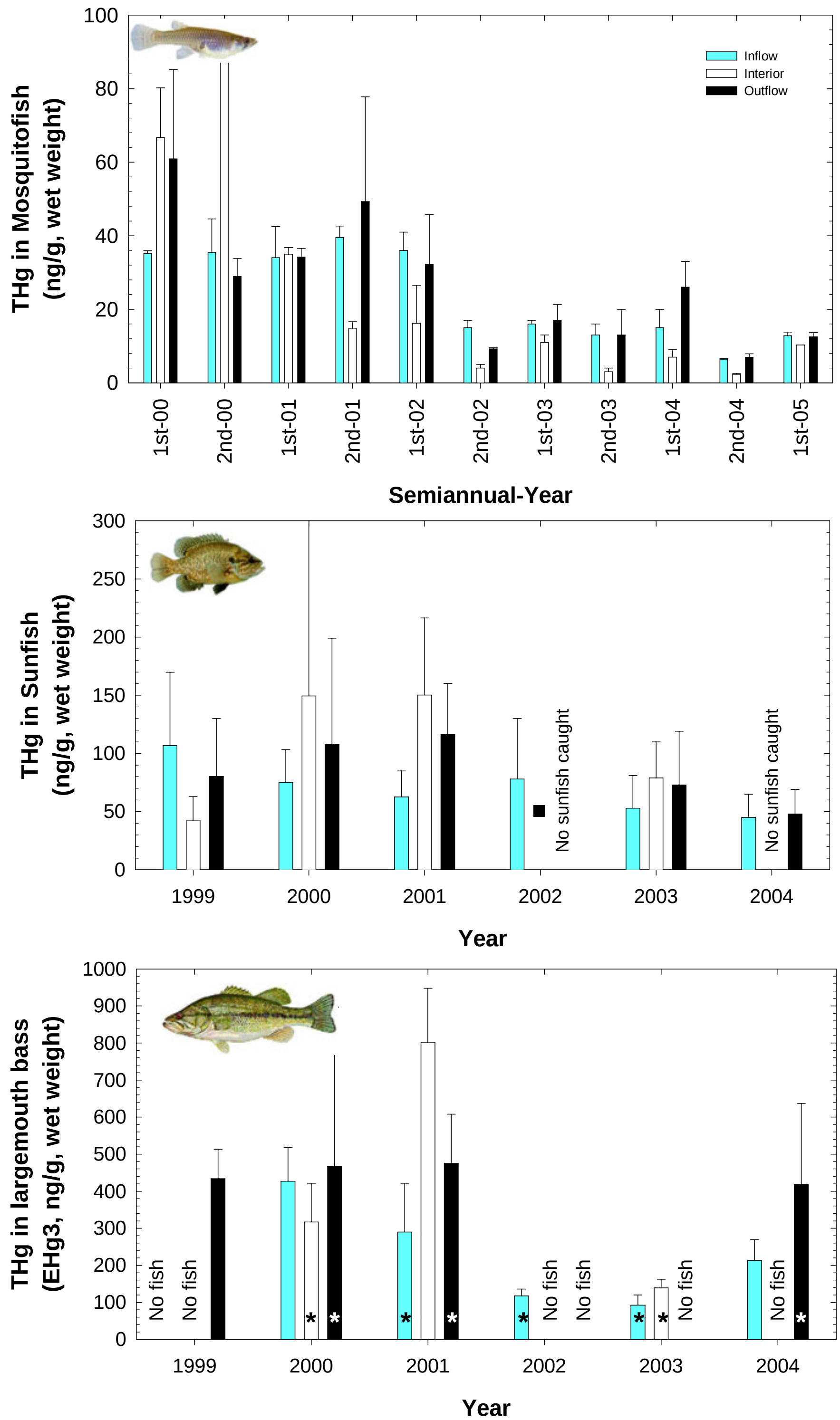


Figure 21. Mercury concentrations (ng/g, wet weight) in (*top*) mosquitofish composites (+ range), (*middle*) whole sunfish (± SD), and (*bottom*) fillets of largemouth bass (± 95% C.I. or, if arithmetic, SD) collected at STA-5.

STA-6, Section 1

STA-6, Section 1 (Cells 3 and 5) met start-up criteria for Hg in November 1997, and began operation in December 1997. Routine monitoring of Hg at STA-6 was initiated in the first calendar quarter of 1998. Results of monitoring prior to May 1, 2004 have been reported previously (SFWMD, 1998c and 1999c; Rumbold and Rawlik, 2000; Rumbold et al., 2001a; Rumbold and Fink, 2002a; Rumbold and Fink, 2003a; Rumbold, 2004a and 2005).

As evident from data tabulated in **Table 2** and graphically presented in **Figure 22**, both THg and MeHg concentrations spiked in STA-6 outflows during the second quarter of 2004. It is noteworthy that there was no period in which THg concentrations exceeded the Class III water quality standard of 12 ng/L, and that these spikes were not as high as those observed in 2001 and 2002 (**Figure 22**). All three periods where water-column concentrations spiked followed dryout and reflooding of the cells (**Figure 23**) are believed to be a result of sediment oxidation and altered sulfur biogeochemistry. Note that sulfate concentration spiked in outflows (relative to inflows) following each rewetting event (**Figure 23**). Similar spikes have also been observed at STA-5 (**Figure 20**). One possibility is that sulfate released from oxidation of organic sulfur pools in the sediments stimulated methylation; however, sulfate loads in the inflow water are not believed to be limiting (**Figure 23**). The more likely scenario here is that the drydowns altered redox conditions and reduced porewater sulfide concentrations that at other times are inhibiting Hg methylation in the STA (for discussion of sulfide inhibition, see Benoit 1999a and b). It is noteworthy that STA-6 also dried down in 1998, 1999, and 2000 (**Figure 23**); however, spikes in THg and MeHg were not observed (**Figure 22**) **—** at least not at the sites that were being sampled at the time (i.e., G607 and G606 located in the discharge canal as opposed to current sites, immediately upstream of the outflow culverts). It is possible that spikes occurred in the marsh but were not captured at the downstream sites.

Following the 2002 spike, the monitoring program was immediately expanded to include monthly sampling. This increased frequency of sampling demonstrated that concentrations of both THg and MeHg declined to more typical levels within a month following the initial spike (**Figure 22**). Likewise, the 2004 spikes in water-column concentration declined by the next (quarterly) sample; percent change across the STA was zero percent for THg and -17 percent for MeHg in the third quarter (**Table 3**). As discussed below, these transient spikes in water-column concentrations do not appear to be having an obvious effect on Hg levels in resident fish populations either within the treatment cells or immediately downstream in the discharge canal.

Visual inspection of **Figure 24** suggests that Hg levels in mosquitofish captured in 2004 were not as high as observed in some of the previous years, but slightly higher than levels in 2003, a year when the STA did not dry out (**Figure 23**). It is important to note that the first semi-annual mosquitofish collection occurred in April 2004, as the water levels in the cells were receding but prior to their drydown; the second semi-annual sampling event occurred in October, almost four months following reflooding. It is therefore conceivable that any spike in tissue-Hg in mosquitofish was missed.

As evident from **Figure 24**, sunfish from the interior marsh contained less Hg than fish from both the supply and discharge canals. When only bluegill (4-7 inches in length) were assessed, fish from Cell 5 (mean = 32 ng/g, n = 8) were found to contain significantly less Hg than fish from supply canal (mean = 47 ng/g, n = 12)($t = -2.628$, $df = 18$, $p = 0.02$). Levels of Hg in Cell 5 sunfish were also low compared to sunfish from downstream Everglades sites (see Figure 13 in Appendix 2B-1 of this volume). To assess what impact, if any, the drydowns and spikes might be having on Hg levels in sunfish, which should integrate their exposure over a longer period of time than mosquitofish (i.e., less chance of missing a spike), data on bluegill (4–7 inches in length) from Cell 5 (bluegill caught in Cell 3 only infrequently) were evaluated and found to exhibit significant temporal trends (ANOVA, df = 5, 37; $F = 11.2$; $p < 0.001$). Mean tissue-Hg in fish captured in 2003 (25 ng/g), a year in which the STA did not dry down, was lower than levels observed in 2000 (58 ng/g), 2001 (53 ng/g), 2002 (40 ng/g), and 2004 (32 ng/g; pairwise comparisons between 2003 and 2001 and 2000 were statistically significant), years in which the STA dried down. However, the levels in 2003 fish were slightly higher than fish collected in 1999, a year in which the STA dried down (**Figure 22**). When bluegill in the discharge canal were assessed, 2003 fish contained the highest tissue concentration (140 ng/g), with 2004 containing the lowest (64 ng/g), this being one of two significant between-year, pairwise comparisons ($H = 24.8$, df = 5, $p < 0.001$; Dunn's Test, $p < 0.05$); the other was between 2004 and 2002 fish (125 ng/g).

Similar to the spatial patterns shown in the mosquitofish and sunfish, largemouth bass collected from the discharge canal of STA-6 contained greater Hg concentrations than fish from both the supply canal (i.e., a positive percent change across this STA) and the interior marsh (**Table 6** and **Figure 24**). This general pattern has been consistent over the seven-year monitoring program (the only exception was when large numbers of bass were collected from Cell 3; e.g., 1998 and 2000). However, Hg levels have been declining in bass in the discharge canal. When temporal trends were assessed, bass from the discharge canal were found to differ among years in age-adjusted Hg levels (ANCOVA, $F = 41$, df = 6, 131, $p < 0.001$). As reported previously, levels in these fish have been on a general downward trend with the exception of a sudden increase in 2001 (**Figure 24**). The LSM calculated for bass collected from the discharge canal in 2004 (287 ng/g) was significantly lower than all other years (Tukey-Kramer post hoc test, 1998 = 594 ng/g, 1999 = 547 ng/g, 2000 = 489 ng/g, 2001 = 604 ng/g, 2002 = 512 ng/g, 2003 = 435 ng/g). This general downward trend, with the interruption in 2001, would seem to suggest the transient spikes in water-column concentrations, each year except 2003, are not having a significant impact. Attempts were made to assess data from the fish from within the marsh, and to eliminate possible lag time by looking only at young-of-the-year bass (otolith age < 1); however, this resulted in a small dataset with some gaps. When one-year-old bass from Cell 5 were assessed, Hg levels were slightly higher in fish caught in 2004 (66 ± 15 ng/g) when compared to fish collected in 2003 (44 ± 12 ng/g); however, this difference was not statistically significant given the variance ($t = -2.2$, df = 6, $p = 0.07$). Further, although these fish were all the same age, 2004 fish were significantly larger than 2003 fish (607 grams and 293 grams, respectively; $t = -28$, df = 6, $p = 0.03$).

In terms of risk to fish-eating wildlife, Hg levels in mosquitofish, sunfish, and bass (whole-body concentration estimated from fillet concentration) from STA-6 were all below the USFWS (100 ng/g) and USEPA (77 ng/g; 346 ng/g for TL 4 fish) predator protection criteria. Therefore, the risk of Hg exposure to fish-eating wildlife foraging preferentially at STA-6 appears to no longer represent a significant threat.

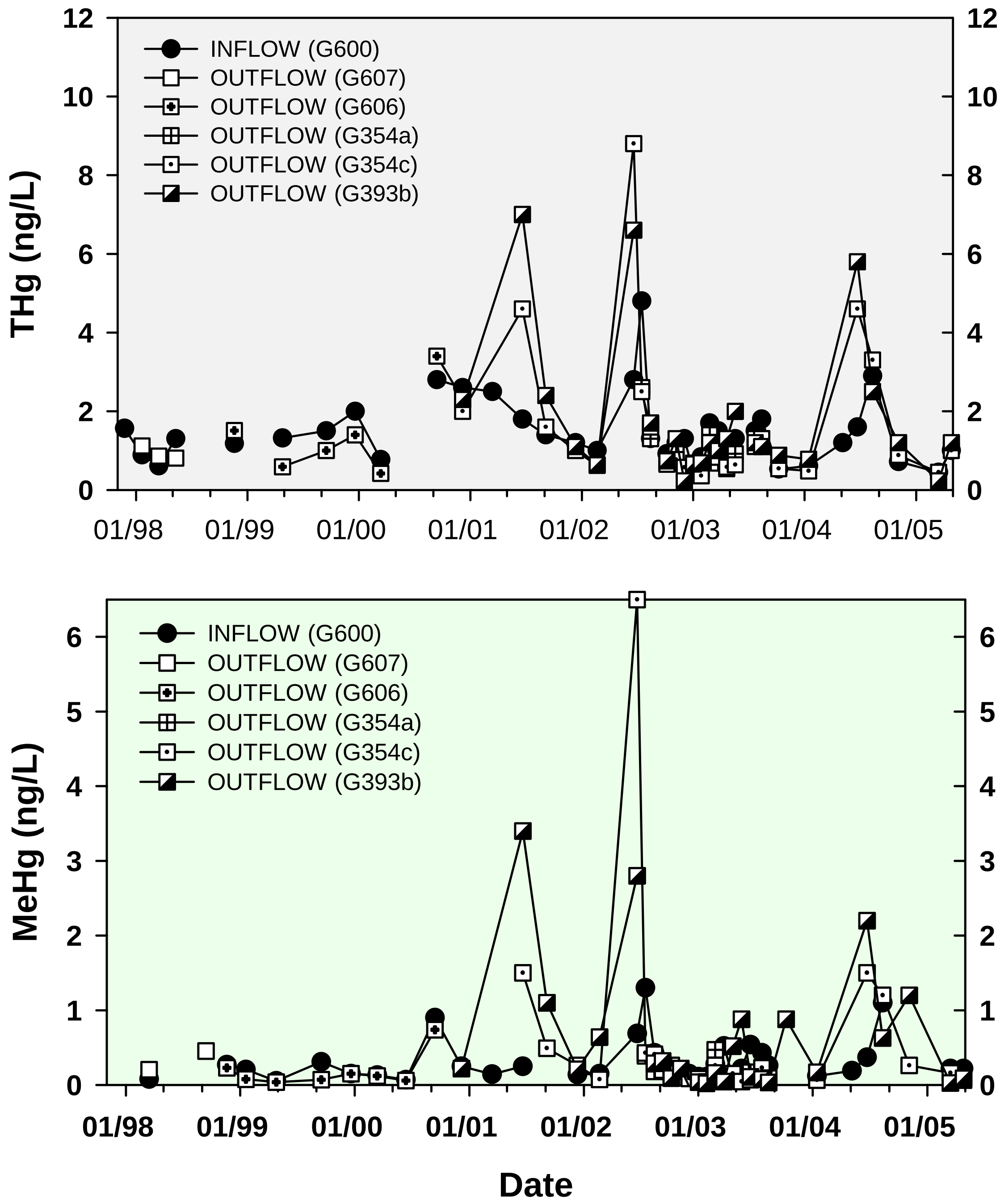


Figure 22. Concentrations of (a) THg and (b) MeHg (ng/L) in unfiltered surface water collected at STA-6, including results from routine and expanded sampling.

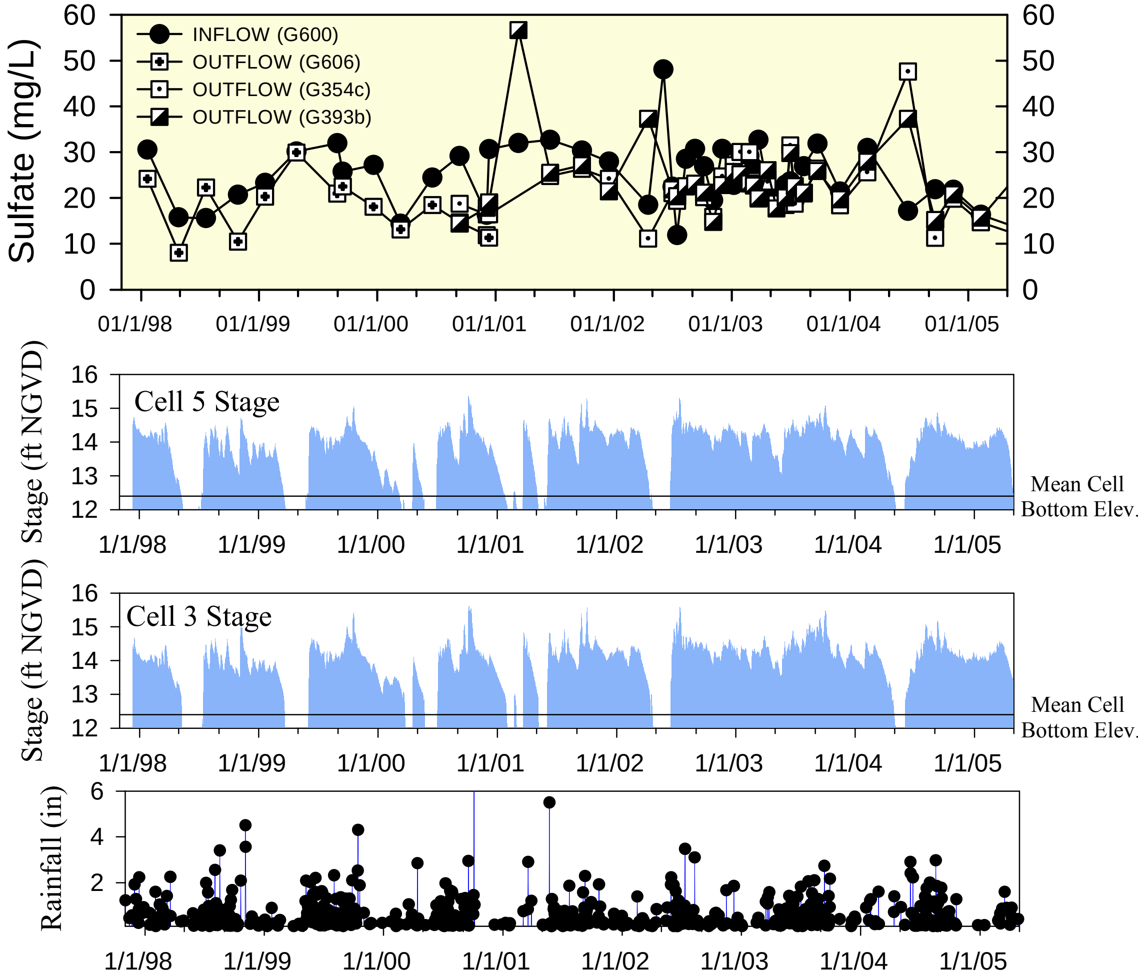


Figure 23. Concentrations of sulfate (top), stage in the two cells (recorded immediately upstream of outflow culvert of cell) and rainfall at STA-6.

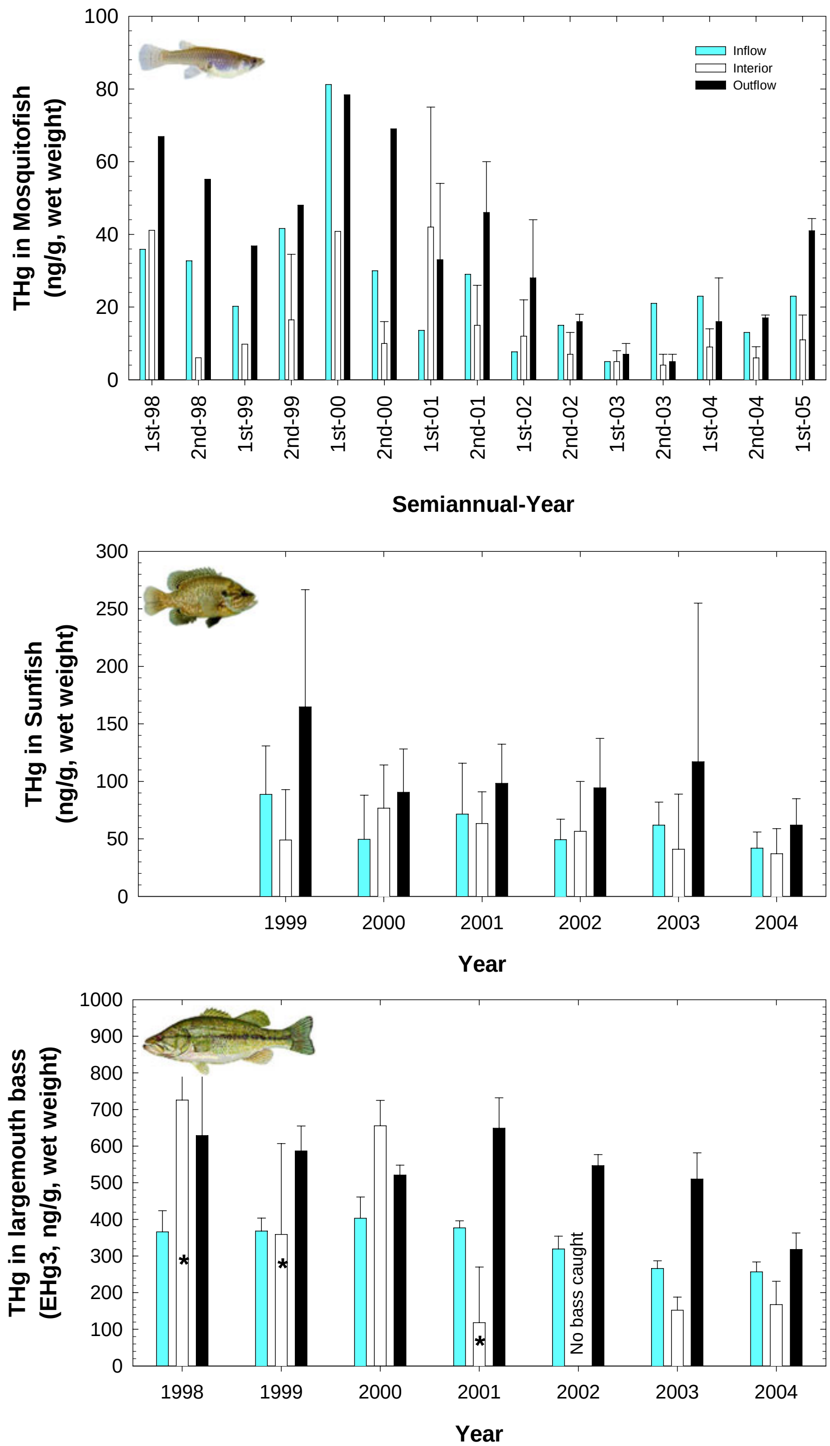


Figure 24. Mercury concentrations (ng/g, wet weight) in (*top*) mosquitofish composites (+ range), (*middle*) whole sunfish (± SD), and (*bottom*) fillets of largemouth bass (± 95% C.I. or, if arithmetic, SD) collected at STA-6.

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