Appendix 4A-6: Report on Expanded Mercury Monitoring at Stormwater Treatment Area-2

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SUMMARY

According to state permit requirements, the flow-through operation of a treatment cell in a Stormwater Treatment Area (STA) cannot begin until the concentrations of total mercury (THg) and methylmercury (MeHg) in the interior are not significantly greater than the corresponding concentrations in the inflow. Methylmercury is a highly toxic form of mercury produced naturally from the inorganic mercury in storm runoff, atmospheric deposition and peat soil. This transformation is carried out by natural bacteria living in the surficial sediment in the presence of an oxidized form of sulfur, sulfate and the absence of dissolved oxygen. Based on biweekly startup monitoring of unfiltered water for total mercury and methylmercury at the inflow and a representative site in each treatment cell, STA-2 and Cells 3 and 2 met their mercury startup criteria on September 14, 2000 and November 9, 2000, respectively. This same monitoring detected an anomalously high concentration of methylmercury in STA-2, Cell-1 water on September 26, 2000. The concentration of methylmercury in adjacent Cell 2 was only 10 percent of that detected in Cell 1. After reporting this anomalous mercury event, expanded mercury monitoring was initiated by the District in STA-2 at the request of the Florida Department of Environmental Protection (FDEP or Department).

In the 90-day follow-up study, the startup Mercury Monitoring Program was expanded to include three sites in Cells 1 and 2 for monthly sampling of filtered water and mosquitofish and one-time sediment sampling. Splitting samples between contract analytical laboratories confirmed the high methylmercury results. The simultaneous collection of filtered and unfiltered samples demonstrated that the high methylmercury concentrations could not be attributed solely to high suspended solids concentrations in the water. Significant fluctuations in unfiltered and filtered methylmercury concentrations within and between Cells 1 and 2 were observed during the follow-up study. These spatial and temporal fluctuations may be a result of differences in soil chemistry or vegetation coverage, the internal recirculation of water via the seepage canal, rapid uptake and release by microscopic plants and animals, or analytical artifacts. By the end of the 90-day study on January 24, 2001, unfiltered methylmercury concentrations in Cell 1 surface water had declined to about five percent of the September 26, 2000, peak of 4.8 ng/L, but still exceeded the inflow concentration, while those in Cell 2 had declined to about three percent of the August 3, 2000 peak of 1.9 ng/L. However, following a significant rainfall event in March 2001, concentrations of both THg and MeHg increased dramatically to near peak levels.

As anticipated, the average concentration of total mercury in mosquitofish increased rapidly from October through December 2000, reaching about the same average concentration as at WCA-3A-15, the Everglades mercury "hot spot." From December 2000 through February 2001, the concentrations appear to have nearly plateaued, but subsequently increased again in March. Anomalously high methylmercury concentrations can also be inferred to be present in fish species at the next step up in the food chain. Such species include sunfish, which are typically consumed by fish-eating wildlife. The inferred magnitude of sunfish methylmercury contamination in STA-2, Cell 1 is likely to represent an unacceptable risk of toxic effects to highly exposed, highly sensitive members of fish-eating wildlife populations foraging there preferentially. Populations at risk include wading birds roosting or nesting in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge) but foraging over a range that includes STA-2.

In April 2001, Cell 1 dried out as a consequence of the worst drought in South Florida's recorded history. Based on the above risk extrapolation and the likelihood that there will be a recurrence of the methylmercury anomaly once Cell 1 is reflooded with wet-season rainfall or runoff, mitigation should be a high priority.

INTRODUCTION

This document reports the results of startup mercury monitoring of Stormwater Treatment Area 2 (STA-2), including results from an expanded sampling program. This monitoring was required by Permit No. 0126704, issued by FDEP under the Everglades Forever Act (EFA). Under this permit, water from the STA is considered acceptable for discharge, and operation may begin when concentrations of total mercury (THg) and methylmercury (MeHg) at the midpoint of a treatment cell are not significantly greater than the concentrations of the corresponding species in the inflow samples.

Biweekly startup sampling for mercury at STA-2 began on July 20, 2000. On September 14, 2000, STA-2, Cell 3 passed startup criteria. On the same date, a surface water sample from the interior of Cell 1 was found to have a MeHg concentration of 2.5 ng/L. While this prompted some concern, this value was not considered anomalously high because it was less than the highest value reported during the startup monitoring of STA-1W, Cell 5. Two weeks later, MeHg concentration in STA-2, Cell 1 had increased to 4.8 ng/L, or 83 percent of the THg concentration of 5.8 ng/L. A confirmed MeHg value of this magnitude had never been reported by the District or the U.S. Geological Survey (USGS) in any water sample collected in a South Florida canal or marsh to date. The U.S. Environmental Protection Agency (USEPA) Region 4 had reported only two higher MeHg values (5.47 and 5.46 ng/L) in the 663 samples of surface water they collected from 1995 through 1999 as part of REMAP (D. Scheidt, personal communication). Based on this information, the concentration of MeHg observed in surface water from STA-2 Cell 1 was considered anomalously high. Accordingly, pursuant to Condition (6)i of EFA permit No. 0126704, immediately following review and validation of the data by the District's Quality Assurance Officer, the Florida Department of Environmental Protection (FDEP) was notified of the high MeHg value.

In response to questions raised by FDEP regarding the representativeness of the collected samples (during the October 16 teleconference), the District proposed a 90-day modification to the startup Mercury Monitoring Program. The modification included expanded sampling of surface water, sediment and fish to obtain more information about the nature and cause of this anomalous condition at STA-2. FDEP formally approved the proposed modifications in startup

monitoring in a letter dated October 30, 2000 (correspondence from Richard Bray to Neal Larson).

BACKGROUND ON THE "RESERVOIR EFFECT"

The creation of new impoundments or reservoirs typically results in large changes in trophic conditions and water chemistry, including changes in dissolved organic carbon (DOC), pH, temperature and nutrients. In addition, flooded soils can be a source of inorganic mercury (Cox et al., 1979). Moreover, decomposition of flooded terrestrial vegetation and soil carbon in new reservoirs has been found to stimulate sulfur-reducing bacteria (SRB) that methylate inorganic mercury (Hg) to the more toxic and bioaccumulative methylmercury (MeHg) species (Kelly et al., 1997, Paterson et al., 1998). For example, Paterson et al. (1998) found that annual fluxes of MeHg increased 10 to 100 times through a zooplankton community after impoundment. Likewise, fish in newly created reservoirs often accumulate high concentrations of MeHg (Abernathy and Cumbie, 1977, Bodaly et al., 1984, Bodaly and Fudge, 1999). Abernathy and Cumbie (1977) suggested that elevated MeHg levels in fish were a transitory phenomenon in newly impounded reservoirs (also see Cox et al., 1979). However, this so-called "reservoir effect" has on occasion resulted in mercury contamination problems spanning several decades after impoundment 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 in northern pike went from 0.61 to 2.99 ppm and was 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 wetlands, which contain larger quantities of organic carbon than uplands, should be avoided.

However, applying these observations directly to the Everglades is problematic. In a report to the District at the startup of the Everglades Nutrient Removal (ENR) Project, 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 subsequently found no evidence that a "reservoir effect" had occurred at the ENR Project. Initial collections of surface water (PTI, 1994 attributed to KBN, 1994a; Watras, 1994) and fishes (mosquitofish and largemouth bass; PTI, 1994 attributed to KBN, 1994b) found no evidence of MeHg spikes during the early phases of the project. All collected mosquitofish and many of the bass had Hg concentrations below the level of detection (< 0.02 ppm, PTI, 1994 attributed to KBN, 1994b). Further, no "reservoir effect" has been observed in the ENR Project at any trophic level following six years of flooding (SFWMD, 1998; Rumbold et al., 2001a). Moreover, with the exception of STA-1W, Cell 5, which exhibited moderately elevated MeHg concentrations in surface water and mosquitofish immediately after flooding (Rawlik et al., 2001), a "reservoir effect" has not been observed following the startup of any of the District's other STAs (e.g., STA-6, STA-5; Rumbold et al., 2001a).

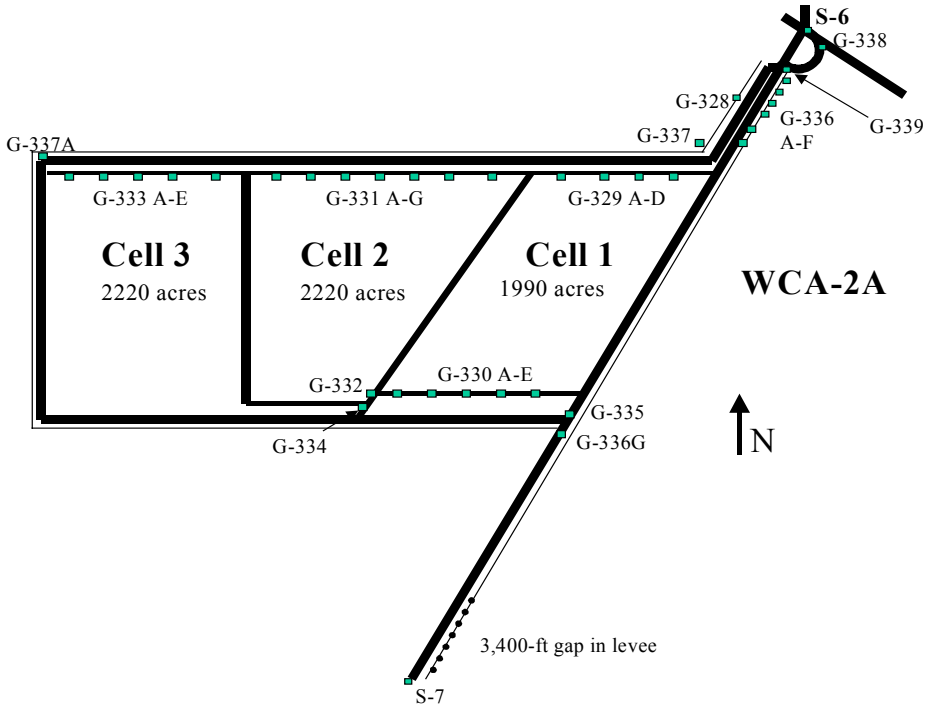

Figure 1. Schematic of STA-2 (Not to Scale)

METHODS

Site Description and Operational History of STA-2

STA-2 is located in Western Palm Beach County near the Browns Farm Wildlife Management Area. STA-2 was developed to provide a total effective treatment area of 6,430 acres (Cell 1 is 1990 acres, Cells 2 and 3 are each 2,220 acres; for additional details, see SFWMD, 1999a). It is intended to treat discharges from the S-6/S-2 Basin, the G-328 Basin, East Shore Water Control District, 715 farms, portions of the S-5A Basin, and Lake Okeechobee via pump station S-6. S-6 and G-328 serve as the primary inflow pumping stations (**Figure 1**). G-328 serves an approximated 9,980 acres of adjacent agricultural lands. Inflows from S-6 and G-328 enter the Supply Canal and are conveyed southward to the Inflow Canal, which extends across the northern perimeter of STA-2. A series of inflow culverts conveys flows from the Inflow Canal to the respective treatment cells (G-329 A-D into Cell 1, G-331 A-G into Cell 2, G-333 A-E into Cell 3). Flows travel southward through the treatment cells and eventually discharge into the discharge canal via culverts or gated spillways (culverts G-330 A-E from Cell 1, gated spillway G-332 from Cell 2, gated spillway G-334 from Cell 3). Flows will then travel eastward in the discharge canal to the STA-2 outflow pump station G-335, which in turn conveys water to a short stub canal leading to the L-6 Borrow Canal. Water in the L-6 Borrow Canal will travel north, then east into WCA-2A through six box culverts (each with a capacity of 300 cfs and an invert of 12 ft NGVD) located south of G-339 between 0.5 and three miles south of S-6. The

area to receive discharge was previously identified as a nutrient-impacted area. Under high-flow conditions, when stage in the L-6 Borrow Canal exceeds 14.25 ft, treated discharges in the L-6 Borrow Canal will spill into five 72-inch culverts and travel south toward S-7. Approximately 0.75 miles north of S-7, the eastern levee has been degraded to ground elevation (approximately 12 feet) that will allow water to sheetflow into WCA-2A. Here again, the area to receive discharge was previously identified as a nutrient-impacted area.

Portions of STA-2 were still being farmed immediately prior to construction. Cell 3 had about 30 percent in sugarcane and 45 percent in sod production. Cell 2 had about 10 percent in sod production (in the northwest corner). Construction activities for STA-2 began in January 1998 (N. Larson, personal communication). The only site preparation occurred in Cell 3, where a portion of the cell was disked to remove remnant cane.

The treatment cells received differing amounts of water during construction and up to the present. Dewatering was required for construction and installation of spillways and culverts. Cell 1 received most of the water from dewatering operations, except for a short period during Cell 1 construction, at which time Cell 2 received dewatering volumes. Construction of the interior works was completed in June 1999; at that time inflow gates to Cells 1 and 2 were opened for a brief period, then closed in that the primary operational objective was to raise water depths in Cell 3 to approximately 1 m to prevent growth of emergent vegetation. Cell 3 inflow gates remained open for several months, including for the duration of Hurricane Irene (October 15, 1999). The inflow gates to Cells 1 and 2 were reopened briefly in December 1999 through January 2000; however, the cells may have partially dried out during the dry season of 1999 through 2000. The final operational testing of the outflow pump station, G-335, was completed in October 2000, and a small amount of water was discharged offsite at that time. In addition to rainfall, source water for the treatment cells through early 2001 originated from G-328 and G-337, i.e., the seepage pump. During the severe drought of 2000 through 2001, STA-2, Cell 1 went dry in April 2001, and Cell 2 went dry around May 10, 2001. Supplemental water deliveries were made during April and May 2001 to Cell 3 to prevent dryout of the submerged aquatic vegetation (SAV). Following local rains, Cell 2 was reflooded around June 1. A summary of water levels since July 2000 is presented in **Figure 1A**.

With the anomalously high mercury concentrations in Cell 1, discharges from Cell 1 during the G-335 pumping tests were discontinued and the outflow culverts blocked off. At the present time, metal plates are in place to prevent discharges from Cell 1.

Original Sampling Scheme for Startup

As originally designed, startup mercury monitoring at STA-2 consisted of biweekly unfiltered surface water sampling for THg and MeHg at the following sites: G-328B (just downstream of G-328 pump, representing inflow water), Cell 1s6 (later designated C1-A) in the interior of Cell 1, Cell 2s5 (later designated C2-A) in the interior of Cell 2, and Cell 3s4 in the interior of Cell 3. Initial site names corresponded to sediment core sampling locations (and thus the "s" prefix). Because the S-6 pump station was intended to be the primary inflow, it was added as a second inflow location on September 14, 2000.

Biweekly startup sampling for mercury at STA-2 began on July 20, 2000. STA-2, Cells 3 and 2, met startup criteria on September 14, 2000 and November 9, 2000, respectively.

Modifications to Startup Sampling

The following modifications to startup sampling were formally approved by the FDEP on October 30 (correspondence from Richard Bray to Neil Larson).

The District would add filtered sampling and would increase the number of water collection sites from one to three in Cells 1 and 2.

The District would initiate monthly collection of mosquitofish at the Cell 1 and 2 interior sites as a standard indicator of MeHg bioavailability and bioaccumulation. If the mosquitofish results warrant it, followup sunfish and largemouth bass collection could be instituted at the FDEP's direction.

The District would collect sediment and pore water from 0 to 4 cm cores at each interior-monitoring site. Sediment and pore water were to be analyzed for THg, MeHg and the pore water for known influential constituents (i.e., pH, DO, $SO_4^{=}$, $S^{=}$, Fe and DOC). Concurrent collection of 0 to 10 cm cores at each of the original six sampling sites in STA-2 was also to be carried out.

Finally, the District would split samples with a reputable laboratory to verify results.

The expanded sampling program was subsequently modified for two reasons. First, because of inherent difficulties encountered in sampling and filtering pore water under inert atmosphere (i.e., to maintain redox potential and concentration of redox sensitive constituents; for review, see Mason et al., 1998), attempts at pore water collection and analysis were put on hold. Second, the FDEP laboratory was unable to assimilate many of these additional samples for ultratrace THg and MeHg analysis. Consequently, the District was forced to send samples from the expanded program (i.e., above and beyond standard QC splits) to a contract laboratory (Frontier Geoscience Inc.), which, due to interlaboratory variability, added a degree of uncertainty to data interpretation.

RESULTS AND DISCUSSION

Surface Water Mercury Levels

Concentrations of THg and MeHg in unfiltered surface water collected at STA-2 from July through April 2001, as reported by the FDEP laboratory, are graphically summarized in **Figure 2** and **3**, respectively. Because sample load exceeded the capacity of the FDEP laboratory (i.e., samples from the expanded STA-2 program, in addition to samples from the District's routine ECP and Non-ECP monitoring programs), water samples collected in January and February were analyzed only by the contract laboratory. Interlaboratory comparability was assessed by having each laboratory independently analyze split water samples collected during three events at STA-2 (**Figure 4**). Results reported by the contract laboratory for samples collected during November 2000 through February 2001 are summarized in **Figure 5**.

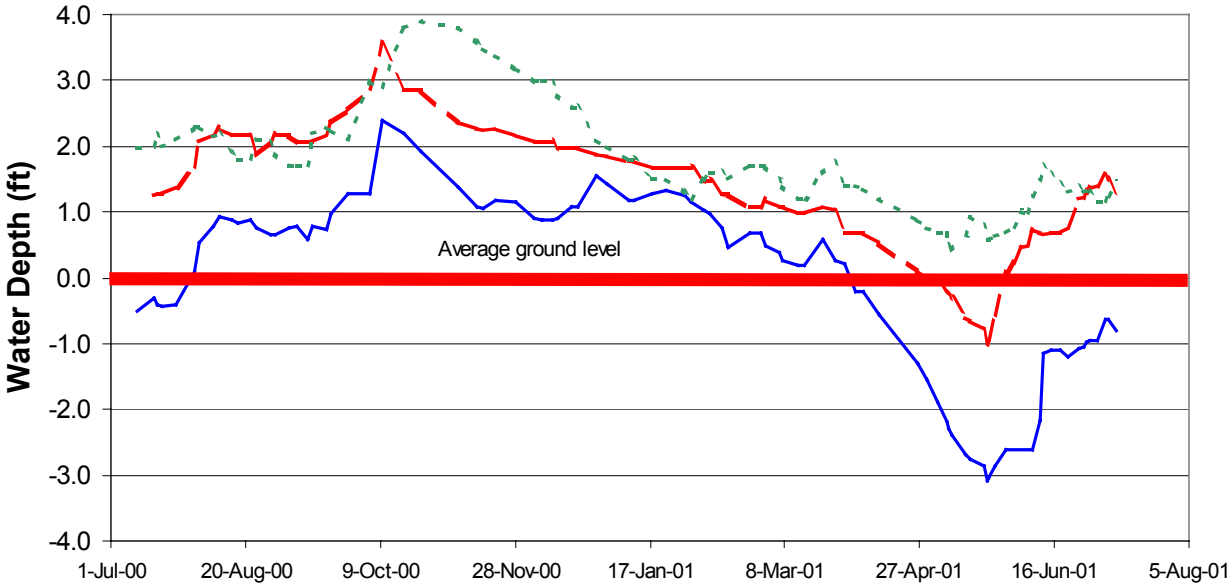

Figure 1A. STA-2 Treatment Cell Water Depths

THG IN WATER

As is evident from **Figure 2**, THg concentrations in surface water were variable and ranged from 0.7 to 7.6 ng/L. As previously stated, STA-2, Cell 3 met startup criteria for THg (and MeHg) on September 14, 2000; Cell 2 met startup criteria on November 9, 2000. However, it should be noted that subsequent samples showed increased concentrations of THg (and MeHg in Cell 2) in both cells, which was not unexpected given the inherent variability in environmental conditions, sampling and ultratrace analyses. As of the date of this Report, STA-2, Cell 1 has not met startup criteria.

The peak THg concentration that occurred in a surface water sample taken from STA-2, Cell 1 on April 5 was high compared to levels observed in the interior of the ENR Project (period of record: monthly sampling from January 1998 through April 1999; maximum concentration was 5.18 ng/L). However, samples collected on April 5 did not meet QC criteria for THg (i.e., THg found in trip blank >2xMDL), and were estimates only. Average THg concentration in inflows to STA-2 (i.e., G328B and S6) during this period was 1.9 ±0.61 ng/L (n=22; median=1.75 ng/L). This concentration was comparable or lower than levels observed previously at Non-ECP structures (cumulative average for samples collected during the third quarter from 1997 through 1999 was 2.4 ±1.13; Rumbold et al., 2001a). Alternatively, average surface water THg concentration in the interior marshes of the STA, 2.98 ±1.4 ng/L (unqualified FDEP data, three cells pooled, n=33; median=2.9 ng/L), was higher than average inflow to the STA, and relatively high compared to the mean concentration observed at interior marsh sites of the ENR Project (POR: 1/1998 – 4/1999; 1.02 ±0.69; n=167; median=0.81 ng/L).

THg concentrations in surface waters of STA-2 declined from September 2000 through March 8, 2001 (**Figure 2** and **Figure 5a**), particularly in Cell 1. Although results from split-water samples revealed interlaboratory variability in ultratrace THg determination (paired t-test, df=26, t=2.39, p=0.025; **Figure 4a**), the data set reported by the contract laboratory confirms the downward trend in THg concentration (**Figure 5a**). However, following significant rainfall on March 18 and 19, 2001 (**Figure 6**), THg in Cell 1 increased dramatically and peaked at 7.6 ng/L (**Figure 2**, estimated value).

MEHG IN WATER

As is evident from **Figure 3**, MeHg concentrations in surface water were even more highly variable than THg. As previously discussed, the peak MeHg concentration of 4.8 ng/L, was anomalously high compared to levels previously observed by the District or USGS (USEPA observed two higher values in 663 REMAP samples). During this period, average MeHg concentration in inflows was 0.3 ±0.3 ng/L (i.e., unqualified data from G328B and S6; n=20; median=0.2 ng/L). Like THg, levels of MeHg in inflows were comparable to what has been observed previously at Non-ECP structures (POR: 1998-1999, n=20; cumulative average for third-quarter samples is 0.22 ±0.2; Rumbold et al., 2001a). Alternatively, average surface water MeHg concentration in the interior marshes of the STA, 1.1 ±1.1 ng/L (three cells pooled, n=32; median=0.72 ng/L), was markedly higher than average inflow to the STA and also much higher than the mean surface water MeHg concentration in interior marshes of the ENR Project (POR: January 1998 through April 1999; 0.09 ±0.2; n=206; median=0.04 ng/L).

Similar to the temporal trends observed in THg, surface water MeHg concentrations declined in STA-2 from September 2000 through March 8, 2001 (**Figure 3** and **Figure 5b**). With the exception of two apparent outliers (**Figure 4b**), interlaboratory comparison in ultratrace MeHg determination appeared satisfactory (Wilcoxon Signed Rank test, W= -21.0, p=0.83). More importantly, the data set reported by the contract laboratory again confirms this dramatic downward trend in MeHg concentrations (**Figure 5b**). However, similar to THg, MeHg concentration in surface water of STA-2, Cell 1 spiked to 4.2 ng MeHg/L following a significant rainfall event in late March (**Figure 6**).

Percent MeHg in Water

The percent of THg that was MeHg was highly variable in water, ranging from three percent to 88 percent (mean was 32 ±24 percent, median=27 percent; FDEP data set). The maximum percent MeHg occurred in a sample from Cell 1, collected on November 9, 2000 (peak absolute MeHg value observed on September 28 was 83 percent MeHg). Both the mean and maximal percent MeHg were outside the range previously reported from the ENR Project (up to 58 percent; median 5 percent), other STAs (4 to 28 percent, Rumbold et al., 2001a), Non-ECP structures (range: 5 to 58 percent; median 12 percent, Rumbold et al., 2001a) and WCA-2 (3 to 32 percent, Hurley et al., 1998). The percent MeHg reported here for STA-2 is also very high relative to published reports for other areas. For example, Hines et al. (2000) reported percent MeHg ranging up to only 4.5 percent in the Idrija River, and in one instance reported only 0.6 ng MeHg/L in a water sample containing 322 ng THg/L. Clearly, the observed anomaly at STA-2 was not a result of high levels of THg, but rather how much of the inorganic mercury, Hg (II), was being converted to MeHg.

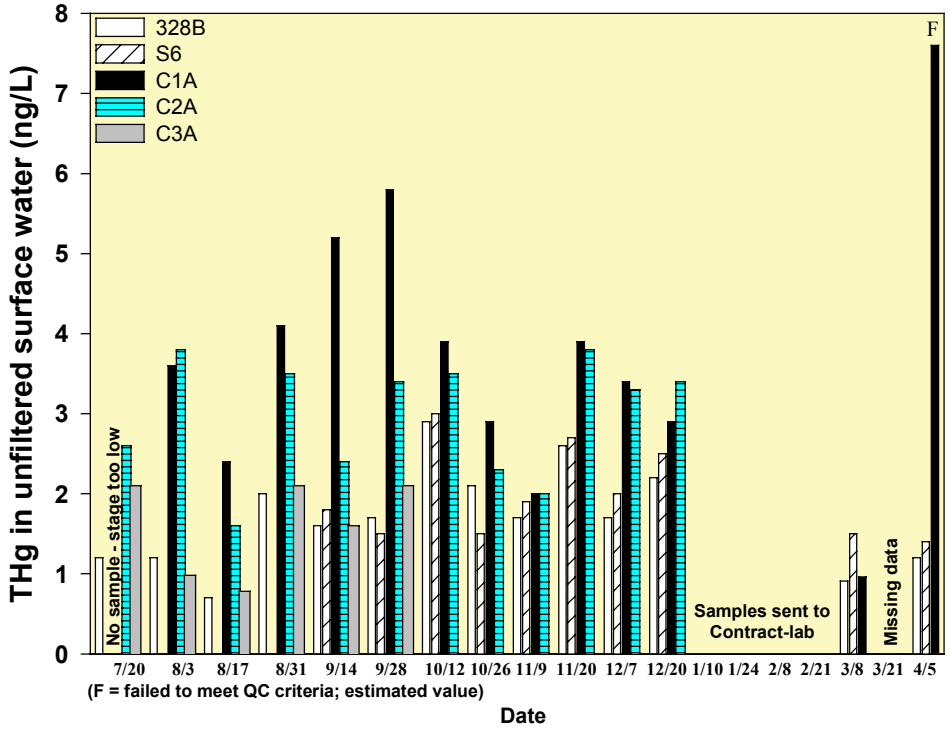

Figure 2. Total Mercury concentrations in surface water during STA-2 startup (FDEP Laboratory)

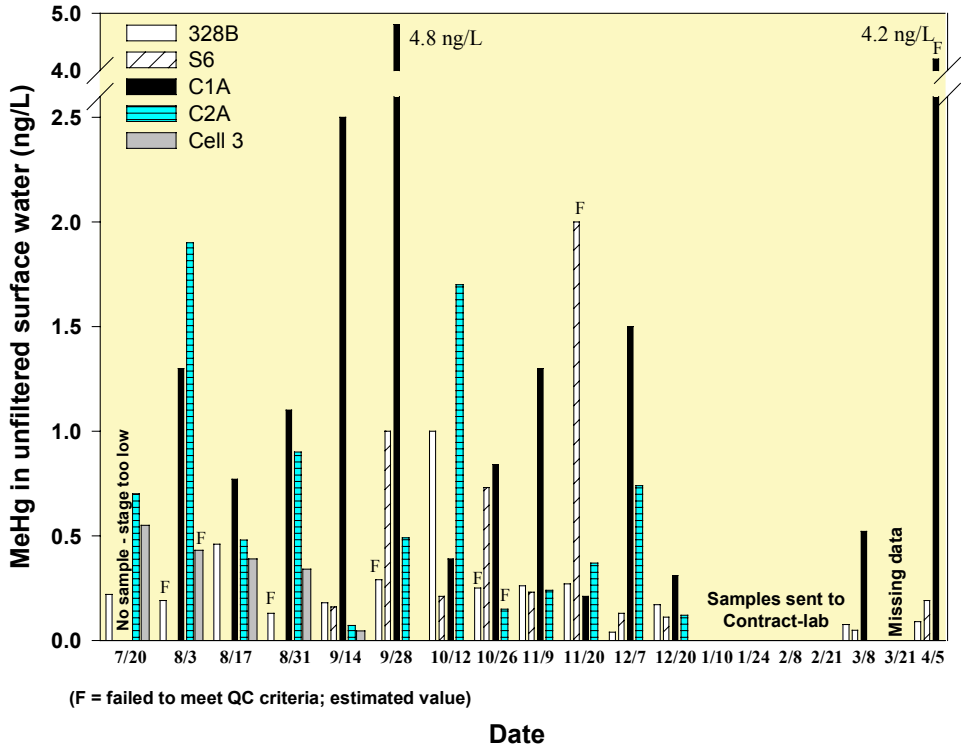

Figure 3. Methylmercury concentrations in surface water during STA-2 startup (FDEP Laboratory)

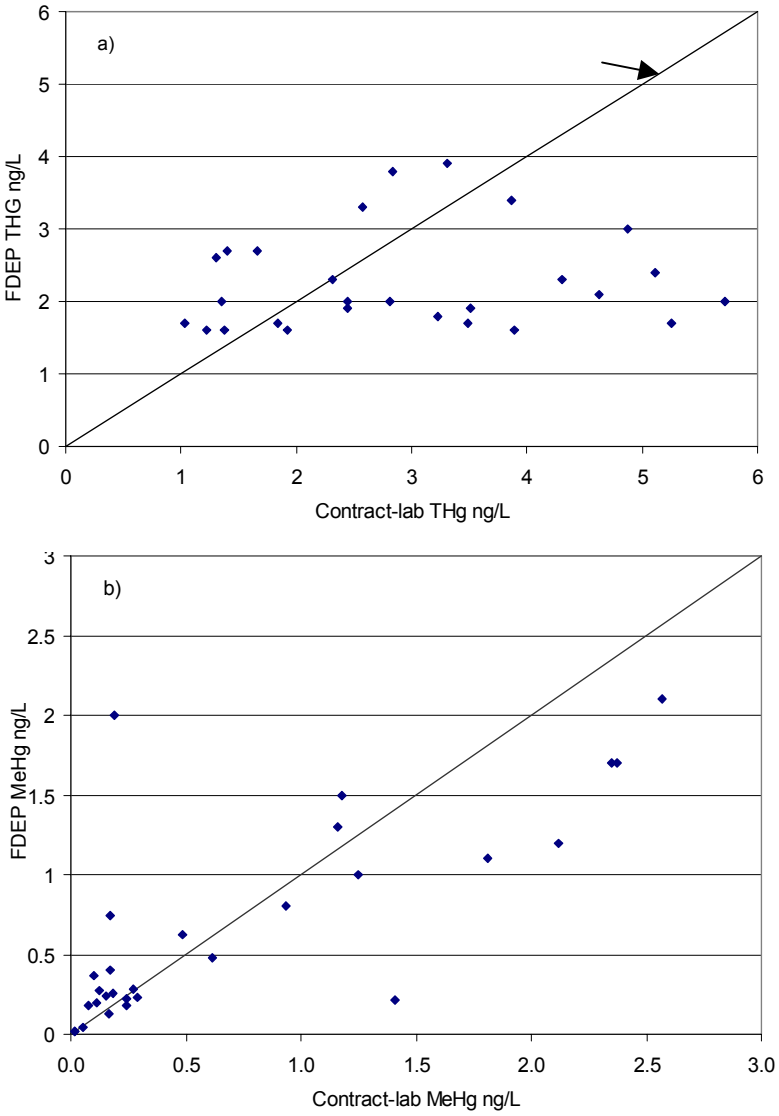

Figure 4. Interlaboratory Comparison for THg (a) and MeHg (b) determination in surface water

THG AND MEG CONCENTRATION IN FILTERED SURFACE WATER

Because only unfiltered samples were collected on September 28, 2000, it is uncertain whether suspended solids contributed to the elevated levels of mercury in the water column. However, filtered samples were collected on two subsequent sampling events (November 9, 2000 and January 10, 2001) and showed dissolved species (i.e., operationally defined as passing through a 0.45 µ filter) dominated over particulate-bound species. Percent dissolved THg ranged from 67 to 94 percent (n=10 unqualified values, mean ±1SD: 85 ±8 percent); percent dissolved MeHg reportedly ranged from 46 to 196 percent (n=16, median=82 percent). The apparent reversal, i.e., filtered concentration greater than unfiltered, may be a result of temporal variability in serially collected samples (filtered, and then unfiltered), the analytical uncertainty at ultra-trace levels (the 196 percent dissolved MeHg was estimated from 0.107 and 0.21 ng/L in unfiltered and filtered samples, respectively) or a combination of both. Despite the reported reversal, it is clear that high levels of dissolved THg and MeHg occurred in surface waters of STA-2 and that the high concentrations of MeHg in surface water were not due to persistent re-suspension phenomenon.

SOURCES OF THG AND MEHG TO SURFACE WATER

Potential sources of inorganic Hg to STA-2 surface water included: (1) inflows (see discussion above); (2) groundwater exchange; (3) atmospheric deposition; and (4) THg released from flooded soils.

THg concentration in rainfall collected at the ENR Project as part of the Mercury Deposition Network averaged 10.94 ng/L in 1999 (Rumbold et al., 2001a). Atmospherically deposited Hg, which is primarily inorganic, is rapidly processed and assimilated as it enters the marsh (e.g., oxidation, sorption, deposition, methylation, etc.), or quickly evades back to the atmosphere. Consequently, THg is generally at a lower concentration in stormwater runoff than in rainfall. Yet it is not surprising that temporal patterns in surface water THg concentrations were visibly correlated, albeit weakly, with rainfall events (**Figure 6**). For instance, THg concentration in Cell 2 increased from July 20 to August 3, 2000 following a large rainfall event that occurred in the area on August 1, 2000. Concentrations of THg then decreased in all cells over the next two weeks during a period of little rainfall. The decline in THg from September through March also coincides with a period of little rainfall. Then, following six inches of rain in late March, THg concentrations spiked. Not surprisingly, correlation between surface water THg and rainfall was confounded by among-cell differences in initial stage and inflows (i.e., due to dilution). For instance, the magnitude of the April spike was likely due to the small amount of standing water in Cell 1 to dilute the THg in the rain. Nevertheless, neither inflows nor atmospheric deposition can account for the marked differences observed in surface water THg concentration among cells (**Figure 2**). These among-cell differences were more likely attributable to as yet unmeasured differences in THg release from soils (Cox et al., 1979; Kelly et al., 1997) or in the way new Hg was assimilated within each cell.

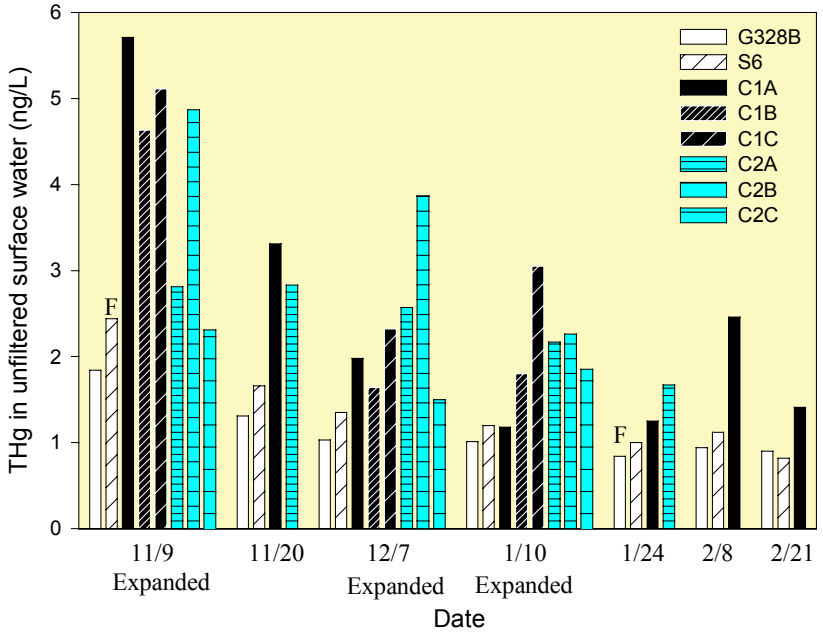

Fig. 5a. Concentrations of THg in surface water collected in expanded sampling at STA-2 (contract-laboratory). (F = failed to meet QC criteria; estimated value).

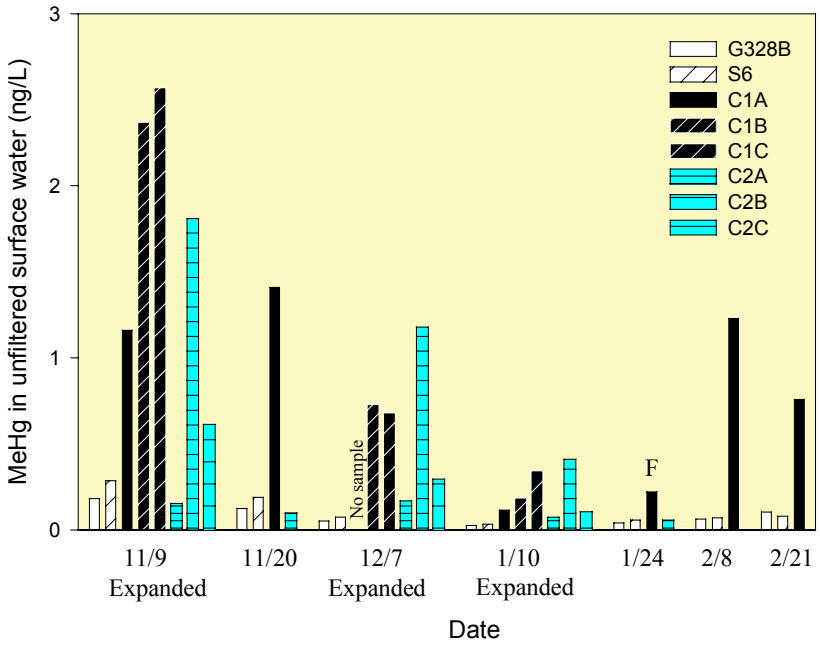

Fig. 5b. Concentrations of MeHg in surface water collected in expanded sampling at STA-2 (contract-laboratory).

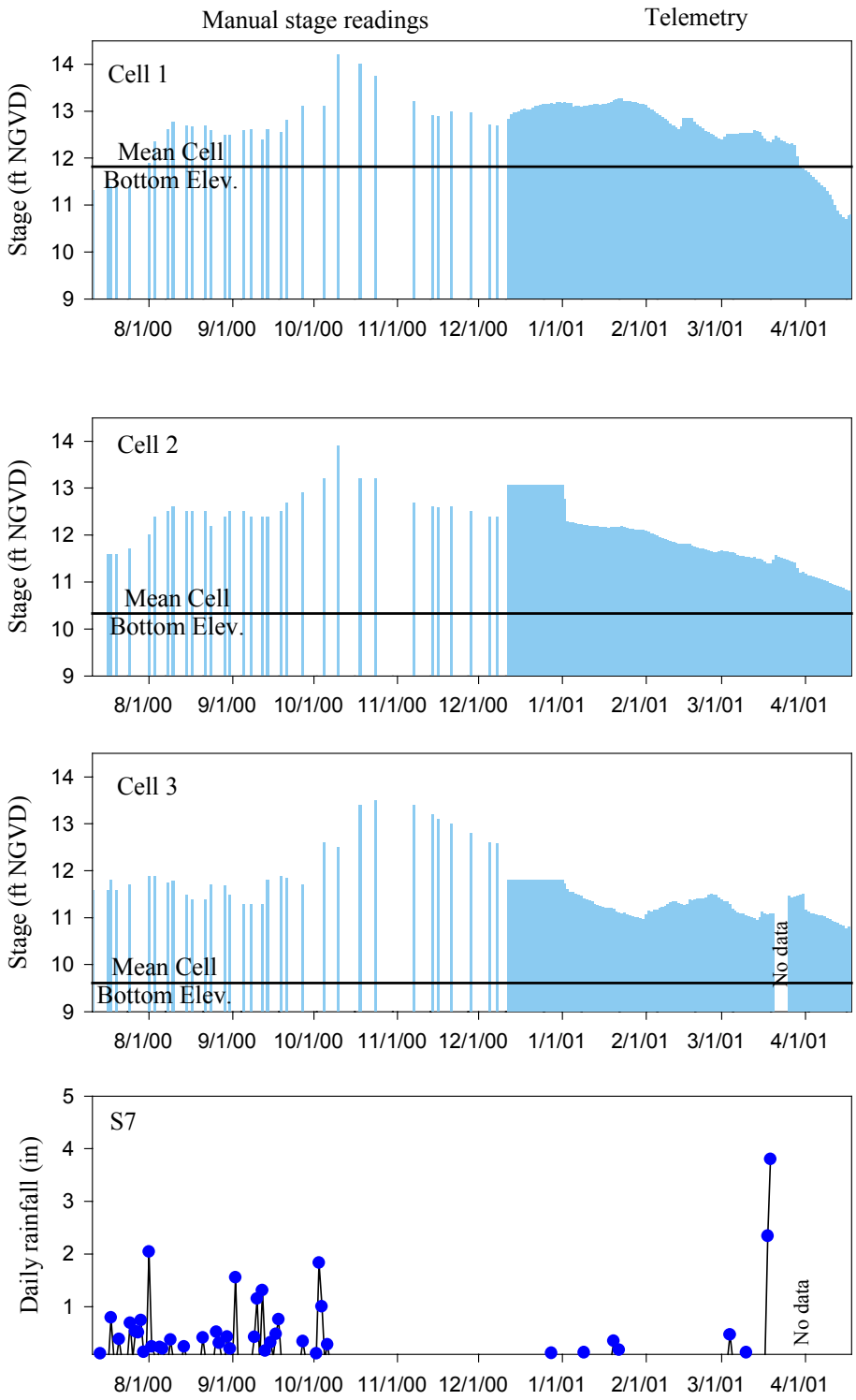

Figure 6. Rainfall in Vicinity of STA-2 and Stage in Cells 1-3

Sources of MeHg inputs to STA-2 are quite different than sources of inorganic Hg. Obviously, similar to inorganic Hg, MeHg loads in surface water inflows must be considered. However, a cursory examination of concentrations and estimated inflows suggests that loading of MeHg in surface water was not substantial. Further, unlike inorganic Hg, concentrations of MeHg in South Florida rain are generally considered environmentally insignificant (E. Prestbo, Frontier Geosciences, personal communication, 1996; Guentzel, 1997). Like inorganic Hg, MeHg can also be released from newly flooded soils (Hans Hultberg, personal communication); however, measurements of this release are scarce. As discussed in the ensuing sections, the primary determinant of MeHg input, and thus local surface water concentrations (as well as local rates of bioaccumulation) in the Everglades, is *in situ* methylation of inorganic Hg, i.e., *de nova* production.

Sediment

THG IN SEDIMENT

The Mercury Restoration Evaluation Plan for the Everglades Construction Project (submitted to the USACE, USEPA and FDEP, March 31, 1999 revision date) requires that surface sediments be collected as 0-to-10 cm cores in each of the STAs prior to flooding, and triennially thereafter to ensure that mercury is not accumulating in accreting peat to hazardous concentrations. This frequency was determined to be appropriate for a system that is accreting peat at a rate of between 0.5 and 2.5 cm/yr.

Accordingly, six 0 to 10 cm sediment cores were collected from STA-2 on April 21, 1999, and were homogenized and analyzed for THg and MeHg. The mean concentration of THg in all six cores was 91 ±33 ng/g dry weight. The three cores taken from Cells 1 and 2 in 1999 had a slightly higher mean concentration of 109.3 ±26.7 (**Figure 7**). Nevertheless, THg levels were within the expected range for surficial sediments of the Everglades (Delfino et al., 1993, Gilmour et al., 1998; for review, see FTN 1999) and were also similar to THg concentrations in soils collected from the ENR Project (SFWMD 1997).

As part of the expanded STA-2 startup monitoring, three 10 cm cores were collected in December 2000 at the same locations previously sampled in Cells 1 and 2 in 1999. Mean THg concentration in these 10 cm cores was 117.5 ±12.46 (n=3, range 105.8 to 130.6; **Figure 7**). The between-year difference in THg concentration in 10 cm cores was not significant (Mann-Whitney rank sum test; T=9, n=3, p=0.7). While the power of the test was low due to small sample size, visual inspection of the data (**Figure 7**) tends to confirm that the observed difference was trivial in light of the high variability typically seen in sediments.

As part of the expanded sampling program, 4 cm cores were also collected from three sites within Cells 1 and 2 in December 2000. As will be discussed below, 4 cm cores were collected because methylation rates tend to be maximum near the sediment surface. Although two of the 4 cm cores were co-located at sites where 10 cm cores were collected, the other four cores were taken at sites B and C, i.e., collection locations for expanded sampling of surface water. Mean THg concentration in 4 cm cores was 126.8 ±29.64 ng/g (n=6, range 90 to 168.3, **Figure 8**). These levels were within the range that Delfino et al. (1993) reported for average mercury concentrations in recent Everglades sediment (i.e., 4 cm depth: ranged from 58 to 243 ng/g).

Between-cell differences in THg concentration in 4 cm cores were not significant (ANOVA, df=1,4; F=0.57; p= 0.49); however, the power of the test was again compromised by small sample size. Likewise, THg concentrations did not appear to differ between the 4 and 10 cm cores (t-test, df=7, p=0.63). Therefore, based on these data no major differences are demonstrated in sediment THg in STA-2 cells.

MEHG IN SEDIMENT

The rate of *in situ* (*de nova*) production of MeHg is the key factor in mercury bioaccumulation at any given site. Isotopic studies have found that MeHg concentrations in bulk sediment are highly correlated with methylation rates, and are a good surrogate for MeHg production (Gilmour et al., 1998). As noted in the 2001 ECR (Rumbold et al., 2001a), concentrations of MeHg in the six 10 cm cores collected in 1999 (2.235 ±1.79 ng/g dry weight, n=6; 3.6 ±1.2 ng/g for 3 cores from Cells 1 and 2) were highly variable and varied outside the range of what was observed in similar cores taken from the ENR Project (SFWMD 1997b). The maximum MeHg concentration, 5 ng/g dry wt, which occurred in the core from Cell 1, was also at the extreme range of concentrations previously reported for cores taken from the WCAs (4 cm cores; Gilmour et al., 1998).

MeHg concentrations in the 0-to-10 cm cores collected in 2000 (mean =2.17 ±1.63 ng/g dry wet; maximum 4 ng/g) were slightly lower than 1999 levels. Similar to THg, the between-year difference was not statistically significant (T=12, n=3, p=0.4); however, as stated above, the power of the test was low. Unlike THg, visual inspection of the data hints at a true decrease in MeHg, i.e., all three cores showed a decrease.

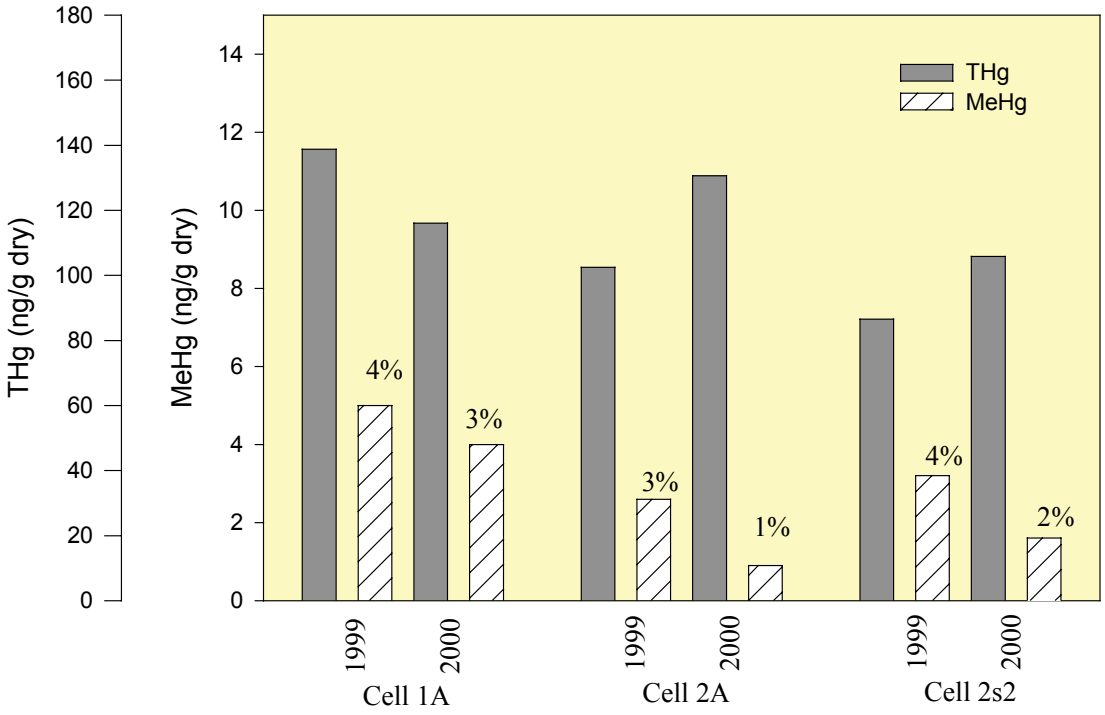

Fig. 7. THg and MeHg concentrations in 10-cm sediment cores collected from STA-2 in 1999 and 2000. Note, two different scales on y-axis. MeHg as a percent of THg in sediment (%MeHg) is also noted for each core.

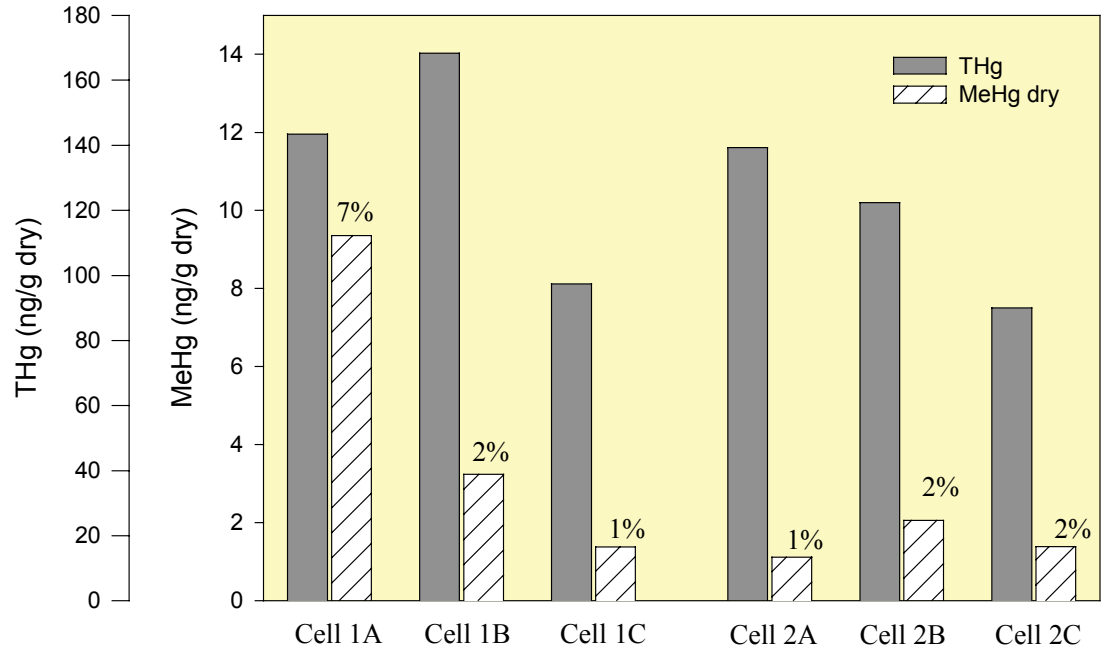

Fig. 8. THg and MeHg concentrations in 4-cm cores collected from STA-2 in 2000. Note, two different scales on y-axis. MeHg as a percent of THg in sediment is also noted for each core.

A between-year difference in sediments is illustrated more clearly by the decrease in the percentage of THg that was MeHg (**Figure 7**). Sediments from Cell 1A decreased from four percent MeHg in 1999 to three percent MeHg in 2000 and percent MeHg decreased two-fold in Cell 2 sediments. Percent MeHg is considered to be a measure of in situ production; where percent MeHg is relatively high the increase in absolute MeHg concentration is thought to be driven by factors other than THg concentration, i.e., the net methylation rate as influenced by variables other than inorganic Hg is the problem, not the quantity of inorganic Hg.

Methylation rates and, not surprisingly, MeHg concentration are generally highest at or within a few cm of the surface (4 cm; Gilmour et al., 1998). MeHg concentration in the 4 cm cores from STA-2 ranged from 1.12 to 9.35 ng/g dry weight (n=6, mean=3.09 ±3.2, **Figure 8**). Average MeHg concentration in 4 cm cores from various Everglades locations has been reported to be 1.6 ng MeHg/g dry weight, with a maximum concentration of 11.58 ng/g (n=131, unpublished data, Gilmour personal communication). Interestingly, the three highest sediment-MeHg concentrations occurred in cores from WCA-1, which is where REMAP recorded the two-highest MeHg concentrations in surface water (5.47 and 5.46 ng MeHg/L).

Mean concentration of MeHg was 4.6 ±4.2 ng/g in Cell-1 sediments and 1.5 ±0.5 in Cell-2 sediments. Like THg, this between-cell difference was not statistically significant (df=1,4; F=1.67; p=0.27); however, the power of the test was again compromised by small sample size. Interestingly, significant between-cell differences in sediment-MeHg concentrations have been observed in other STAs (e.g., STA-6) and are thought to account for between-cell differences in fish THg levels (Rumbold et al., 2001a, 2001b).

As is evident from **Figure 8**, percent MeHg in the 4 cm cores averaged 2 percent, but ranged up to 7 percent. The average value compares favorably with the published report from Gilmour et al. (1998). She has unpublished data that percent MeHg averages about 1.3 percent (n=128), but can be as high as 7.5 to 8.2 percent (at TS7, a site in ENP). These results suggest that percent MeHg in sediments from STA-2 were relatively high and that the geochemistry of the sediments was extremely favorable for mercury methylation; however, the levels and the conditions reported here were not unprecedented.

OTHER CONSTITUENTS OF BULK SEDIMENTS ASSOCIATED WITH MEHG LEVELS

As previously mentioned, sulfate-reducing bacteria (SRB) are stimulated by biodegradable organic carbon, nutrients and sulfate. However, sulfate in the form of sulfide has also long been known to inhibit mercury methylation (Compeau and Bartha, 1984; 1987, Craig, 1986; Berman and Bartha, 1986). Craig (1986, pg. 84) stated, "for environments of similar total mercury content but with varying sulfide levels that methylmercury concentrations initially increase with increasing sulfide but after 1.8 mg/g sulfide they decrease sharply." Initially, investigators believed this was due to the precipitation of HgS, thus making it unavailable for methylation (Craig, 1986; Compeau and Bartha, 1984; Choi and Bartha, 1994; and references therein). Subsequent research by Benoit et al. (1999a, 1999b, 2000) and Jay et al. (2000) provides compelling evidence that MeHg production is inhibited in high-sulfide sediments, including those found in the Everglades (Benoit et al., 1999a) because Hg forms charged disulfide complexes ($HgHS_2^{-1}$). The disulfide complex has a lower rate of passive uptake by the SRBs because of its charge (i.e., relative to neutral Hg-monosulfide complex). Consequently, less Hg is bioavailable for methylation.

It is the relative amounts of various sulfur pools that are the major determinant of net methylation rate. Cindy Gilmour (Academy of Natural Sciences Estuarine Research Center) states, "Too little sulfate, and the bacteria do not go into action; too much sulfate, and the bacteria produce excess sulfide, which inhibits mercury methylation" (i.e., the so-called "sulfide brake"). An area or region where the sulfate/sulfide levels are just right for mercury methylation has been termed a "Goldilocks" area (William H. Orem; USGS; for discussion of Everglades sulfate issues see Renner 2001).

Because geochemistry is critical to understanding MeHg production, sediments were analyzed for total iron and total sulfur. Iron can influence methylation in two ways. In oxic sediments, a significant fraction of Hg is adsorbed onto detrital iron oxyhydroxides (Dmytriw, 1995). However, when the detrital iron oxyhydroxides are buried and reduced, this Hg is released to pore waters. Reactive Fe phases can also buffer sulfides and keep levels of H_2S low in sediments (Gilmour, 1992; Marvin-DiPasquale and Capone, 1998).

Total iron in STA-2 sediments (4 cm cores) ranged from 1.3 to 3.7 mg/g dry sediment (mean ±1SD=2.4 ±0.8). Total sulfur (TS) in these cores ranged from 2.6 to 7.0 mg/g (4.8 ±1.4). As is evident from **Figure 9**, on a percent dry-weight basis, sediments from Cell 1 contained greater amounts of sulfur, but lesser amounts of iron than sediments from Cell 2 (in both 4 and 10 cm cores). By comparison, sediments from the ENR Project and WCA-2A, which are thought to have lower MeHg due to a "sulfide brake," contained much higher amounts of TS.

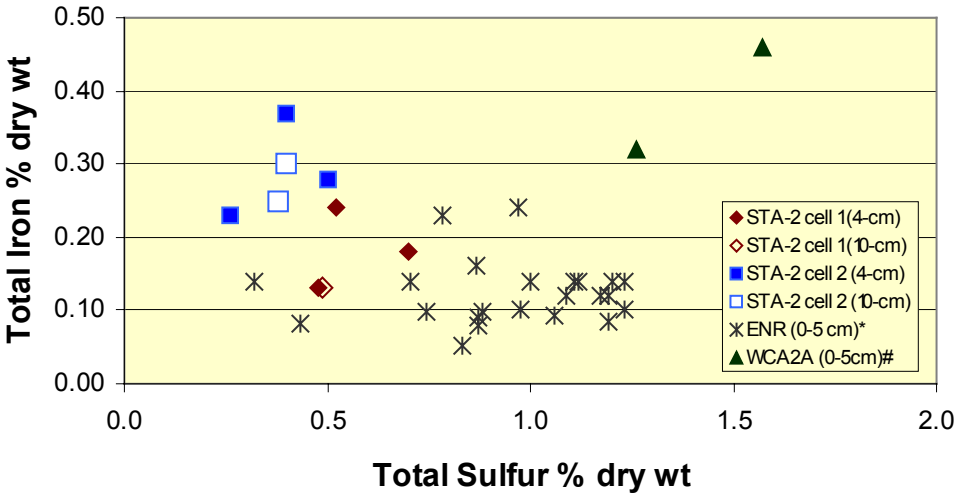

Figure 9. Total Sulfur and iron in Bulk Sediments from STA-2 and reference

Typically, sulfur pools in Everglades sediments consist of, by order of decreasing percent dry weight: organic sulfide, disulfides – pyrite, sulfate and acid volatile sulfides. The speciation of sulfur in sediment is determined by redox potential (E_h), pH, organic productivity, rate of microbial sulfate reduction and the availability of reactive iron (Compeau and Bartha, 1984; for review, refer to Bates et al., 1998). Our ability to predict sulfur speciation in STA-2 sediments was hampered by the lack of pore water data (i.e., cannot predict HS^- based on TS in bulk sediments). It is critical that this data gap be filled because pore water sulfide levels have been found to be the best predictor of methylation rate (i.e., even better than THg concentrations). With this uncertainty in mind, there does not appear to be any evidence in **Figure 9** that would suggest TS in Cell 1 or 2 (as a surrogate for sulfide) is sufficient to inhibit methylation. If this is the case, and the "sulfide brake" has yet to be reached, then increased load of sulfate to STA-2 could continue to stimulate SRB and methylation. In other words, methylmercury production and bioaccumulation could continue to rise (particularly in Cell 2, which has less TS than Cell 1). Moreover, based on previous studies (Krabbenhoft and Fink, 2001), sulfide pools were likely oxidized when these sediments were allowed to dry (**Figure 6**). Upon reflooding, conditions in the sediments will likely again be optimal for SRB and methylation.

Even when pore water data become available, accurately predicting Hg methylation and bioaccumulation in STA-2 will be problematic for a number of reasons. First, given the nutrient load in the source water and the abundance of periphyton in Cell 1, we cannot discount the possibility of mercury methylation within periphyton mats (for review, refer to Cleckner et al., 1999). Further, we must not overlook community dynamics of the SRB as another control of MeHg production. Methylation rates have been shown to exhibit seasonality, with 30-to-50 percent higher MeHg production during the summer months (Gilmour et al., 1998). Therefore, unless there is a change in geochemistry that begins to inhibit methylation (i.e., the "sulfide brake"), MeHg production and bioaccumulation in STA-2 could conceivably increase during the next few months, when warmer temperatures are coupled with an influx of fresh inorganic Hg in summer rains.

THg in Fish

As evident from **Figure 10**, results reported by the FDEP laboratory show Hg levels increased in mosquitofish from certain areas of STA-2 from October through March 2001. This data set also shows fish from Cell 1 consistently had substantially greater concentrations of tissue Hg than fish from Cell 2 or the two inflow sites. In October 2000, the concentration of Hg in Cell 1 mosquitofish was 2.6-to-13 times higher than levels in mosquitofish from Cell 2 or the inflows, respectively. In November 2000, average concentration of mercury increased in mosquitofish from all areas. At that time, concentration of Hg in Cell-1 fish was 2.8-to-7 times higher than levels in fish from Cell 2 and inflows, respectively (i.e., levels in the inflow mosquitofish increased disproportionately compared to Cell 1). In December 2000, Hg concentrations again increased in mosquitofish from all areas, but with relative concentrations among cells remaining constant, i.e., concentrations in Cell-1 fish were 2.8-to-7 times higher than fish from Cell 2 and inflows, respectively. Mosquitofish collected in January were sent to the contract laboratory, and results will be discussed below. In February, THg levels in mosquitofish varied within Cell 1, but appear to have plateaued, overall. At the same time, it is important to note that mercury burdens in Cell 3 mosquitofish, collected for the first time in February, were very low, supporting earlier water management decisions (i.e., passing startup). In March, levels of Hg increased again in mosquitofish from Cell 1, with average concentration reaching 321 ng/g wet weight (346 ng/g at Cell 1-c, a record-high concentration observed in mosquitofish collected by the District).

Statistical analysis of this data set was hampered by the observed variance and by gaps in the data (i.e., no sample taken from certain sites on certain dates) and should be interpreted cautiously. When Cell 1 was assessed alone, the change in tissue-Hg concentration over time was not statistically significant (ANOVA; df=4,7; F=1.8, P=0.23). However, it seems clear that temporal trends in tissue Hg were significant both biologically and statistically in mosquitofish at one or more individual sites (e.g., Cell 1-C), but overall these differences were masked by the spatial variance.

As part of the District's quality assurance program, where sample mass was sufficient, splits of mosquitofish homogenate were sent to a contract laboratory (Frontier Geoscience, Inc.) for analysis. Mean values of replicate analyses (n=3) of each split sample did not differ significantly between laboratories (**Figure 11**; paired t-test; df=18, t=-1.028, p=0.32). However, as is evident from **Figures 10** and **12**, results from the two laboratories were not in complete agreement, especially for high level mosquitofish, i.e., fishes from Cell 1. The contract laboratory tended to report lower Hg concentrations in mosquitofish. While it is possible that Hg levels were not as high as FDEP reported, without additional information we must assume the higher levels (i.e., worst-case) reported by the primary laboratory were more accurate. Regrettably, insufficient sample material was collected in January 2001 for splitting between laboratories, and samples were sent only to the contract laboratory. Visual inspection of data reported by the contract laboratory (Figure 12) supports the results of the statistical analysis of the FDEP data.

Bioaccumulation factors (BAF) from water or sediment are gross oversimplifications of the real-world situation. Nevertheless, these indices provide another means by which to assess mercury monitoring data for average net accumulation. The biota-sediment accumulation factor (BSAF) is a specialized form of the BAF that refers to the THg concentration in fish flesh divided by the concentration of MeHg in sediments. Exposure and accumulation of MeHg in fishes is subtly complex and is influenced by numerous biotic and environmentally mediated factors. Predominant exposure to MeHg for fishes is through diet, with direct uptake of MeHg from water across the gills, providing minimal exposure (Norstrom et al., 1996; Hall et al., 1997), especially in high DOC environments (Choi et al., 1998). Consequently, a BSAF is believed to be a better index of Hg levels in benthic zooplankton and conditions at a particular site than a BAF based on water concentration (D. Krabbenhoft, personal communication). Calculated BSAFs were 281 and 289 for STA-2, Cells 1 and 2, respectively (based on average wet weight MeHg concentrations in 4 cm cores and levels of THg in mosquitofish collected in December 2000, as reported by FDEP). These BSAFs are within the range of similar estimates reported for other areas in South Florida, particularly values observed in 1999, which were associated with recent flooding of marshes following drydowns (**Table 1**).

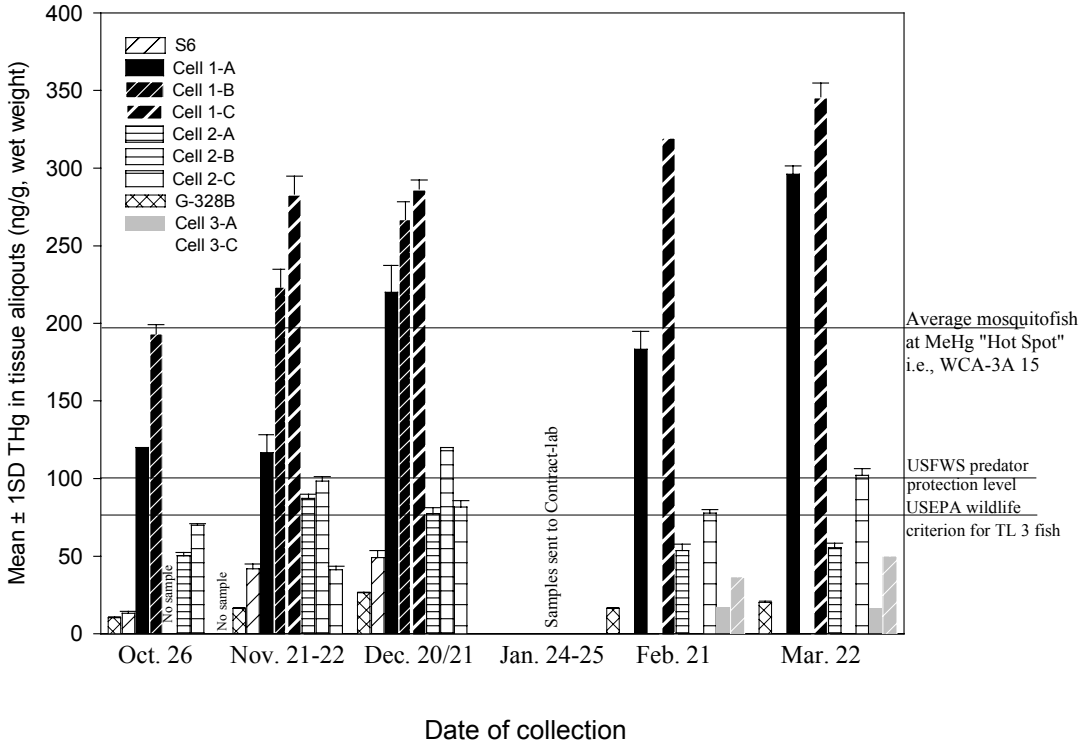

Figure 10. Results of Expanded Startup Sampling at STA-2: Mosquitofish (FDEP Laboratory)

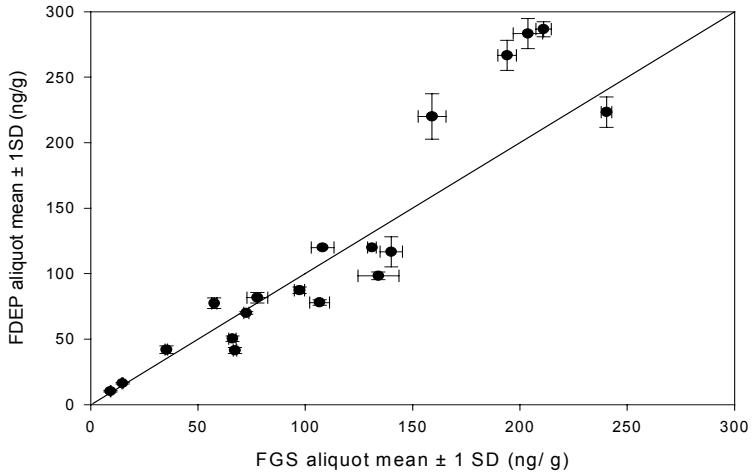

Figure 11. Interlaboratory comparison in determination of THg concnetration in mosquitofish vollected from STA-2

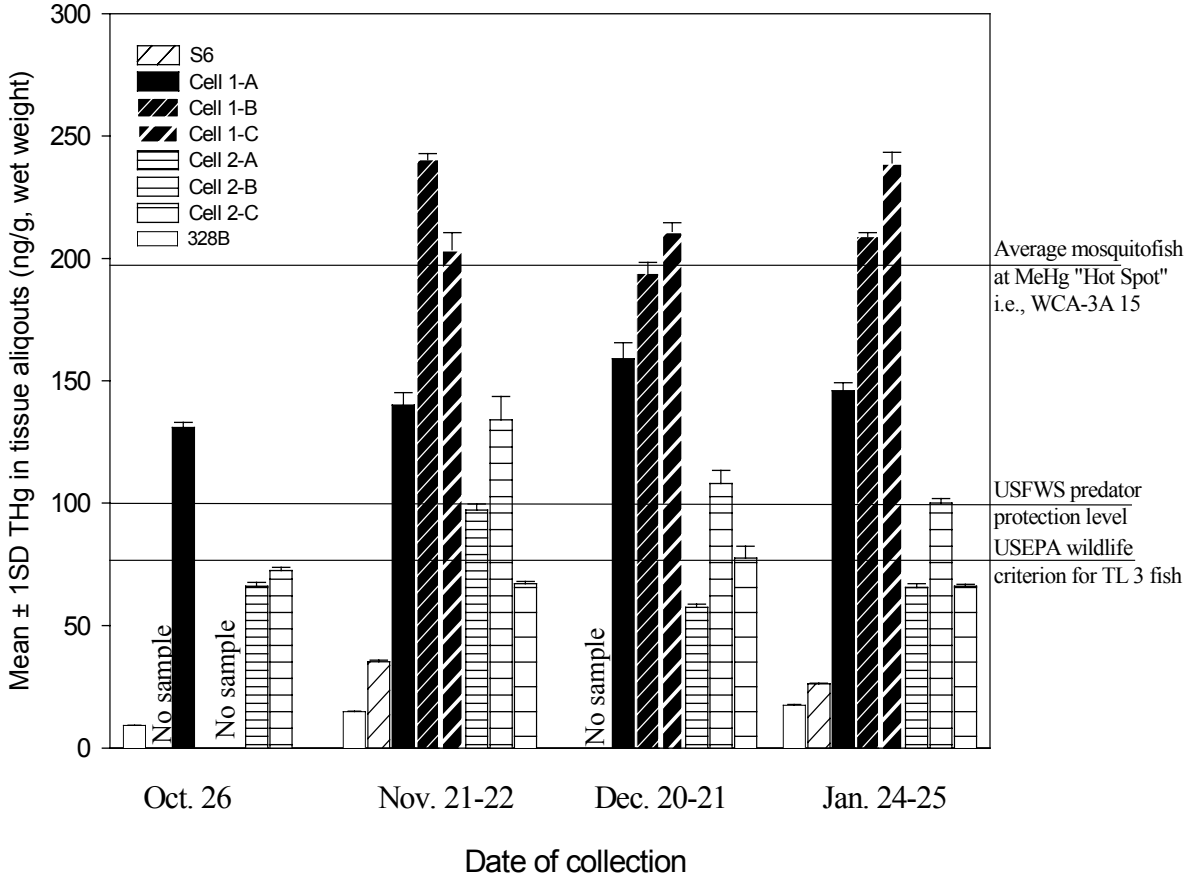

Fig. 12. Results of expanded start-up sampling at STA-2: mosquitofish (contract-laboratory).

Table 1. Biota-Sediment Accumulation Factors (BSAF, Based on Wet Wt.) Observed at Downstream Everglades Marsh Sites (Adapted from Rumbold et al., 2001)

	MeHg	
	1998	1999
Mosquitofish		
STA-6	NA	86
CA2F1	140	100
CA2U3	2,038	389
CA315	5,789	2,705
Sunfish		
CA2U3	4,077	214
CA315	19,737	3,533
Largemouth bass EHg(3)		
STA-6	NA	1,941
CA2U3	20,038	915
CA315	NA	9,648

The among-site variability evident in the BSAFs listed in **Table 1** is likely attributable to locational differences in MeHg bioavailability and trophic dynamics (i.e., zooplankton and benthic fauna), as well as an error component introduced in combining data collected by different studies at different times (for details refer to Rumbold et al., 2001a). Thus, the similarity in BSAFs between cells of STA-2 is noteworthy and suggests that sediment-MeHg concentration is the principal factor in determining exposure. At this point it is too early to predict the trajectory of STA-2 BSAFs, i.e., how net methylation rate, MeHg bioavailability and trophic dynamics will equilibrate in determining the long-term mercury levels in STA-2 biota.

MEHG IN FISH

MeHg concentrations reportedly ranged from 11 to 176 ng/g in mosquitofish collected in October (only fish where MeHg was determined). On average, MeHg as a percent of THg (percent MeHg) was 129 percent. The fact that this value was greater than 100 percent indicates that MeHg determinations by the contract laboratory were biased high. Accordingly, archived samples have been resubmitted for MeHg determination. However, 85 to 99 percent of the THg in mosquitofish is often found to be in the form of MeHg (Grieb et al., 1990; R. Jones, FIU, personal communication, 1995; L. Cleckner, University of Wisconsin, personal communication, 1996; SFWMD, unpublished data). While biased high, the observed percent MeHg in the STA-2 mosquitofish indicates that THg concentrations measured routinely in these fish represents bioaccumulated MeHg through diet, and not inorganic mercury from sediment ingestion.

COMPARISON TO MOSQUITOFISH FROM OTHER STAS

At the request of FDEP, we examined the effect of stage (i.e., with a focus on drydown) and rainfall on mercury levels in mosquitofish from other STAs (**Figures 13** through **15**). The longest period of record, and therefore the largest data set, was from the ENR Project that was later subsumed by STA-1W. As discussed previously, initial collections of fishes following flooding

of the ENR Project in 1994 found no evidence of MeHg spikes (mosquitofish and largemouth bass; PTI 1994 attributed to KBN 1994b); all collected mosquitofish and many of the bass had Hg concentrations below the level of detection (< 0.02 ppm; PTI, 1994 attributed to KBN, 1994b). As is evident from **Figure 13**, routine collections carried out by the District since 1996 show some variability in THg concentrations in ENR mosquitofish, but levels have generally remained low compared to mosquitofish from STA-2 or Everglades marshes (Rumbold et al., 2001a). However, during this period of record, the District was able to maintain water levels in the ENR Project and prevented drydown (**Figure 13**). Moreover, as previously stated, methylation in ENR sediments is likely inhibited by high sulfide levels. A preliminary examination of the influence that rainfall had on mercury levels in the mosquitofish showed a weak association with greatest concentrations occurring in the fall following the rainy season (**Figure 13**). As previously discussed, elevated mercury levels were observed during startup of STA-1W Cell 5. In response to the elevated MeHg concentrations in surface water during the second week of routine startup monitoring (1.8 ng/L on May 26, 1999), sampling was expanded to include mosquitofish (collection began in June 1999). Levels of tissue mercury were elevated in the first sample of mosquitofish from the interior of Cell 5 (105 ng/g), but declined by the next month (54 ng/g in July 1999) and never reached levels observed at STA-2. Note how rapidly THg levels declined in mosquitofish collected from Cell 5 (**Figure 13**). Tissue-mercury concentrations have remained only slightly higher in Cell-5 mosquitofish compared to mosquitofish from other, older parts of the ENR. Thus the "reservoir effect" in STA-1W, Cell 5 appears to have been very short lived.

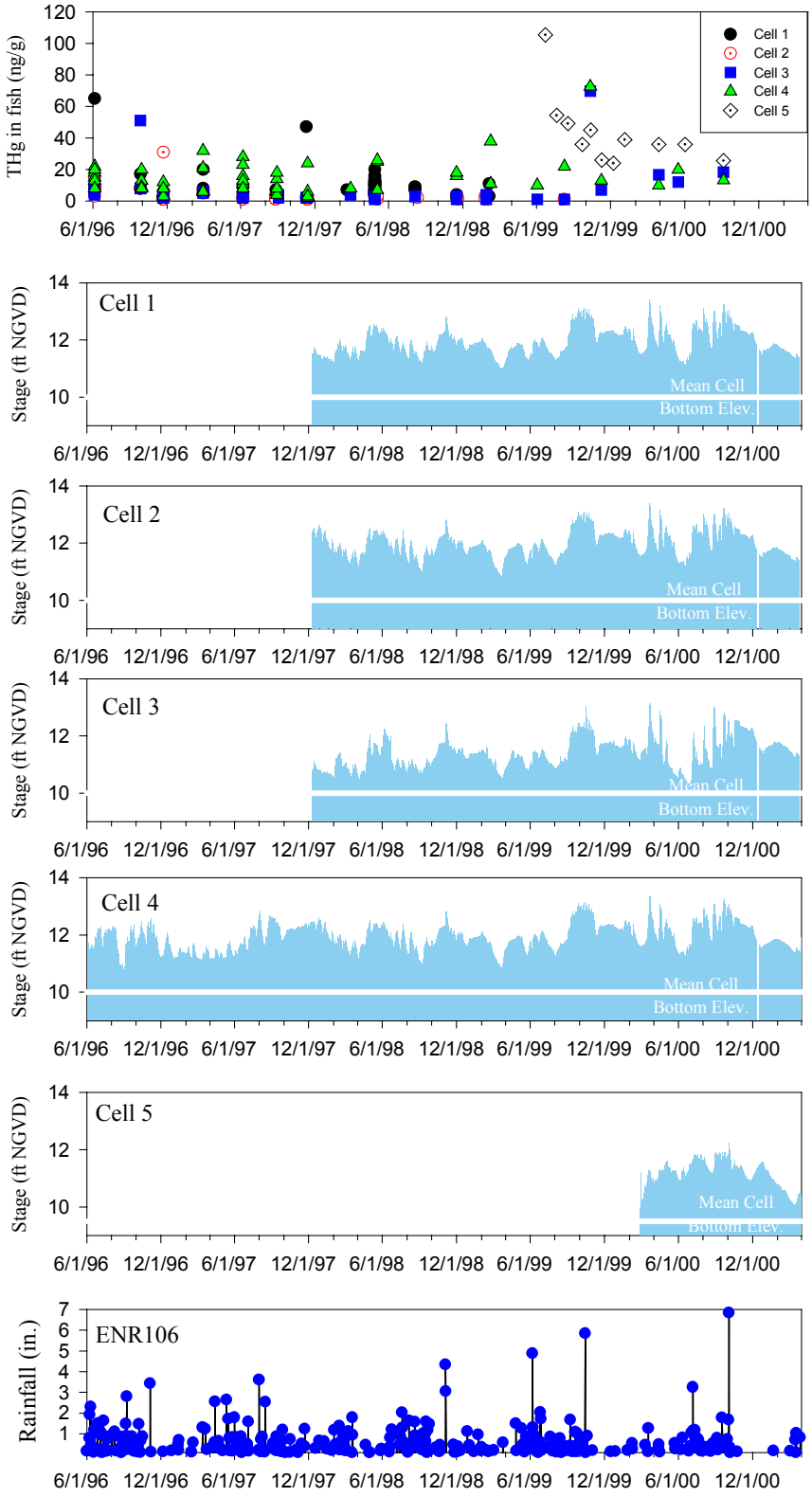

Figure 13. Rainfall, Stage, and THg in Mosquitofish from ENR/STA-1W

Mosquitofish from the ENR Project have also exhibited between-cell differences, albeit to a much lesser extent than STA-2 mosquitofish. As is evident from **Figure 13**, from June 1996 through August 1999 greater concentrations of THg occurred in mosquitofish from Cell 4 relative to mosquitofish from Cell 3. As is discussed in more detail in **Appendix 7-15** of the 2001 ECR (Rawlik 2001), the District initiated a followup study at the ENR Project and concluded these between-cell differences in mercury burdens resulted primarily from differences in food web structure.

Figure 14 summarizes rainfall, stage and mercury concentrations observed at STA-6. STA 6, Section 1 met startup criteria for mercury in November 1997 (i.e., in the first startup sampling event) and began operation in December 1997. Operational monitoring began in January 1998 for surface water (i.e., quarterly at inflows and outflows) and in June 1998 for mosquitofish (i.e., semiannual collection at inflow, interior and outflow). Initial results showed that concentrations of THg and MeHg at the outflow exceeded levels at the inflow. In June 1999 the District initiated a followup study to reduce uncertainties regarding the spatial patterns observed at STA-6 (data included in **Figure 14**; for details see Rumbold et al., 2001b). As is evident from **Figure 14**, unlike the ENR Project, STA-6 dries out frequently. Although we are constrained in our assessment by the routine monitoring sampling scheme (e.g., frequency and collection location), levels of mercury in STA-6 mosquitofish appear to follow a pattern of higher concentrations during and immediately following a drydown. Alternatively, levels of THg in STA-6 mosquitofish were observed to decrease, sometimes rapidly (i.e., during fall 1999), following extended periods of flooding.

Similar to what is reported here for STA-2, mosquitofish at STA-6 exhibited between-cell differences in THg levels, with fishes from Cell 3 having much greater concentrations than Cell 5 fishes. As discussed in more detail in Rumbold et al. (2001a, 2001b), these differences in mercury bioaccumulation were thought to be a result of between-cell differences in in situ production as evidenced by significant between-cell differences in sediment-MeHg concentrations (i.e., in cores collected in 1997 and 2000). As discussed elsewhere (Rumbold et al., 2001a, 2001b), conditions in STA-6, Cell 3 appear to be evolving toward those in Cell 5.

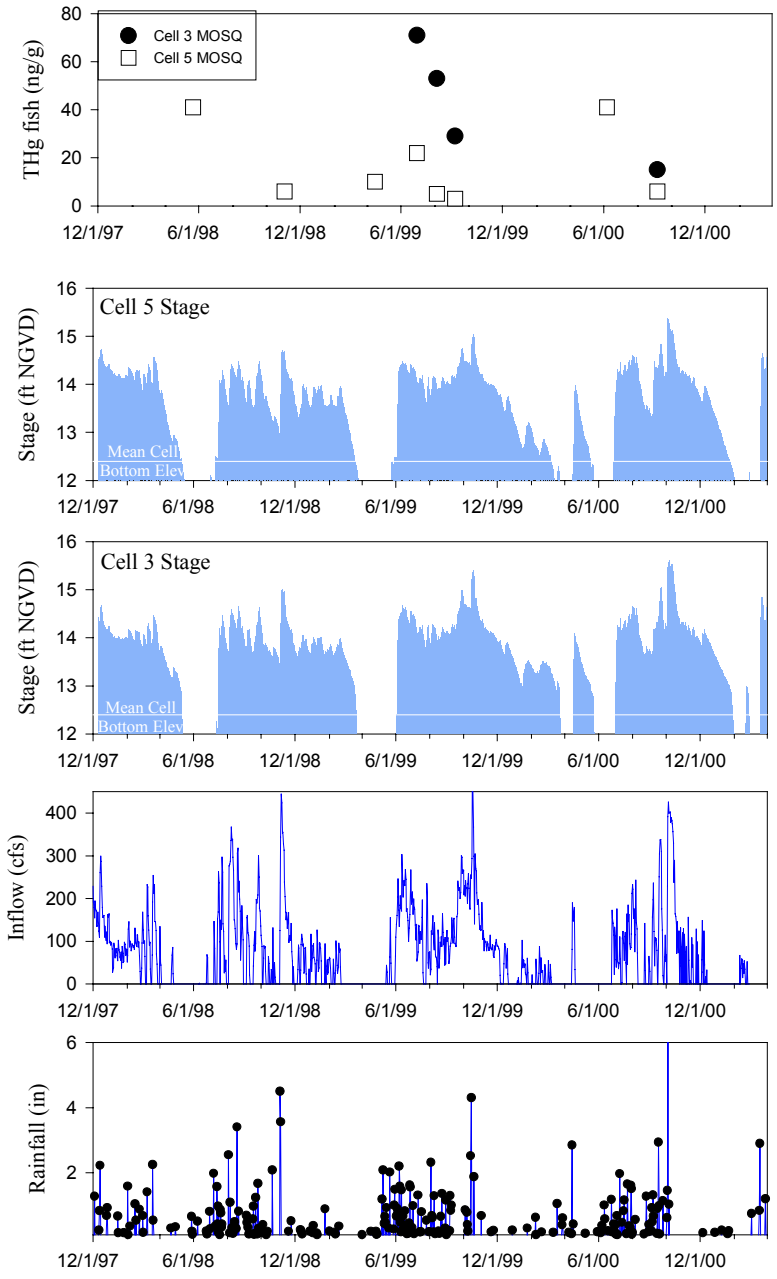

Fig. 14. Rainfall, stage and THg in mosquitofish from STA-6.

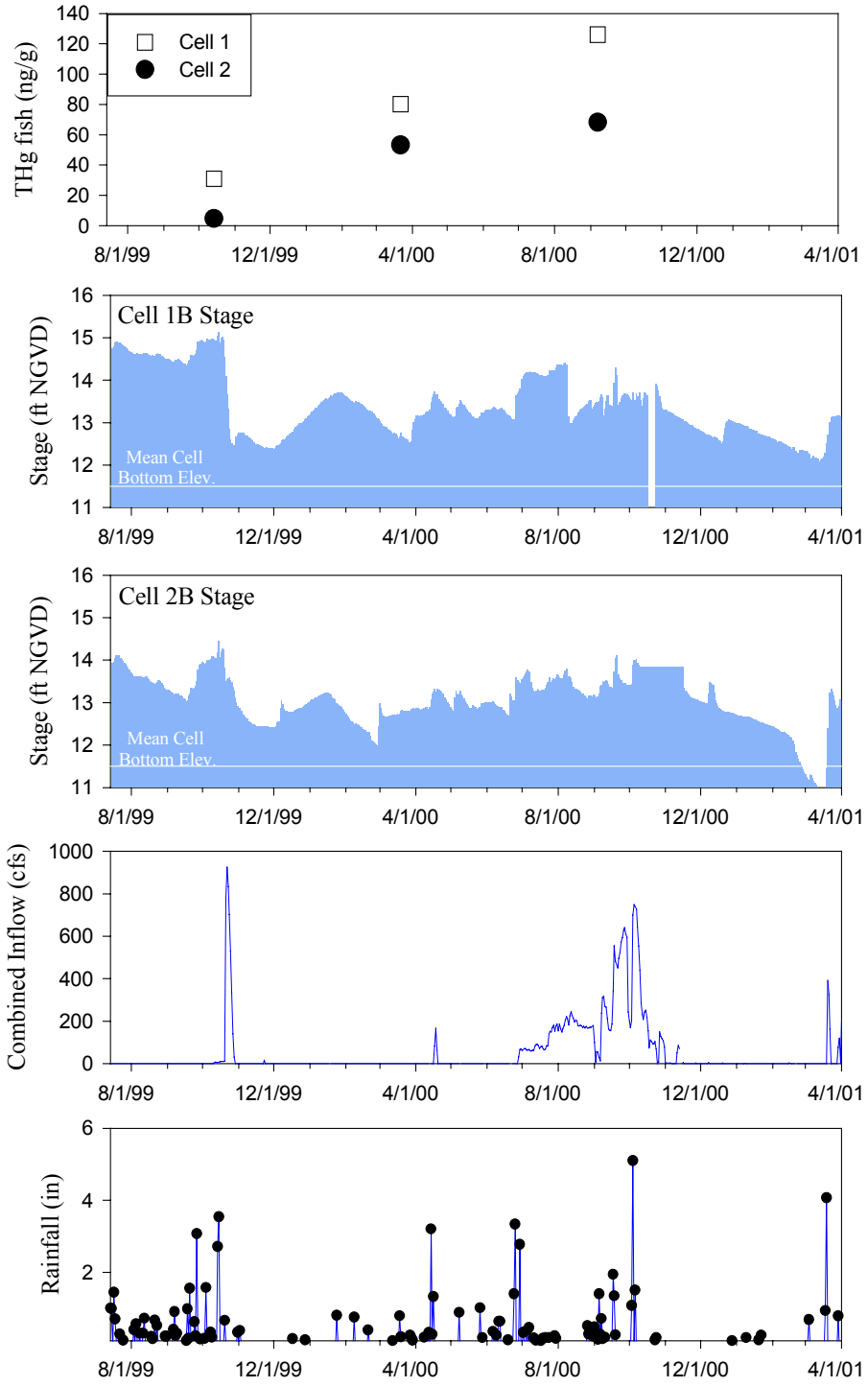

Figure 15. Rainfall, stage and THg in mosquitofish from STA-5

Based on the spatial patterns observed at STA-6, routine monitoring, which had required only a single interior sampling site for fish, was revised to include samples from each treatment train until such time as they were shown to be comparable.

Figure 15 summarizes rainfall, stage and mercury concentrations observed at STA-5. STA-5 met startup criteria for mercury in September 1999 (first startup sampling event following a single nonroutine sampling event in July 1999), but did not begin flowthrough operation until July 7, 2000. A nonroutine preoperational sample of mosquitofish was collected in October 1999 during the annual fish collection at STAs and downstream waters. Operational monitoring began in March 2000 for surface water (i.e., quarterly at inflows and outflows) and mosquitofish (i.e., semiannual collection at inflows, two interior sites and outflows). As is evident from **Figure 15**, THg has steadily increased in concentration in mosquitofish from the interior marsh of STA-5. Unlike STA-6, where mosquitofish from the interior marsh had much less THg than fishes from either the inflow or outflow, interior mosquitofish from STA-5 contained higher concentrations, on average, than inflow or outflow fishes (data not shown). The temporal pattern does not appear to be related to season or drydown; however, interpretation is difficult from data limited to three semiannual events. Like the other STAs, STA-5 also exhibits between-cell differences in bioaccumulation, with mosquitofish from Cell 1b having higher THg concentrations than fishes from Cell 2b. As discussed elsewhere (Rumbold and Rawlik, 2000), there were no between-cell differences in sediment-MeHg concentrations at STA-5.

In addition to monitoring the STAs, the District has also monitored conditions along a transect through WCA-2A. **Figure 16** summarizes rainfall, long-term stage in WCA-2A and stage and THg in mosquitofish from WCA-2A, U3. As is evident from **Figure 16**, THg levels in mosquitofish were high in 1997 following a drydown, decreased during an extended period of flooding from late 1997 through early 1999, and then dramatically increased following the 1999 drydown. Researchers from the USGS reported similar increases in mercury in mosquitofish collected from WCA-2A in October 1999. USGS was in WCA-2A conducting a collaborative study with the District on the effect of sediment drying and fires on mercury speciation and bioaccumulation. At the time of the first post-burn sampling (July 1999), USGS found levels of MeHg in surface water, pore water, sediment, periphyton and mosquitofish about 2x, 18x, 11x, 1.5x and 0.7x higher, respectively, in the burned areas versus nonburned locations. Monitoring at these sites showed burdens of MeHg in mosquitofish and periphyton continued to build throughout Fall 1999, reaching maximums in October. Peat oxidation from burning or intense drying could potentially enhance methylation of Hg by increasing the availability of sulfate, labile carbon, Hg(II) or all three. Of these three parameters, USGS found only sulfate at demonstrably higher levels (about 2.4x) in response to the drying and burning. While the precise biogeochemical mechanism remains uncertain, USGS scientists concluded that drydown, extended dryout and subsequent oxidation (with fires being the extreme oxidation event) altered soil and water chemistry influencing the rate of net methylation of inorganic mercury. They hypothesized that oxidized sulfate was a primary driving factor for increased methylation (for details, refer to Krabbenhoft and Fink, 2001).

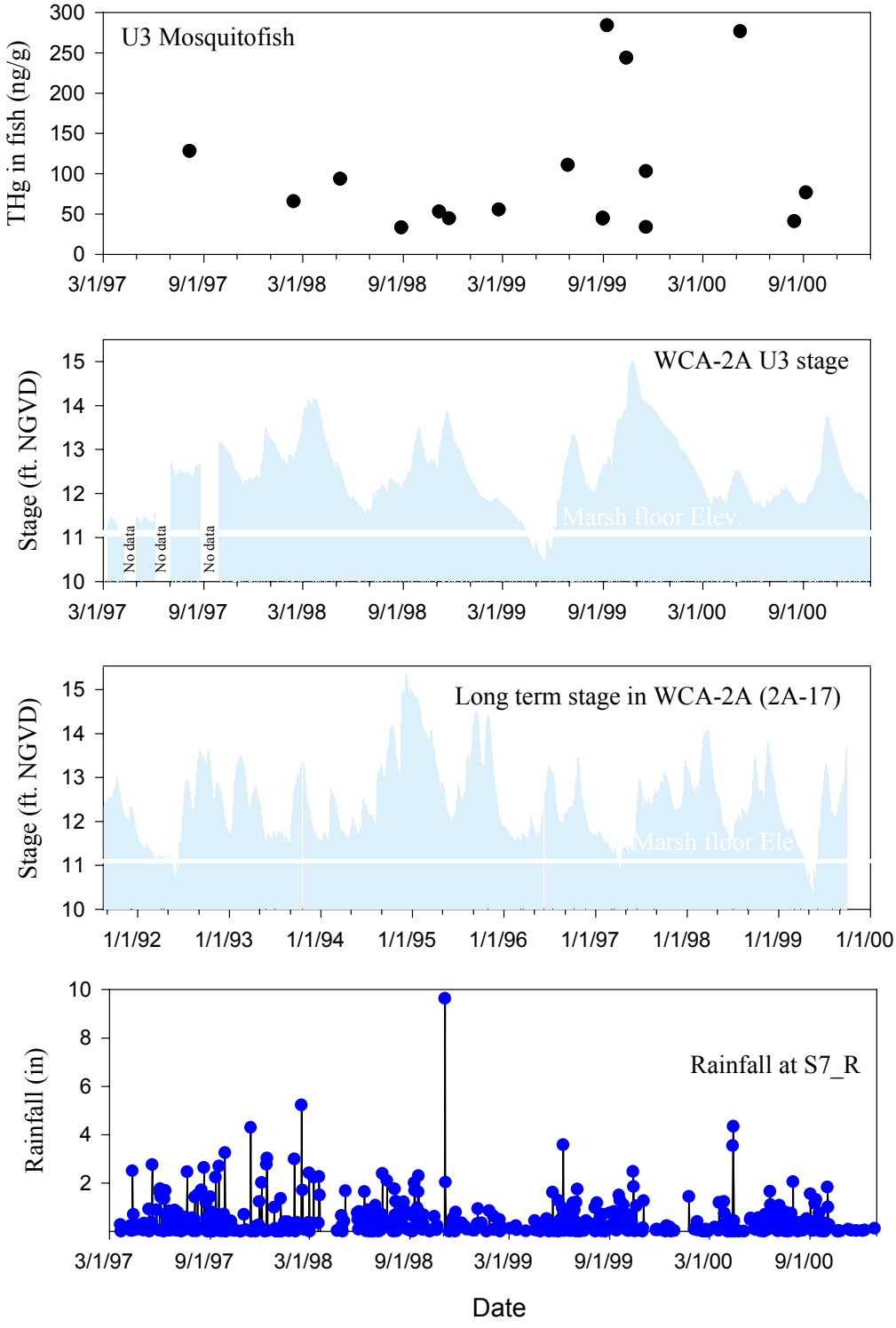

Figure 16. Rainfall, Stage, and THg in Mosquitofish from WCA-2A U3

The implications of the USGS research for STA startup, when oxidized peat soils are initially flooded, or for STAs that dry out was initially discussed in Rumbold et al. (2001a).

Following 10 months of flooded conditions, concentrations of THg have declined in WCA-2A, U3 mosquitofish to relatively low levels (**Figure 16**). Nonetheless, the pulse of mercury following the 1999 drydown has moved up the food chain to sunfish and first-year largemouth bass at WCA-2A, U3 (Rumbold et al., 2001a).

Assessing Risk to Wildlife

Levels of mercury in mosquitofish tissues can also be put into perspective and evaluated with regard to mercury risk to wildlife. The U.S. Fish and Wildlife Service (USFWS) has proposed a predator-protection criterion of 100 ng/g THg in prey species (Eisler, 1987). More recently, in its "Mercury Study Report to Congress," USEPA proposed 77 and 346 ng/g THg for trophic level (TL) 3 and 4 fish, respectively, for the protection of piscivorous avian and mammalian wildlife (USEPA, 1997). As is evident from **Figure 10** (and **Figure 12**), mosquitofish from STA-2, Cell 1, which are considered to be at TL 2-to-3, depending on age (Loftus et al., 1998), exceeded both the USFWS and USEPA criteria. Based on these guidance values, populations of piscivorous avian and mammalian wildlife appear to be at risk of adverse effects from mercury exposures if feeding in Cell 1 for any length of time.

Superimposed on **Figure 10** (and **Figure 12**) is a reference line for the two-year average tissue-Hg concentration in mosquitofish collected at WCA-3A 15 (POR: 1998 -1999, Rumbold et al., 2001a). This site is representative of the largest MeHg "hot spot" first identified in the Everglades by REMAP (USEPA, 1998). Because it likely represented the worst-case exposure scenario within the basin, the District used WCA-3A 15 as a positive-control reference site in its assessment of MeHg risk to Everglades wading birds (Rumbold, 2000). Probabilistic derived hazard quotients (HQs) from that assessment ranged from 2.0 for the 50 percentile for wood storks, to 5.3 for the 95 percentile exposure to great egrets. Egrets at the high-end exposure would be receiving more than five times what is considered a "safe dose" when feeding at this site. Based on this, MeHg risks to wading birds feeding exclusively within the WCA-3A "hot spot" were considered a potential concern warranting further studies. Risk was considered only a potential concern because, as pointed out in the assessment (Rumbold, 2000), we have yet to find clear evidence linking MeHg to impaired reproduction (i.e., fledging success) or population declines in the vicinity of the "hot spot." Nevertheless, the potential for deleterious effects, which may manifest as subtle effects to individuals (including endangered species), could not be dismissed. As evident from **Figure 10** (and **Figure 12**), Hg concentrations in mosquitofish from STA-2, Cell 1 currently exceed average concentrations in mosquitofish collected from the "hot spot" over the last two years.

Table 2. Biomagnification factors (BMF) observed at downstream interior marsh sites (adapted from Rumbold et al., 2001).

Location	Mosquitofish to Sunfish		Mosquitofish to Bass EHg(3)		Sunfish to Bass EHg(3)	
	1998	1999	1998	1999	1998	1999
LOX4	3	0.9	9	3	3	3
L39F1	NA	0.6	NA	3	NA	4
L38F1	NA	0.4	NA	2	3	4
Holey Land	1	0.3	9	2	7	6
CA2U3	2	0.5	10	2	5	4
L5F1	1	0.4	3	NA	5	NA
CA3F1	7	0.9	NA	5	NA	5
CA315	3	1.3	NA	4	NA	3
CA3F2	4	1.2	NA	NA	NA	NA
P33	6	2.0	NA	NA	NA	NA
L67F1	NA	2.7	NA	NA	3	NA
Mean	3	1	8	3	4	4

However, mosquitofish are not a preferred prey item of long-legged wading birds. Accordingly, the risk assessment at WCA-3A, 15 relied heavily on concentrations observed in sunfish, which are a preferred prey item (Kahl, 1963; Ogden et al., 1976; Smith, 1995; Frederick et al., 1997, 1999). At present, we have no data on mercury levels in sunfish at STA-2. Using biomagnification factors (a BMF is the factor by which THg concentration in the organisms at one trophic level exceed the concentration in the next lower trophic level) observed elsewhere in the Everglades (**Table 2**), we can predict that STA-2 sunfish would have Hg levels 0.3-to-7 times higher than the mosquitofish (i.e., 96 to 2,247 ng/g).

The range in BMFs summarized in **Table 2** is likely attributable to differences in trophic dynamics among sites and a time lag from exposure to collection and measurement (i.e., where on the uptake curve the two species of fish are positioned relative to an antecedent perturbing event, e.g., dryout and reflooding, etc.). Because they are small-sized and short-lived, mosquitofish are used to monitor short-term changes in environmental concentrations of mercury through time. Sunfish, on the other hand, are larger and longer-lived and represent average exposure conditions over a longer time period, possibly up to a year. Consequently, following high exposures mercury levels tend to peak earlier in mosquitofish than in sunfish. As a result of MeHg pulses associated with drydowns and reflooding in 1999, mosquitofish from many downstream areas had higher levels of Hg in their tissues than sunfish (BMF < 1.0, **Table 2**). A similar situation may be occurring in STA-2 as a result of the "reservoir effect." If so, then sunfish may currently have THg levels only 0.3 times that of mosquitofish. The amount of mercury that is ultimately integrated and biomagnified within the sunfish will be determined by the duration of the MeHg peak in the mosquitofish and other preyfish. However, until we have evidence to the contrary we must assume that all the mercury has biomagnified or will biomagnify up the food chain in the

proportions more typical of near steady-state conditions. Based on this, the only conclusion that can be reached is that populations of piscivorous avian and mammalian wildlife are at potential risk of chronic adverse effects from mercury exposures if feeding in Cell 1 for any length of time.

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