Chapter 3B: Mercury and Sulfur Environmental Assessment for the Everglades

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SUMMARY

This chapter provides an assessment of the sulfur and mercury status within the Everglades Protection Area (EPA), and Holey Land and Rotenberger wildlife management areas (WMAs) during Water Year 2018 (WY2018; May 1, 2017–April 30, 2018). The report fulfills the requirements of the Everglades Forever Act (EFA), Subparagraph 373.4592(4)(d)13, Florida Statues. The information provided in this chapter is an update to Chapter 3B of the *2018 South Florida Environmental Report* (SFER) – *Volume I* (Julian et al. 2018).

The analysis and summaries provide a synoptic view of mercury and sulfur in the EPA and surrounding areas on a regional scale and include the Arthur R. Marshall Loxahatchee National Wildlife Refuge (LNWR or Water Conservation Area [WCA] 1), WCA-2, WCA-3, and Everglades National Park (ENP). This chapter updates the status of mercury and sulfur monitoring in the Everglades region and summarizes mercury concentrations in biota; mercury atmospheric deposition; and surface water sulfate (SO_4^{2-}) concentrations, loads, and atmospheric deposition to the EPA. Analytical data are reported for WY2016 for wildlife—mercury in fish tissue for largemouth bass (LMB; *Micropterus salmoides*), sunfish (*Lepomis* spp.), and mosquitofish (*Gambusia* spp.)—and for surface water sulfate.

Key highlights for this year's reporting period are as follows:

- WY2018 total mercury (THg) concentrations in mosquitofish from 13 monitoring sites ranged from 0.006 milligram per kilogram (mg/kg) at site CA2NF to 0.132 mg/kg at site CA315, with a median value of 0.028 mg/kg. Mosquitofish THg concentrations during WY2018 exceeded the United States Environmental Protection Agency (USEPA) trophic level 3 (TL3) criterion (0.077 mg/kg) for protection of piscivorous wildlife at three of the 13 sites.
- In WY2018, mean THg concentrations in TL3 sunfish from 13 monitoring sites ranged from 0.046 mg/kg at CA2NF to 0.296 mg/kg at CA315. Mean annual sunfish THg

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concentration during WY2018 exceeded the USEPA protection of wildlife criterion at 9 stations across the monitoring network. Annual mean THg concentrations in sunfish species from all sites were highest in spotted sunfish (*L. punctatus*; 0.247 mg/kg), intermediate in bluegill (*Lepomis macrochirus*; 0.195 mg/kg), and lowest in redear sunfish (*L. microlophus*; 0.126 mg/kg).

- During WY2018, THg concentrations in LMB were determined from 9 of the 13 locations within the EPA. Annual mean THg concentration from EPA sites ranged from 0.183 mg/kg at site CA2NF to 0.949 mg/kg at site L67F1 (ENP), with a median value of 0.360 mg/kg. During WY2018, five locations exceeded the USEPA recommended criterion for the protection of human health (0.350 mg/kg).
- Trophic position of LMB across the EPA is variable potentially linked to food web and habitat dynamics within and across the ecosystem.
- Evidence from a sulfate-amended incubate suggests that mercury methylation can be facilitated by non-sulfate-dependent microorganisms (syntrophs) in the Everglades ecosystem. Furthermore, another result of the sulfate amended incubation is that sulfate addition has the potential to facilitate deep soil methylmercury (MeHg) linked to abiotic incorporation of reduced sulfur compounds. Additional study is needed to further study mercury methylation and sulfur biogeochemistry.
- During WY2018, annual mean inflow sulfate concentrations ranged from 7.8 milligrams per liter (mg/L) for WCA-3 to 57.6 mg/L for LNWR. The annual mean sulfate concentrations at interior marsh regions ranged from 3.9 mg/L for ENP to 38.4 mg/L for WCA-2.

AREA OF INTEREST

The greater Everglades is a vast mixed wetland ecosystem that stretches from Lake Okeechobee to Florida Bay and the Gulf of Mexico (DeAngelis et al. 1998). The EPA and Holey Land and Rotenberger WMAs are situated within this immense ecosystem. The EPA is a complex system of marsh areas, canals, levees, and inflow and outflow water control structures that covers almost 2.5 million acres (1 acre = 4,047 square meters) of former Everglades marsh and currently is divided into separate distinct shallow impoundments, or WCAs (Bancroft et al. 1992). In addition to rainfall inputs, surface water inflows regulated by water control structures from agricultural tributaries, such as the Everglades Agricultural Area (EAA) to the north and the C-139 Basin to the west, feed the EPA. The EPA also receives surface water inflows originating from Lake Okeechobee to the north and from predominantly urbanized areas to the east. The timing and distribution of the surface inflows from the tributaries to the EPA are based on a complex set of operational decisions that account for natural and environmental system requirements, water supply for urbanized and natural areas, aquifer recharge, and flood control. The Holey Land and Rotenberger WMAs are located just north of the EPA, and together span 64,000 acres and consist of remnant Everglades' marsh with scattered small tree islands (Newman et al. 1998). The major features of the EPA and surrounding area are illustrated in Figure 1-1 in Chapter 1 of this volume.

METHYL MERCURY FORMATION IN THE EVERGLADES

Over the past several decades, multiple research studies have been done regarding the factors that influence the formation of MeHg in the aquatic and semi-aquatic environments, particularly within the Everglades ecosystem. As a result, a suite of peer reviewed and technical publications have been produced exploring the underlying biogeochemical regulation of MeHg production within natural systems. The majority of these with relevance to South Florida focus on the hypothetical unimodal relationship of S/sulfate and MeHg production (Gilmour et al. 1992, Benoit et al. 1999a, b, 2003, Axelrad et al. 2008, 2013, Orem et al. 2011). Since the evolution of the theoretical relationship between sulfate and MeHg production, which states that MeHg production follows a unimodal curve with respect to sulfate along the sulfate concentration gradient, early sulfur and mercury studies and large-scale biogeochemical surveys have informed our understanding of S and the role it plays in wetland biogeochemistry. However at the landscape scale, mercury methylation is subject to large unexplained variations and appears to be influenced not only by sulfate but a combination of many environmental factors (Gilmour 2011, Julian et al. 2014). Due to this complexity and variability, the sulfate-mercury unimodal relationship is not spatially or temporally consistent within the Everglades (Julian et al. 2014, 2015a). The proof of an ecological concept lies in its predictive capability in nature and direct evidence of the sulfate and MeHg linkage has proven elusive. Rigorous analysis of the plethora of ambient monitoring data from the Everglades in combination with decades of research have yet to yield satisfactory models to develop an empirically rigorous relationships to explain MeHg formation and bioaccumulation dynamics in a predictable manner.

MERCURY IN EVERGLADES FISH AND WILDLIFE

Elevated mercury concentrations in fish and biota have been a concern for the Everglades regions since the 1970s (Ogden et al. 1973). Subsequently, elevated mercury levels were reported in other wildlife species including American alligators (*Alligator mississippiensis*), blue crayfish (*Procambarus alleni*), Florida softshell turtles (*Apalone ferox*), pig frogs (*Rana grylio*), mottled ducks (*Anas fulvigula*), white-tailed deer (*Odocoileus virginianus*), and the endangered Florida panther (*Puma concolor coryi*) (Ware et al. 1991). More detailed synoptic monitoring programs identified elevated and variable mercury concentrations in piscivorous wildlife within the EPA including raccoons (*Procyon lotor*), alligators, wading birds, and Florida panthers (Roelke et al. 1991, Spalding et al. 2000, Rumbold et al. 2002, Porcella et al. 2004).

Because of its large size, extensive wetlands, and relatively high rates of mercury deposition, the Everglades is considered sensitive to mercury methylation and subsequent bioaccumulation into piscivorous wildlife (Wiener et al. 2003). Methylation of inorganic mercury in Everglades wetlands leads to the formation of MeHg, a potent neurotoxin. MeHg in aquatic biota is of human health and ecological concern due to its ability to bioaccumulate and biomagnify in food webs to concentrations that may pose a potential health threat to wildlife and humans that consume fish (Lange et al. 1993, Rumbold et al. 2001, Frederick et al. 2004, Hammerschmidt and Fitzgerald 2006). Because fish are the main MeHg exposure pathway to both human and wildlife consumers (Sunderland 2007), monitoring is necessary to understand the ecological significance of the spatial and temporal patterns in THg bioaccumulation in the Everglades. This section summarizes the research on the status and trends of mercury in native fish and wading birds from the Everglades region.

SPATIAL AND TEMPORAL TRENDS IN MERCURY LEVELS IN EVERGLADES FISH

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As discussed above, the Everglades ecosystem continues to experience elevated concentrations of Hg in various wildlife species. For the majority of these wildlife species, fish are an integral part of their diet either directly or indirectly. As such, biological monitoring of fish species is very important in the Everglades system. Fish can be useful indicators of aquatic ecosystem health, as various fish species have the ability to integrate ecological processes of the system across both temporal and spatial scales (Joy and Death 2002).

This section presents an update to Hg tissue concentrations in native Everglades fish of multiple trophic levels and provides an opportunity to evaluate spatial and temporal trends in MeHg exposure levels for both wildlife and humans. Mercury data from fish representing three distinct trophic levels and with varying life histories allow for assessment of various bioaccumulation and health assessment endpoints. Mosquitofish represent short-term changes in bioaccumulation due to their relatively short life span and limited home range, although they are widely distributed throughout the Everglades. Mosquitofish become sexually mature at approximately three weeks of age and have an average life span of only four to five months (though some individual females are thought to live up to 1.5 years).

Sunfish (bluegill, redear sunfish, and spotted sunfish) and all Centrarchid species are also common in the canal and marsh complex and provide a longer-term environmental exposure estimate over a more expanded spatial scale. Sunfish are thought to have an average life span of four to seven years in the wild, but the size classes selected are typically in the age class of 1 to 3 years. These three centrarchid species overlap with diverse diets and may compete across species and age classes for prey items. Larger bluegill feed on a broad array of invertebrates and small fish and may appear higher in the food web structure than redear or spotted sunfish (Loftus 2000). Overall, both mosquitofish and sunfishes represent intermediate links within the Everglades aquatic food web and are preferred prey items for several fish-eating species; therefore, whole body mercury concentrations of these species are utilized to assess potential wildlife health risks.

THg concentrations in LMB provide a spatially integrated measure of exposure to a long-lived top predator and, as such, are primarily utilized to assess human exposure to MeHg. Mercury concentrations in axial muscle tissue (fillets) from individual bass are used to assess human health risks associated with mercury exposure. Mercury effects on Everglades fish health from actual environmental exposure have been documented (Scheuhammer et al. 2007, Wiener et al. 2003).

Methods

Fish Sampling within the Greater Everglades Ecosystem

To assess THg concentrations and trends in fish, samples were collected at 13 monitoring stations within the EPA and Holey Land and Rotenberger WMAs (**Figure 3B-1)**. These stations are part of the long-term mercury monitoring projects of the South Florida Water Management District (SFWMD or District) and the Florida Fish and Wildlife Conservation Commission (FWC).

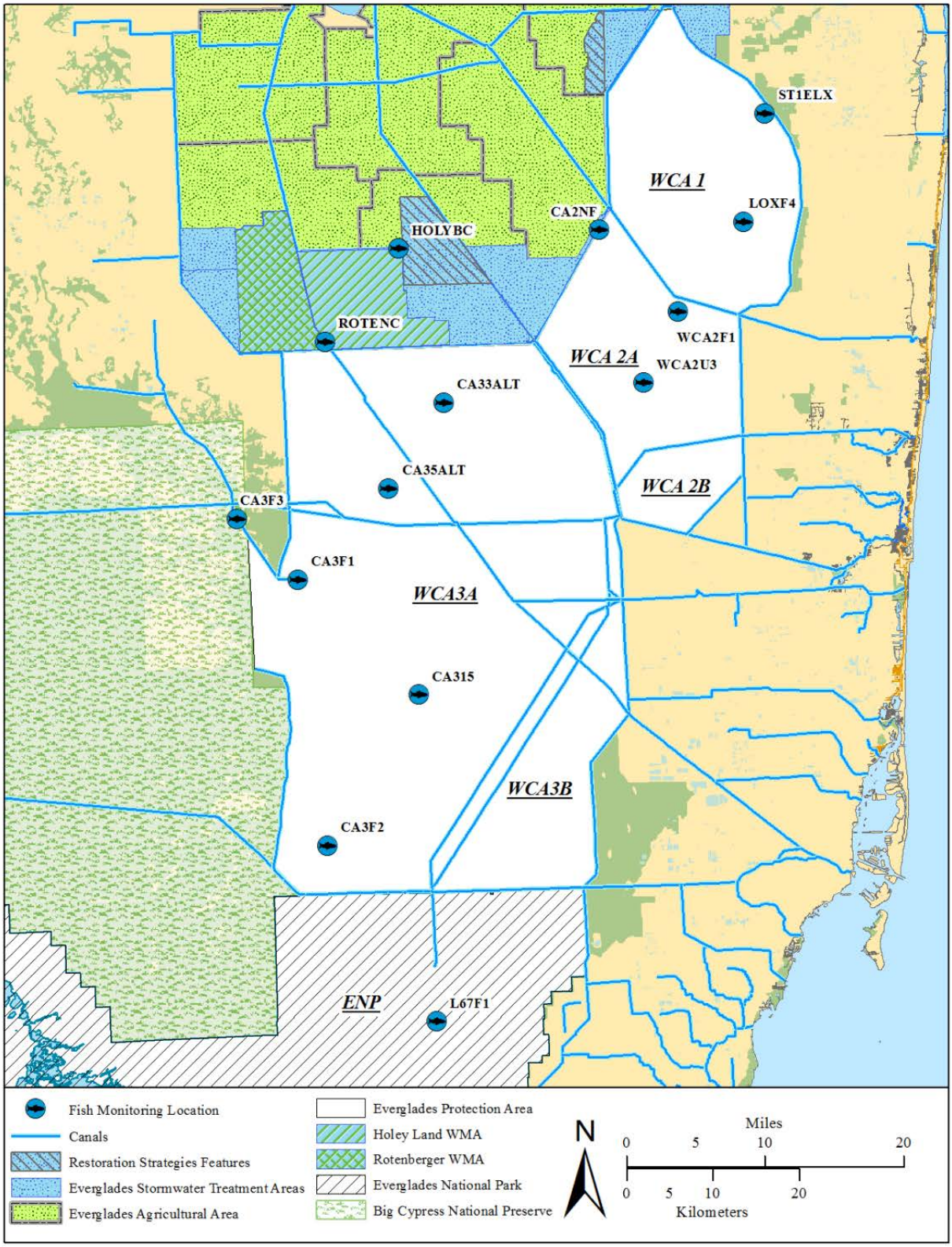

Figure 3B-1. Location of fish tissue monitoring locations within the EPA and Holey Land and Rotenberger WMAs. (Note: Station CA3F1 is an inactive station with fish sampling activity suspended since October 2009. CA3F3 is used to replace CA3F1 since October 2010.)

Annual fish collections generally occur during September and November. Mosquitofish were collected using a dip net to obtain a grab sample of between 100 and 250 mosquitofish from each site. After collections, mosquitofish were homogenized and subsamples were analyzed for THg. Sunfish and bass were collected using direct-current, electrofishing equipment mounted on either an airboat or Jon boat at each site. For sunfish, up to 10 individuals in the target size range of 102 to 178 millimeter (mm; i.e., 4 to 7 inches) total length (TL) were collected at each station, while the remaining sunfish were divided among the common species encountered at each site. A total of 20 sunfish were targeted for collected at each location. Similarly, 20 bass ranging in size between 200- and 500-mm TL were targeted for collection at each site and collected concurrently with sunfish. In the laboratory, sunfish and bass were weighed, measured, sexed, and, for bass only, the sagittal otoliths were removed for determination of age. Whole sunfish and whole axial muscle (fillet) samples of bass were preserved at 4 degrees Celsius in plastic bags.

Homogenized samples of mosquitofish, sunfish, and bass axial muscle tissue collected from the EPA were analyzed by the District using USEPA *Method 7473: Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry* (USEPA 2007), with a method detection limit (MDL) of 0.005 mg/kg. All results are reported as THg on a wet weight basis as mg/kg. Because more than 85% of the mercury found in fish is in the form of MeHg (Bloom 1992, Grieb et al. 1990), it is assumed that THg concentrations are considered to be representative of MeHg concentrations in fish tissue samples.

Both mosquitofish and sunfish were processed as whole-body homogenates to assess potential ecological risk from MeHg exposure to fish-eating wildlife. These data were evaluated against the USEPA TL3 MeHg criterion of 0.077 mg/kg for protection of wildlife (USEPA 1997). Human exposure to MeHg occurs primarily through consumption of fish; therefore, axial muscle tissue (fillets) from bass are utilized to assess human health risk from MeHg exposure. The USEPA-recommended MeHg criterion for the protection of human health (0.35 mg/kg in fish tissue) provides a baseline for these assessments (USEPA 2001). All results are reported as THg on a wet weight basis as mg/kg.

Data Screening and Handling

Mercury data evaluated in this section of the chapter were retrieved from the District's corporate environmental database, DBHYDRO. Similar to water quality analysis within this section and Chapter 3A of this volume, fish tissue data were screened based on laboratory qualifier codes. These qualifiers are consistent with the Florida Department of Environmental Protection's (FDEP's) Quality Assurance Rule (Chapter 62-160, Florida Administrative Code [F.A.C.]). Any datum associated with a fatal qualifier (e.g., G, H, J, K, N, O, V, Q, Y, Z, or ?) indicating a potential data quality problem was removed from the analysis. Fatal qualifiers are used both by laboratories for sample analyses and data users for reporting to indicate that the quality or accuracy of the data may not be suitable for water quality evaluations.

Quantitative Analysis

Fish tissue THg concentrations were summarized by station, region, and species using basic descriptive statistics for the current water year, Water Year 2018, and the entire period of record (POR; WY1999–WY2018; May 1, 1998–April 30, 2018). Mosquitofish THg concentrations were assessed between climatic years (i.e., wet versus dry years) and habitat (i.e., canal and marsh) separately using the Kruskal-Wallis rank sum test. Wet and dry years were determined based on the historical rainfall amount observed at long-term rainfall monitoring stations in the EPA. THg concentrations of TL3 sunfish species (i.e., bluegill, redear sunfish, and spotted sunfish) were compared using the Kruskal-Wallis rank sum test and Dunn's test of multiple comparisons for both the entire POR and current water year. Trend analysis of tissue THg for all fish species was performed using Kendall's correlation analysis. All statistical operations were performed with SigmaPlot 17 and R with the critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Mosquitofish

Mosquitofish composite samples from across the EPA have been collected for THg analysis since WY1999 (**Table 3B-1**). Since WY2017, two alternative sites were used to replace WCA2F1 due to a site access issue. In WY2018, THg concentrations in mosquitofish from 13 monitoring sites ranged from 0.006 mg/kg at site CA2NF to 0.132 mg/kg at site CA315, with a median value of 0.028 mg/kg (**Figure 3B-2**), which is on average 29% greater than the median value reported in WY2017 (0.020 mg/kg). Average mosquitofish THg level in WY2017 decreased in all but 3 stations compared to WY2018 where increases were observed at 9 stations in WY2018 relative to the prior water year. Average mosquitofish THg at WCAU3 during WY2018 displayed the greatest decrease of 83% while this site displayed the greatest increase by >100% in WY2017 than WY2016 (0.228 and 0.036, respectively). In fact, 6 of 12 sites in WY2018 were below their median POR values (**Figure 3B-2**). While some sites experience significant year-to-year variability, significant decreases have been observed at three sites and a significant increase in one site (**Table 3B-1**).

Table 3B-1. Temporal trend analysis: Kendall Tau Correlation of median annual mosquitofish THg concentration at the 13 active monitoring locations within the EPA for WY1999–WY2018 (May 1, 1998–April 30, 2018).

Area	Station	Kendall's τ	p-value	Sample Size	Trend Direction
WCA-1	ST1ELX	0.05	0.84	12	Not statistically significant
	LOXF4	-0.56	<0.01	19	Decrease
WCA-2	CA2NF	-0.33	0.13	13	Not statistically significant
	WCA2F1 [a]	0.43	<0.05	17	Increase
	WCA2U3	-0.02	0.92	20	Not statistically significant
WCA-3	CA33ALT	-0.17	0.41	14	Not statistically significant
	CA35ALT	0.02	0.96	16	Not statistically significant
	CA3F1/F3	-0.07	0.67	20	Not statistically significant
	CA315	-0.32	<0.05	20	Decrease
	CA3F2	-0.41	<0.05	20	Decrease
ENP	L67F1	-0.17	0.34	18	Not statistically significant
WMAs	ROTENC	0.08	0.69	15	Not statistically significant
	HOLYBC	-0.13	0.42	20	Not statistically significant

a. Since WY2017, site WCA2F1 is represented by two alternative sites (CA2F1ALT1 and CA2F1ALT2).

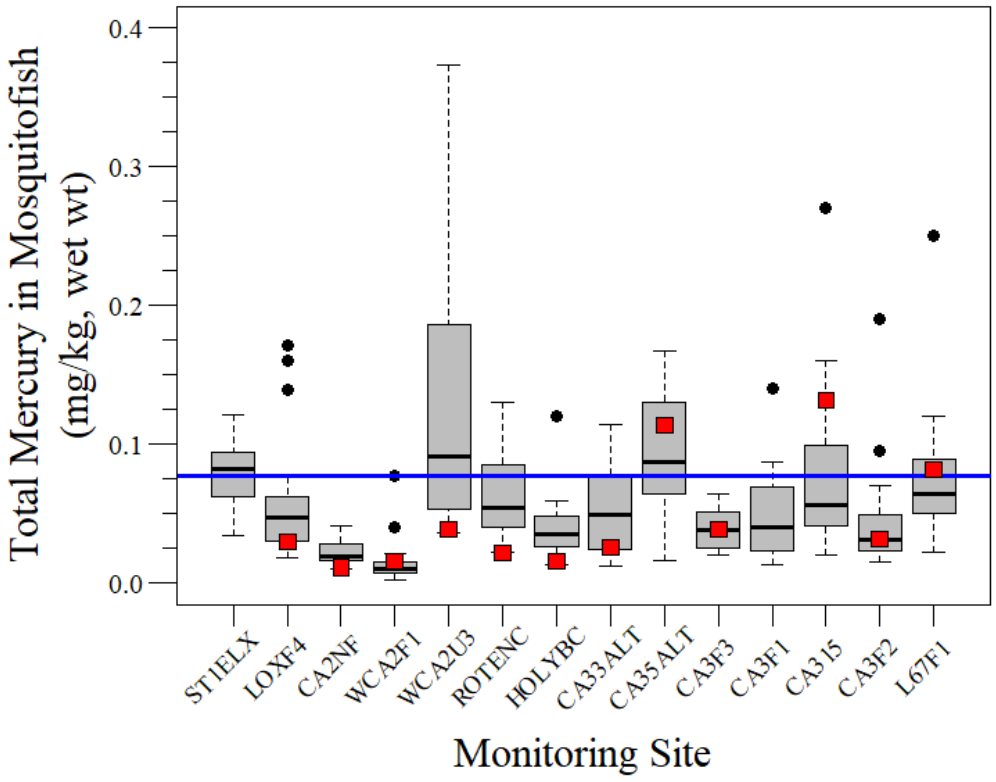

Figure 3B-2. Box plots of THg concentrations in mosquitofish in mg/kg, wet weight (wt), at each monitoring site in the EPA for WY1999–WY2018. Red boxes indicate WY2018 mean THg concentrations and the blue line denotes the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of piscivorous wildlife.

Mosquitofish THg concentrations in WY2018 exceeded the federal criterion of 0.077 mg/kg MeHg for TL3 fish at 3 of the 13 active monitoring sites (CA35ALT, CA315, and L6F1) (**Figure 3B-2**). For the POR, the median value of THg in mosquitofish is 0.049 mg/kg and 23% of the data exceeded the federal criterion. The highest value of mosquitofish THg throughout the POR was 0.373 mg/kg observed at WCA2U3 (WY2011), while the lowest observed value of 0.003 mg/kg was recorded at WCA2F1 (WY20005) (**Figure 3B-3**). Site WCA2F1 (and its alternative sites) near the Hillsborough Canal and site CA2NF near the L-6 Canal had no exceedance for the entire monitoring period and displayed the lowest mosquitofish median THg values of 0.008 and 0.019 mg/kg, respectively. It is noteworthy that WCA2F1 and CA2NF are located in the northern portion of WCA-2A near the Stormwater Treatment Area 2 (STA-2) outflow and within the nutrient enriched areas of the marsh. Additionally, sites WCA2U3 and CA35ALT, which have relatively elevated mosquitofish THg tissue concentrations are located in the nutrient-poor area on the mid-southern end of the marsh. The differences in tissue mercury concentrations and relative ambient nutrient concentrations could suggest changes in trophic structure and/or conditions relative to mercury uptake and accumulation.

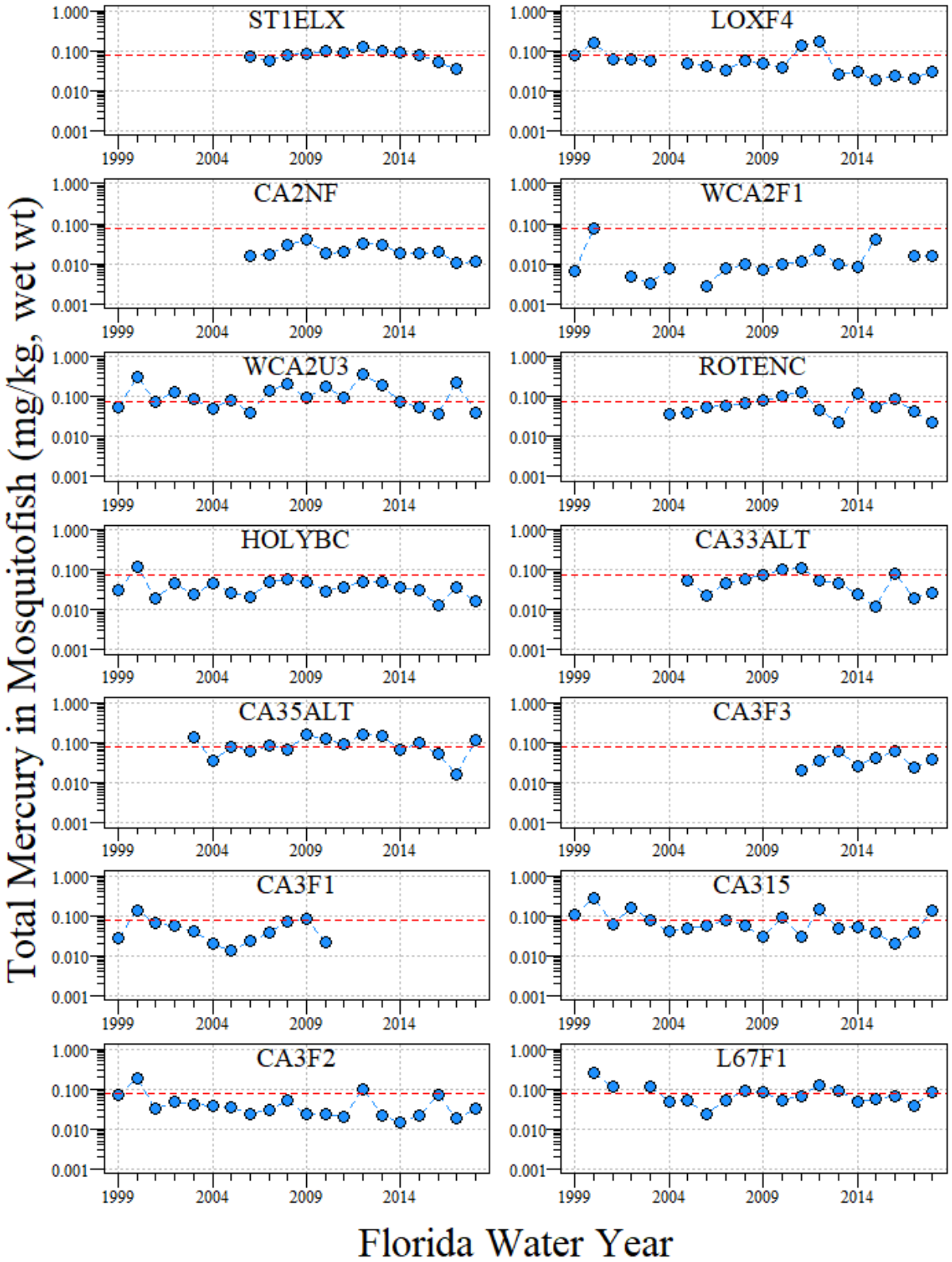

Figure 3B-3. Annual THg concentrations in mosquitofish composite samples at each monitoring site within the EPA for WY1999–WY2018. The red dashed lines indicate the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of piscivorous wildlife.

Interannual and inter-site variations in mosquitofish THg concentrations between consecutive years remains high (**Figure 3B-3**). During WY2017, the highest observed mosquitofish THg concentration occurred at WCA2U3 with a concentration of 0.228 mg/kg. During WY2018, this site reported a THg concentration of just 0.039 mg/kg. This one-year change is over a 5-fold decrease in THg at this site. Compared to WY2017, CA315 and CA35ALT in WY2018 displayed 3- to 9-fold increases in THg concentration, respectively. To date, it is not clear what factor(s) control the dramatic intra-site temporal variations in mosquitofish THg concentration. It is hypothesized that changes in interannual precipitation (i.e., wet years versus dry years) and site-specific biogeochemistry including iron, sulfate, available inorganic mercury (Hg^{2+}), dissolved organic carbon (DOC), and reduction-oxidation (redox) have the ability to influence prey mercury concentrations. Additionally, site-specific trophic dynamics can each can play an important role in controlling THg concentration in mosquitofish. However, a comparison of mosquitofish THg collected between the wet and dry years (**Figure 3B-4**) did not reveal a statistically significant difference (Kruskal-Wallis One Way Analysis of Variance on Ranks, H = 0.74, df = 1, p = 0.39). The median THg values for the wet (0.048 mg/kg, sample size [n] = 122) and dry season (0.049 mg/kg, n = 86) were nearly identical. It is possible that the difference in precipitation between wet and dry years alone was not sufficient to result in significant changes in mosquitofish THg. This could be the result of synergistic and competing interactions involving biogeochemistry, water quality conditions, predation or food sources, and trophic structure.

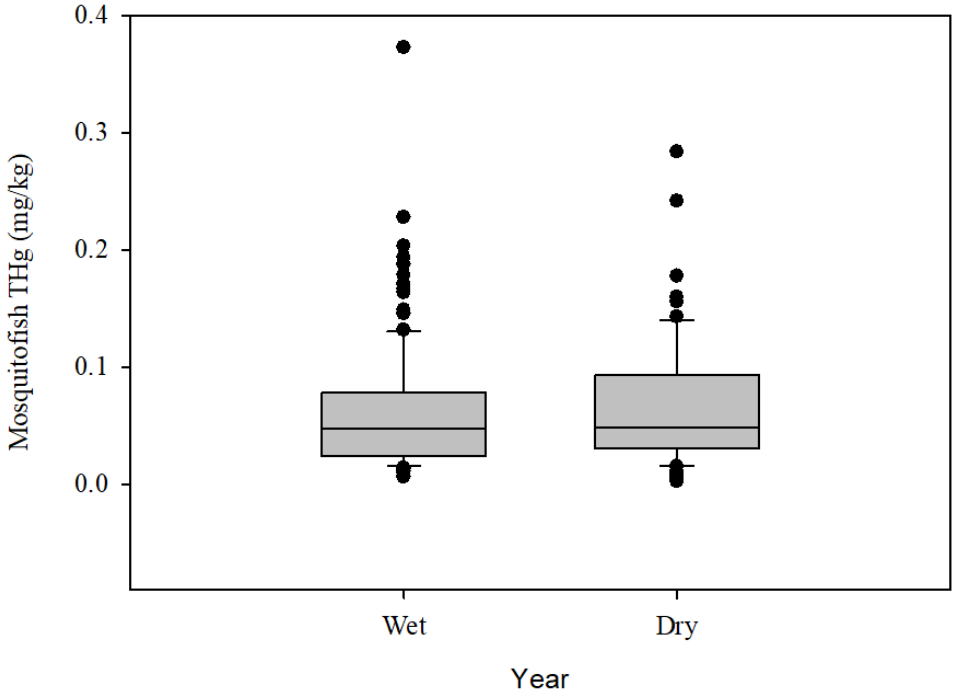

Figure 3B-4. Comparison of mosquitofish THg concentrations collected during wet and dry years for WY1999–WY2018 within the EPA.

Mosquitofish THg concentrations were compared by habitat with stations either characterizing canal or marsh habitat types. Overall variation in mosquitofish THg concentration was greatest in marsh habitat (variance = 0.003) than canal habitat (variance = 0.001). This high degree of variation in marsh habitat could be due to relatively dynamic hydrology (i.e., dry-down, dry-out, water level changes, etc.), the dynamics of marsh trophic structure, and biogeochemistry associated with dynamic hydrology. The median THg concentration is 0.052 mg/kg (n = 177) for marsh area and 0.047 mg/kg (n = 58) for canal and do not show statistical difference between habitat (Kruskal-Wallis Analysis, H = 0.32, p = 0.57) (**Figure 3B-5**).

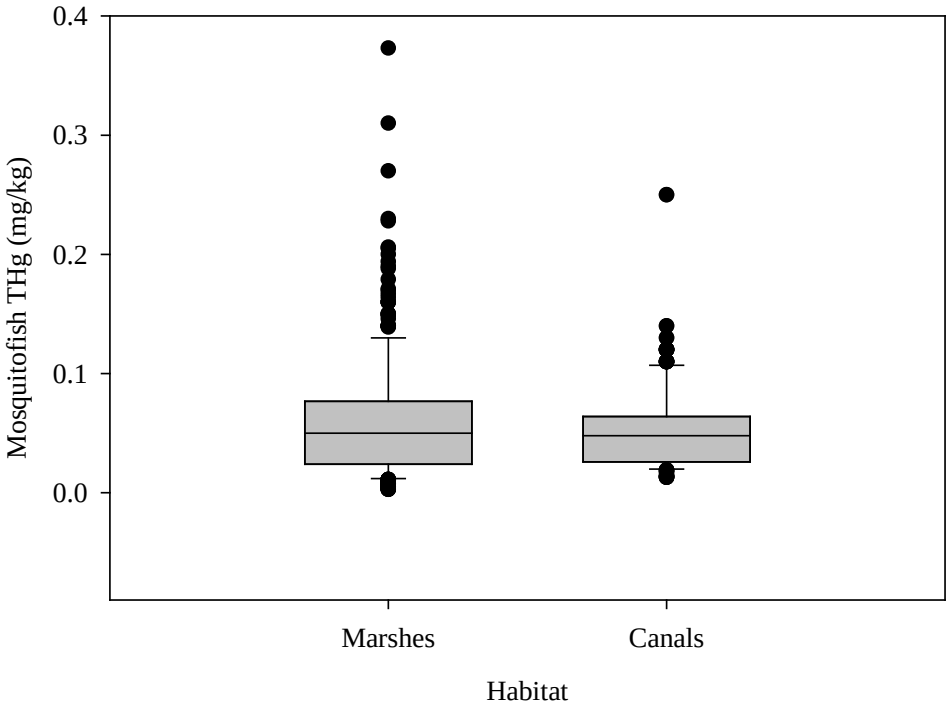

Figure 3B-5. Comparison of mosquitofish THg concentrations between marsh and canal habitats from data collected in the EPA for WY1999–WY2018.

During the entire POR, 3 sites (LOXF4, CA3F2, and CA315) experienced a significantly declining temporal trend in mosquitofish THg while one site (WCA2F1) showed a significantly increasing trend (**Table 3B-1**). Considerable interannual variations of THg concentrations were observed within sites with low nutrient concentrations including interior stations such as LOXF4, WCA2U3, CA3A15, and L67F1 (**Figure 3B-3**). It is not known if the large variations were associated with internal factors such as changes in water chemistry; wading bird feeding, which has the potential to alter trophic structure; dietary sources; internal marsh sources of THg or MeHg from tree islands (Zhu et al. 2014), or other factors.

Sunfish

TL3 sunfish species including bluegill, redear sunfish, and spotted sunfish have been sampled for THg analysis in the EPA since WY1999. The overall average sunfish whole body concentration of THg for data pooled from all sites and years was 0.177 ± 0.003 mg/kg (n = 3,128). Throughout the POR, 75% of annual mean sunfish THg concentrations exceeded the USEPA MeHg criterion of 0.077 mg/kg for TL3 fish for protection of wildlife. Except WCA2F1, all current monitored stations observed annual mean sunfish THg concentration above the USEPA MeHg criterion, with the nutrient enriched WCA2F1 experiencing the least number of exceedances (1) during the POR.

The average THg concentration for the POR was highest (0.352 mg/kg) at the ENP site L67F1 and lowest (0.058 mg/kg) at a WCA-2A site (WCA2F1) near the Hillsborough Canal (**Figure 3B-1**). Several interior sites in WCA-2 and WCA-3 displayed high long-term (i.e., POR) average THg concentrations above 0.200 mg/kg. The THg concentration in sunfish tended to increase from north to south (**Figure 3B-6**). No monitoring station shows a significant increasing or decreasing temporal trend in annual mean sunfish THg concentration throughout the POR (**Table 3B-2** and **Figure 3B-7**).

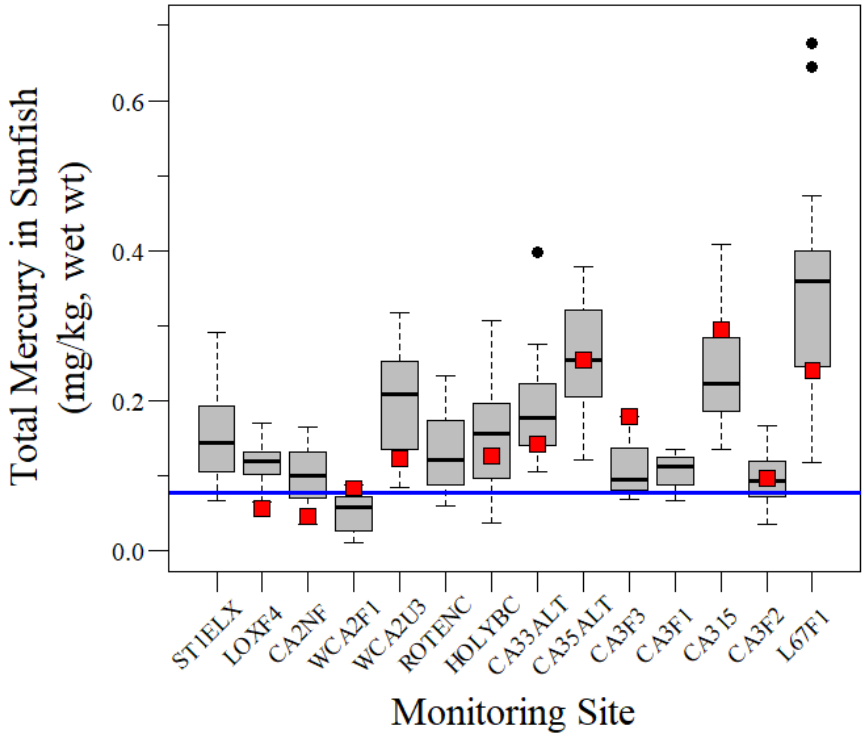

Figure 3B-6. Box plots of THg concentrations in TL3 sunfish at each monitoring site in the EPA for WY1999–WY2018. Black dots are the mean THg collected in WY2018. and the blue line denotes the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of piscivorous wildlife.

Table 3B-2. Kendall trend analysis of TL3 sunfish annual median THg concentration at the 13 active monitoring locations within the EPA for Water Years 1999–2018.

Area	Station	Kendall's τ	p–value	Sample Size	Trend Direction
WCA-1	ST1ELX	-0.11	0.73	10	Not statistically significant
	LOXF4	-0.19	0.26	19	Not statistically significant
WCA-2	CA2NF	-0.35	0.10	13	Not statistically significant
	WCA2F1 [a]	0.14	0.77	7	Not statistically significant
	WCA2U3	0.16	0.31	20	Not statistically significant
WCA-3	CA33ALT [a]	0.09	0.74	12	Not statistically significant
	CA35ALT	0.32	0.10	16	Not statistically significant
	CA3F1/F3	-0.11	0.54	20	Not statistically significant
	CA315	-0.17	0.28	20	Not statistically significant
	CA3F2	-0.31	0.06	20	Not statistically significant
ENP	L67F1	-0.20	0.23	20	Not statistically significant
WMAs	ROTENC	0.00	1.00	12	Not statistically significant
	HOLYBC	0.07	0.67	19	Not statistically significant

a. No data for WY2015 and WY2016.

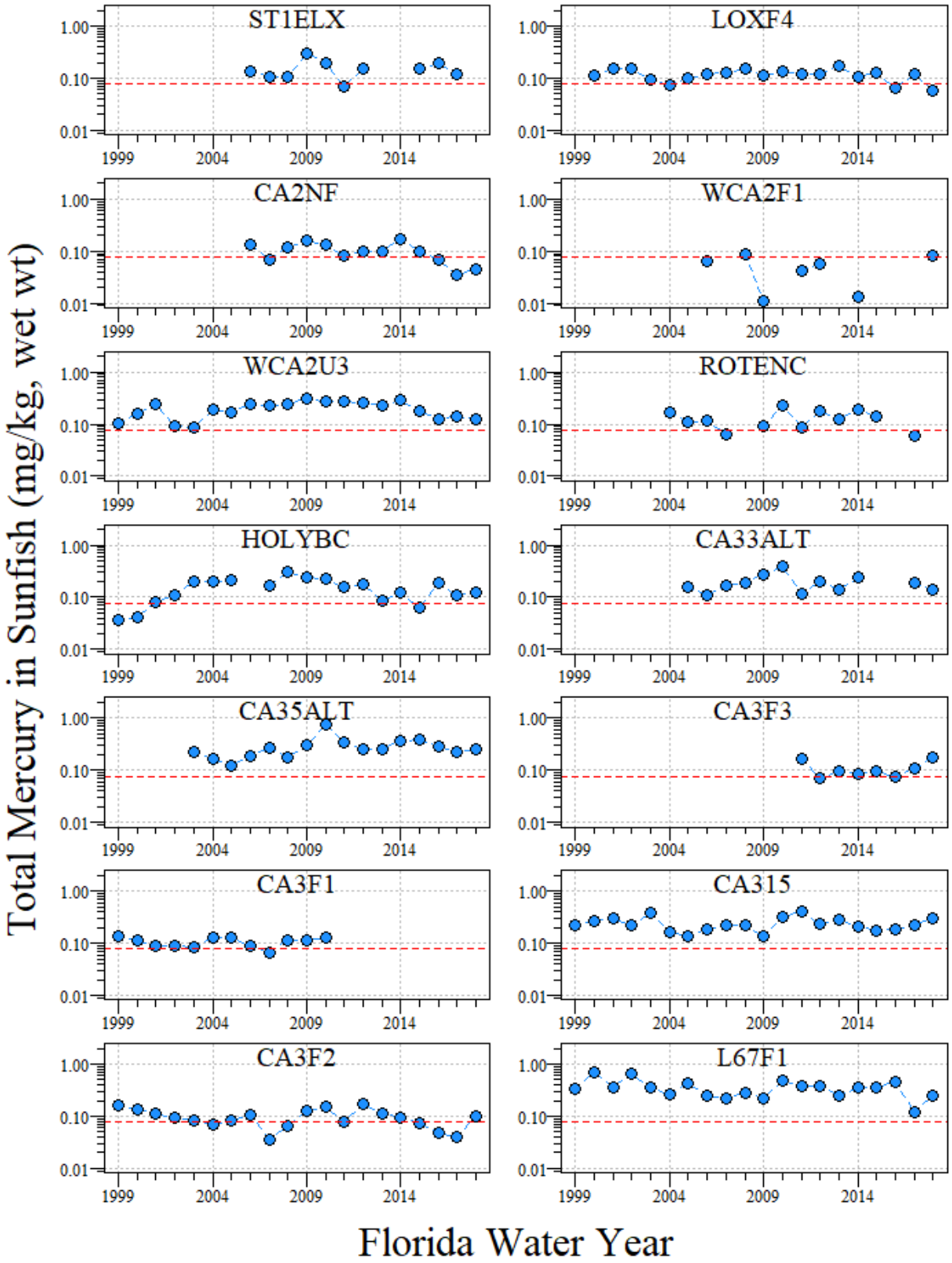

Figure 3B-7. Annual THg in TL3 sunfish whole body samples at each monitoirng site in the EPA for WY1999–WY2018.

During WY2018, the mean THg concentration in the TL3 sunfish species from 11 of the 13 active monitoring sites with data available was 0.149.mg/kg. Annual mean THg concentration range from 0.045 mg/kg at CA2NF to 0.295 mg/kg at CA315. Compared to average (0.120 mg/kg) in WY2017, a 15% increase in mean sunfish THg concentrations occurred.

THg concentration varied significantly between sunfish species during WY2018 (χ^2 = 9.90, df = 2, p < 0.01; **Figure 3B-8)** with spotted sunfish being significantly different from bluegill (z = -2.54, p < 0.01) and redear (z = -3.00, p < 0.01) while bluegill and redear were not significantly different (z = 0.83, p = 0.20). Throughout the entire POR all three species (annual mean) statistically differed in THg concentrations (χ^2 = 47.21, df = 2, p < 0.001) with the highest THg concentration in spotted sunfish (0.247 ± 0.008 mg/kg), followed by bluegill (0.195 ± 0.005 mg/kg), and redear sunfish (0.126 ± 0.003 mg/kg) (**Figure 3B-8**). These differences could be due to feeding preferences among these three species. Depending on size class and hydrologic conditions, bluegill prefer omnivorous invertebrates, redear sunfish prefer herbivorous invertebrates, and spotted sunfish prefer decapods and omnivorous invertebrates (Loftus 2000).

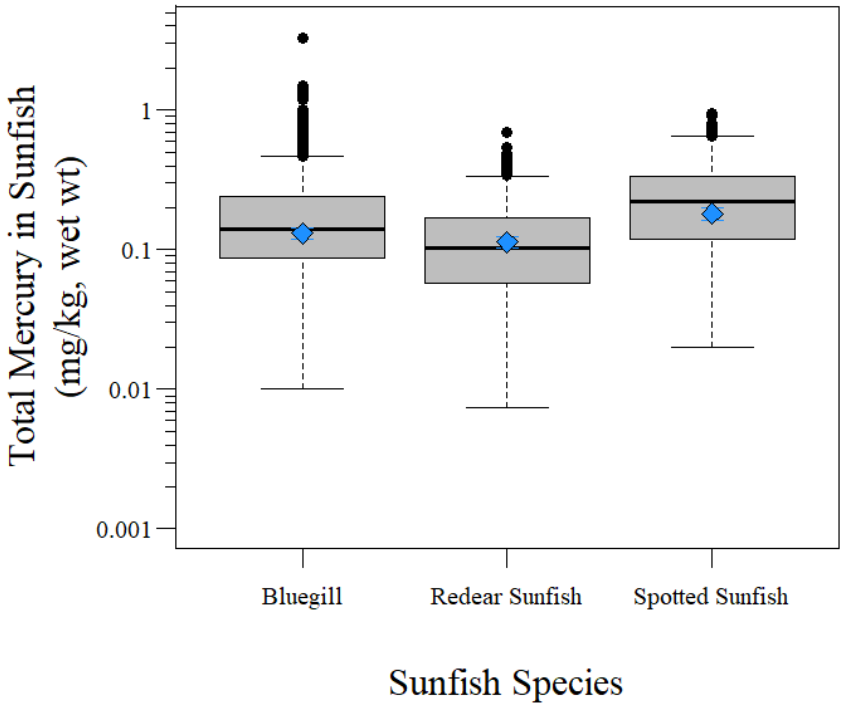

Figure 3B-8. Comparison of TL3 sunfish species collected in the EPA for WY1999–WY2017. Blue diamonds indicate WY2016 mean (± standard error) THg concentrations for each species.

Largemouth Bass

Largemouth bass (LMB) axial tissue fillet samples have been collected across the EPA for THg analysis since WY1999. During WY2018, no fish samples were collected from 5 of the 13 monitoring stations (ST1ELX, WCA2F1, ROTENC, CA35ALT, and CA3F1). Average THg concentrations in LMB ranged from 0.183 mg/kg at site CA2NF (WCA-2) to 0.949 mg/kg at site L67F1 (ENP), with an overall median value of 0.360 mg/kg observed during WY2018. This represents a 29% increase in the reported median value for WY2017 (0.280 mg/kg). Generally, LMB THg concentrations follow a strong north-to-south gradient with concentrations being lower in WCA-1 and WCA-2 and higher in WCA-3 and ENP (**Figure 3B-9**). Along this gradient, several key factors could influence THg conditions including water

quality conditions (pH, alkalinity, nutrient availability, etc.), trophic position, and habitat structure (Julian and Gu 2015).

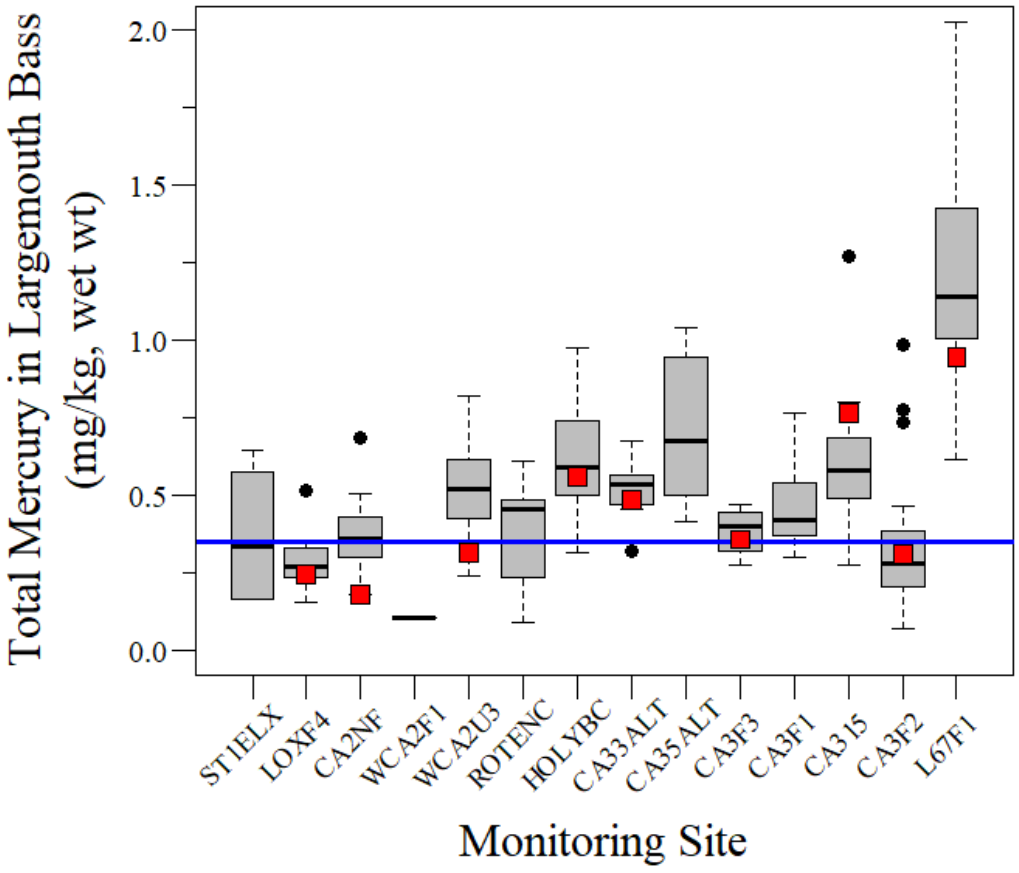

Figure 3B-9. Box plots of THg concentrations in LMB at each monitoring site for WY1999–WY2018. Red boxes indicate WY2018 mean THg concentrations and the blue line denotes the 0.350 mg/kg USEPA MeHg criterion for protection of human health.

During WY2018, 5 of the 9 monitoring stations with data in the region had mean THg concentration above the USEPA recommended MeHg criterion for the protection of human health (0.35 mg/kg; USEPA 2001) (**Figure 3B-9**). In contrast to last water year (WY2017) where 4 sites exceeded criteria out of 11 sites with data. Overall, exceedance rates of the recommended criterion have improved from >80% exceedance to almost 50% exceedance across the monitoring network. These exceedances of the recommend criterion could potentially be driven by hydrologic factors (i.e., wet versus dry years) and/or changes in water quality, quantity, and timing.

Throughout the POR (WY1999–WY2018), station HOLYBC maintained a significant increasing trend. Meanwhile two stations LOXF4 (LNWR) and CA3F1/F3 (WCA-3) exhibit decreasing trends in LMB THg tissue concentration (**Table 3B-3**). All other stations do not have statistically significant trends or enough data to assess trends. The lack of temporal trend in tissue THg could be due to gaps in the data for some stations and/or little to no variation or too much variation in interannual concentrations at some sites, which could be driven by unexplained underlying conditions (discussed above) (**Figure 3B-10**).

Table 3B-3. Kendall trend analysis of LMB annual median THg concentration at the 13 active monitoring locations within the EPA for WY1999–WY2018.

Area	Station	Kendall's τ	p-value	Sample Size	Trend Direction
WCA-1	ST1ELX	0.00	1.00	4	Not statistically significant
	LOXF4	-0.34	<0.05	19	Decrease
WCA-2	CA2NF	-0.28	0.20	13	Not statistically significant
	WCA2F1	---	---	---	Not enough data
	WCA2U3	-0.05	0.77	20	Not statistically significant
WCA-3	CA33ALT	-0.43	0.24	7	Not statistically significant
	CA35ALT	-0.33	0.47	6	Not statistically significant
	CA3F1/F3	-0.44	<0.01	20	Decrease
	CA315	0.03	0.89	19	Not statistically significant
	CA3F2	-0.25	0.12	20	Not statistically significant
ENP	L67F1	0.005	0.97	20	Not statistically significant
WMAs	ROTENC	0.20	0.48	10	Not statistically significant
	HOLYBC	0.38	<0.05	20	Increase

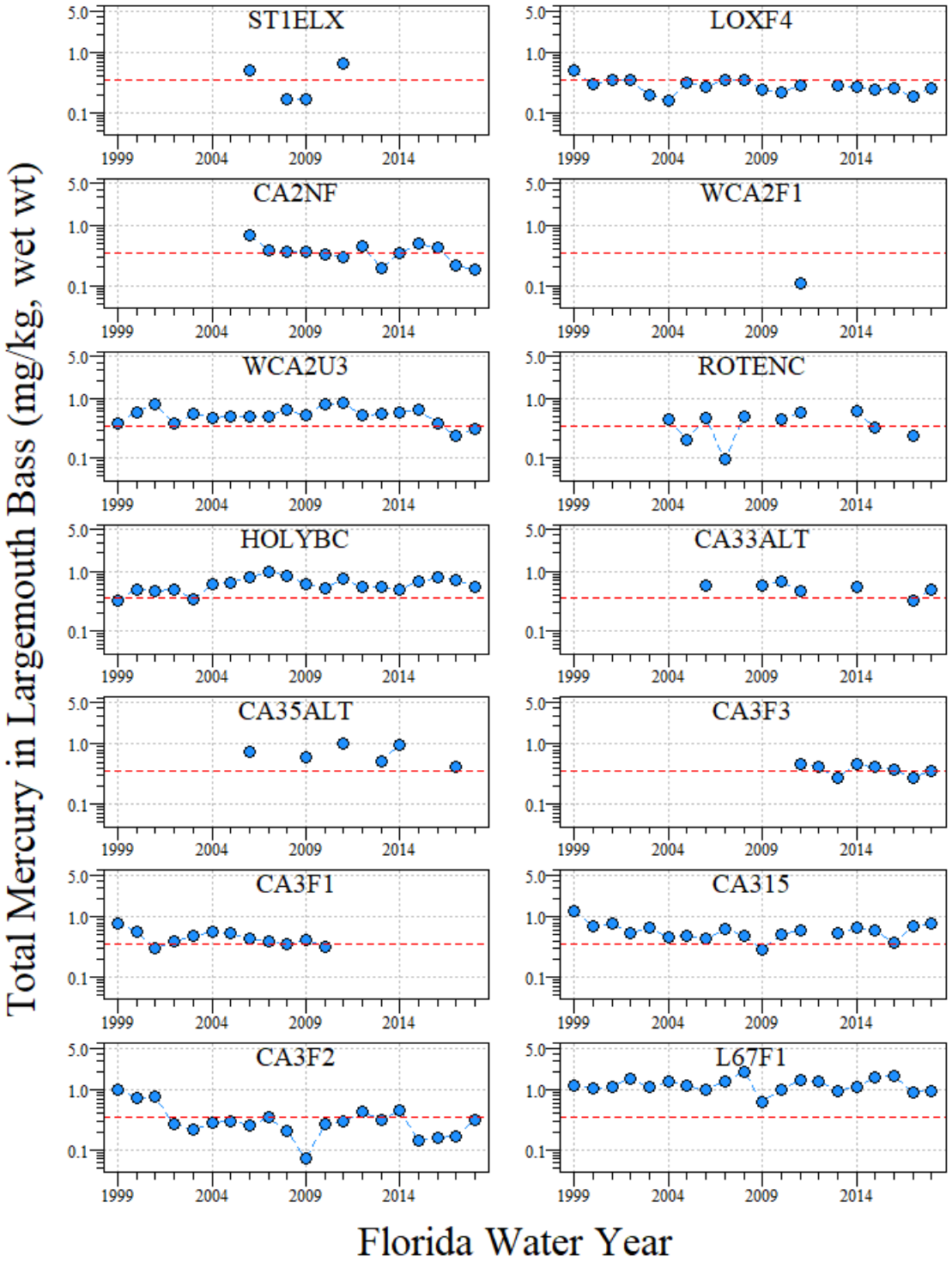

Figure 3B-10. Annual THg concentrations in LMB axial fillet samples at each monitoring site in the EPA for WY1999–WY2017. Red dashed line denotes the 0.350 mg/kg USEPA MeHg criterion for protection of human health

Over the entire POR, the median mosquitofish THg concentration (0.049 mg/kg) has been below the federal criterion for wildlife protection. For WY2018, Mosquitofish THg concentrations exceeded the federal criterion of 0.077 mg/kg MeHg for TL3 fish at 3 of the 13 active monitoring sites. POR median THg concentration for TL3 sunfish (0.130 mg/kg) and LMB (0.450 mg/kg) has been above the wildlife and human health protection criterion, respectively. During WY2018 sunfish THg concentrations exceeded the USEPA criterion in 9 of the 11 sampled monitoring sites while LMB THg concentration exceeded the recommend USEPA criterion in 5 of the 9 sampled monitoring sites. Several sites experienced THg concentration declines in POR annual mosquitofish (LOXF4, CA315, and CA3F2) and LMB (LOX4 and CAF1/F3) THg concentrations while no monitoring sites indicated significant trends in sunfish THg concentration for the POR. Additionally, one site of each indicated significant increasing trends in mosquitofish (WCA2F1) and LMB (HOLYBC) THg concentrations across the POR. It is worth noting that declining trends in fish tissue THg concentration between long lived (LMB) and short lived (mosquitofish) species are shared at LOX4 site indicating significant improvement with respect to THg accumulation in the trophic structure while other areas indicate potential concern with respect to THg accumulation and warrant further study.

Whether THg concentrations in fish are remaining constant over the past decade, as reported in previous SFERs, or whether there are recent increases in fish tissue THg concentrations, mercury bioaccumulation continues to be a significant water quality issue within the EPA and greater Everglades. THg concentrations in higher trophic level fish (i.e., sunfish and LMB) are highly variable across the landscape but continue to exceed criteria concentrations for the protection of piscivorous wildlife and humans at many locations. Future rates of mercury emissions and atmospheric deposition are highly uncertain (Krabbenhoft and Sunderland 2013), the response of fisheries to mercury load reductions could take decades (Munthe et al. 2007), and repeated attempts to gain a better understanding of system controls on methylation and bioaccumulation have found little consistent patterns. These basic findings do not provide a basis to develop a comprehensive strategy to manage the Everglades mercury issue beyond the state's total maximum daily load (FDEP 2013).

HIGH BIOTIC MERCURY IN SOUTH FLORIDA WETLANDS: THE ROLE OF FISH TROPHIC POSITION

Binhe Gu and Paul Julian[1]

Mercury data for fishes across the EPA continue to reflect an important water quality problem. THg levels in fish tissue often exceed USEPA guidelines for human health and wildlife protection (see above). Over the last 20 years, average THg levels in LMB, a top aquatic predator and food source for humans, is 0.404 mg/kg and above the USEPA trophic level 4 (TL4) fish criteria, which is 0.346 mg/kg. Previous studies on the mechanisms leading to high mercury levels in aquatic biota typically focus on factors controlling in-situ mercury methylation. These factors include concentrations of mercury, sulfate, and dissolved organic matter (DOM) along with types of microbial methylators (Gilmour 2011). Although these factors play some important roles in mercury methylation and accumulation in biota, a large portion of variance between these factors and MeHg levels from accumulation in biota remains unexplained (Julian and Gu 2015). From an environmental management perspective, it is the bioaccumulation of mercury that creates a water quality problem regardless of the rate of methylation. Other factors may also influence mercury level in biota and account for some significant variances in the relationship between environmental factors and biotic mercury levels. Prominent among these factors is consumer trophic position.

Metal accumulations by aquatic consumers increase along food chains (Cabana and Rasmussen 1994). This observation is complicated by the fact that the trophic position of a consumer can differ among habitats and ecosystems due to differences in food web structure and changes in trophic position of dietary

organisms. Nowadays, the trophic position of consumers is typically estimated using stable nitrogen isotopes (defined as $\delta^{15}N$). The $\delta^{15}N$ of consumers increases consistently during each trophic transfer (Minagawa and Wada 1984) and can be used as an indicator of a consumer's trophic position (Post 2002). Since the stable isotope signature of consumers reflects the chemical compounds assimilated from their diets over space and time, analyses of a consumer's $\delta^{15}N$ can provide spatially and temporally integrated information on consumer feeding history.

This section presents results of an evaluation of the differences in LMB THg concentrations among wetland habitats from the Everglades and to associate these variations with changes in trophic position. This will be accomplished by comparing the trophic position and THg concentration in LMB collected from multiple habitats in the Everglades. Findings from this work may help explain the variances in LMB THg that cannot be accounted by other ecological and biogeochemical variables.

Methods

All THg data for LMB were retrieved from DBHYDRO (www.sfwmd.gov/dbhydro). Bass fillets and mosquitofish composite samples (for isotope trophic baseline) were collected from 7 monitoring sites in the Everglades STAs and WCAs between 2006 and 2008 and analyzed for THg determination and $\delta^{15}N$ analysis (**Table 3B-4**). Statistical analysis was performed using THg and $\delta^{15}N$ data from each individual of bass. Fish muscle tissue was freeze-dried and powdered prior to analysis and approximately 1 mg of fine powder was loaded into a tin capsule for analysis. All samples were analyzed using a Carlo Erba Elemental Analyzer interfaced to a Finnigan MAT Delta Plus XP stable isotope ratio mass spectrometer. The results were reported in the standard δ notation relative to the atmospheric nitrogen for $^{15}N/^{14}N$ ratios. The analytical precision (based on replicate analyses of lab standards processed with each batch of samples and on sample replicates) was ± 0.1 per mille (‰).

Table 3B-4. Number of fish samples available for $\delta^{15}N$ and THg analysis collected between 2006 and 2008 at sites across the monitoring network.

Area	Station	Mosquitofish [a]	Largemouth Bass
WCA-1	LOXF4	1	20
WCA-2	WCA2U3	5	11
WCA-3	CA3ALT	2	7
STA-1 West	S5A	2	15
STA-1 West	G310	4	5
STA-3/4	L5F1	18	5
Holey Land WMA	HOLYBC	1	13

a. One composite sample consists of at least 100 fish.

Trophic position was estimated using the equation below (**Equation 3B-1**) where $\delta^{15}N_F$ indicates the $\delta^{15}N$ value of LMB tissue (predator) and $\delta^{15}N_B$ represents the $\delta^{15}N$ value of mosquitofish tissue (prey). A value of 3.4‰ was used for the nitrogen stable isotope fractionation factor per trophic transfer (Δ_n) during animal feed as reported by Minagawa and Wada (1984) and Post (2002). The trophic position (λ) of the baseline organism (mosquitofish) is assumed as 3 (TL3).

$$Trophic\ Position = \lambda + \frac{\delta^{15}N_F - \delta^{15}N_B}{\Delta_n} \quad \textbf{(3B-1)}$$

Results and Discussion

The average THg concentration in LMB was 0.434 mg/kg and exceeded the USEPA MeHg criterion for protection of human health (0.350 mg/kg). Trophic position and THg concentration in LMB from 3 STA and 4 WCA sites are presented in **Figure 3B-11** indicating that trophic position was lowest at 2.8 (STA1 S5A) and highest at 3.5 (Holey Land). The average trophic position was 3.3 from all sites and is below the value for TL4 fish. This estimated trophic position suggests that LMB at these study sites relied primarily on consumers such as invertebrates and small fish for their diets. THg concentration in LMB ranged from 0.10 mg/kg at the STA-1 West (STA-1W) site G310 to 0.80 mg/kg at Holey Land. Notably, there is a linear correlation between trophic position and THg concentration. Based on the results from the regression analysis, 55% of the THg variance in LMB can be explained by fish trophic position (**Figure 3B-11**).

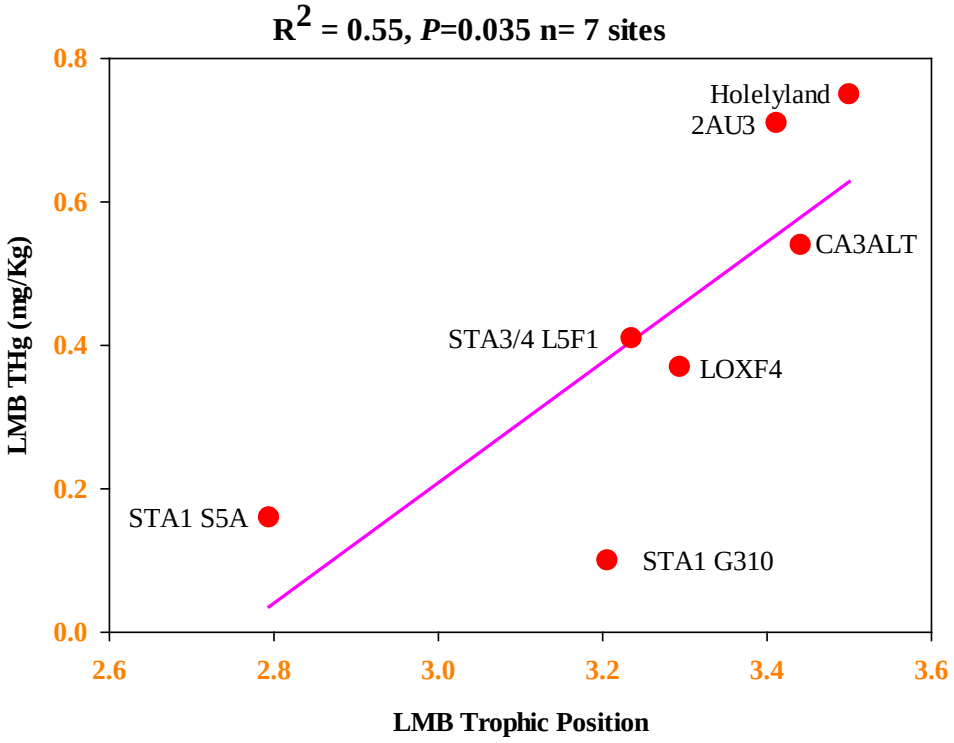

Figure 3B-11. Relationship between trophic position and total mercury in LMB from locations across the EPA between 2006 and 2008.

Food chain length or trophic position of consumers depends on ecosystem size, primary productivity (Post et al. 2000), hydrology (Sabo et al. 2010), and species invasion (Vander Zanden et al. 1999). Prey accessibility is also considered an important factor affecting animal trophic position. The diet of LMB varies among fish and invertebrates from multiple trophic levels. In the STAs with dense vegetation, prey fish such as sunfish with high mobility can use vegetation for refuge. LMB in STAs might have been forced to prey on diets with low trophic level such as invertebrates, leading to lower trophic position. Mason (2006) found that frequency of occurrence and percentage of the fish prey consistently decreased as hydrilla (*Hydrilla* spp.) coverage increased. Bass switched to a more fish-dominated diet after drawdown, but the importance of fishes in the diet decreased quickly as hydrilla beds became reestablished. Fisher et al. (2012) also observe similar macrophyte-mediated predation on benthic invertebrates. Ted Lang (Fish and Wildlife Service, unpublished data) found more benthic invertebrates in bass from STAs than those living in more open water marshes. Savino et al (1992) showed the benthic invertebrate density increased as aquatic plant density increased. These studies demonstrated that ecological habitats may influence prey selection by fish,

which in turn affect trophic position and THg bioaccumulation. These studies also support our finding of lower trophic position and THg in LMB collected from STAs. More information on vegetation biomass and coverage from these study sites are needed to closely link plant density to fish trophic position and THg concentration in LMB.

Results from this analysis demonstrated that the accumulation of THg in LMB could be the consequence of differences in fish trophic position among habitats. This is because metal bioaccumulation in consumers increases along the food web. The trophic position of the same species of fish inhabited at different habitats and ecosystems can change due to dietary preferences and prey availability. Differences in vegetation density in aquatic ecosystems is considered an important factor controlling fish accessibility to diets and the trophic position. Other factors such as drought may affect prey type and availability in the Everglades and deserve further research.

MERCURY BIOGEOCHEMISTRY

SULFATE EFFECTS ON NET METHYLMERCURY PRODUCTION IN SOILS FROM TWO EVERGLADES "HOT SPOTS"

Mike Jerauld[3], Forrest E. Dierberg[3], Thomas A. DeBusk[3], Janelle A. Potts[3], Nichole R. Larson[3], Karen Hileman[3], and Dawn Sierer Finn[3]

Introduction

As elaborated in this and all previous annual versions of this chapter, mercury concentration in Everglades biota are unacceptably high. Common in other wetlands, inorganic mercury deposited from the atmosphere is converted to the neurotoxic, bioaccumulative organic form, MeHg by microorganisms in the Everglades. The first actors definitively identified with microbial mercury methylation were sulfate-reducing bacteria (SRB; Compeau and Bartha 1985). Experimental and ecosystem-level observations have linked sulfate loading to MeHg accumulation (Branfireun et al. 1999, Gilmour et al. 1992, Harmon et al. 2004, Jeremiason et al. 2006, Mitchell et al. 2008) and the converse, MeHg declines linked to sulfate abatement, (Coleman Wasik et al. 2012, Hrabik and Watras 2002), in some ecosystems. Sulfate abatement therefore has been advocated as a strategy to limit mercury methylation and thus mercury bioaccumulation in the Everglades (Orem et al. 2011), given that further reductions of mercury deposition (currently attributed to global emissions sources) may not be achievable by local managers (Atkeson et al. 2005). However, the high cost of implementation requires that the efficacy of sulfate abatement as a mitigation strategy be demonstrated *in the Everglades* since inferences of biogeochemical processes drawn from observations in other ecosystems have not been reliably transferred to the Everglades. For example, while sulfate has been linked to internal eutrophication (release of soil phosphorus) elsewhere (Lamers et al. 1998, Smolders et al. 2006), the biogeochemical conditions of the Everglades (low phosphrus, low iron-bound soil phosphrous, and high carbon:phosphorus) dampen the sensitivity of soil phosphorus to sulfate enrichment (Dierberg et al. 2011).

Here, we briefly describe the results of a laboratory experiment testing the effect of sulfate amendments on MeHg production in waters and surficial soils from two Everglades sites with histories of high mercury levels in biota. Results from portions of this experiment were previously published in Appendix 3B-1 of the 2014 SFER (Dierberg et al. 2014).

Methods

North-central WCA-3A and Shark River Slough in ENP have long been recognized as hot spots of Hg accumulation in biota (**Figure 3B-12**). Surficial soils (top 0 to 5 centimeters (cm), inclusive of any floc present) and surface water were collected from DB-15 in WCA-3A and P-36 in ENP (**Figure 3B-12**). In the laboratory, 100 milliliters (mL) fresh soils were incubated with 900 mL site water in 1-liter borosilicate glass vessels. Amendments of sulfate (as sodium sulfate [Na_2SO_4]) and inorganic mercury (as mercury II cation [Hg(II)] chloride [$Hg(II)Cl_2$)) were applied to the waters of the vessels in an incomplete factorial design (**Table 3B-5**). All controls and treatment levels were conducted in triplicate. The experiment was initiated with gently shaking the vessels to slurry the soils, water, and amendments. All vessels were purged with 0.03% balance nitrogen gas (N_2) for the first 24 hours after the initial set up of the incubation, and then 2 to 5 hours daily thereafter to promote anoxia and maintain a more consistent pH for the duration of the experiment. Waters were sampled from each vessel at time = 0 (after mixing), 7, and 14 days and analyzed for a suite of parameters including sulfate, dissolved MeHg, and dissolved THg. Soils were sampled at time = 0 (sacrificial vessels) and 14 days, and analyzed for MeHg, THg, and other constituents.

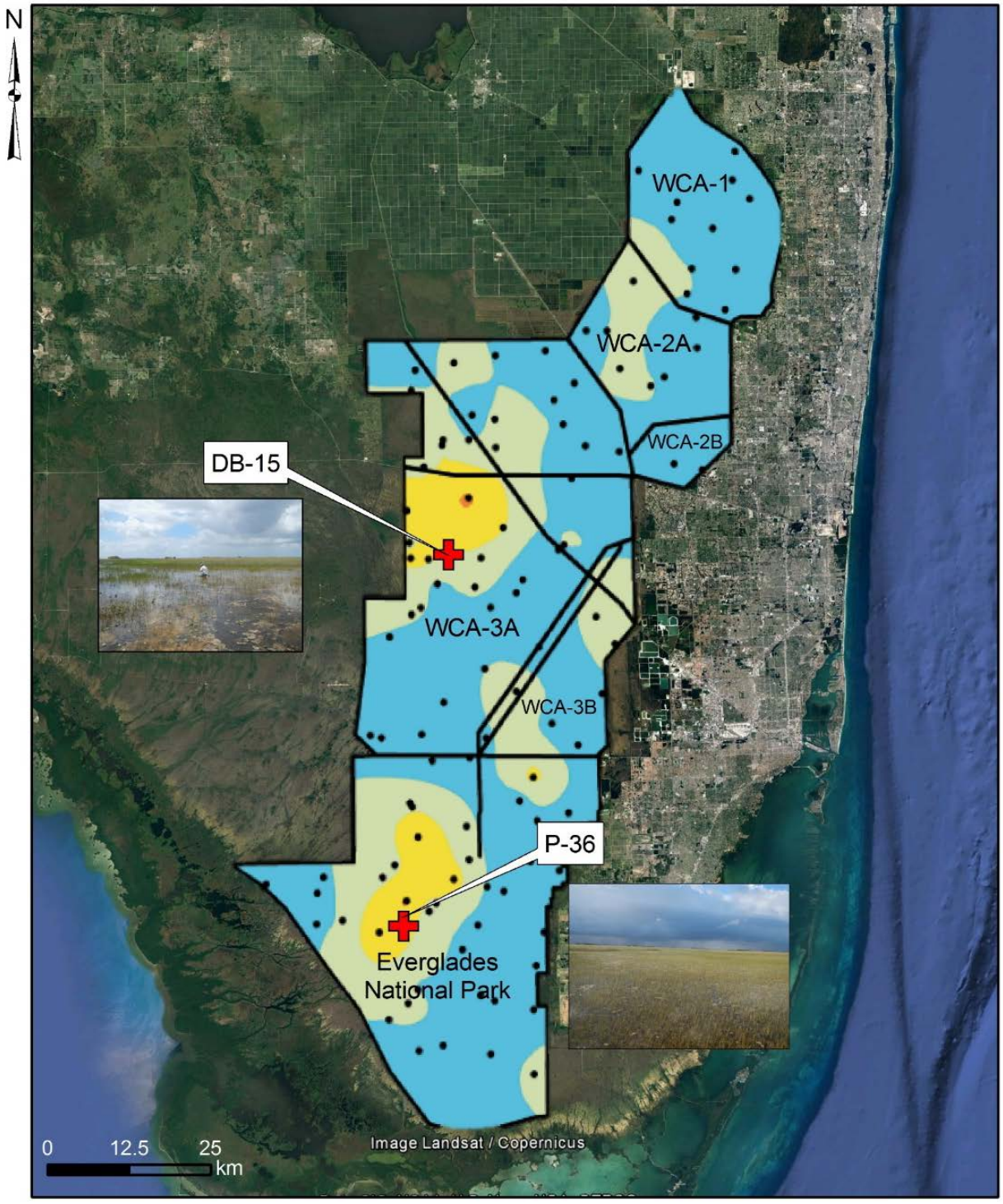

Figure 3B-12. Stations DB-15 and P-36 within the EPA. The shaded surface shows interpolated mosquitofish tissue THg concentrations from the 2005 Regional Environmental Monitoring and Assessment Program (REMAP) collection cycle (Scheidt and Kalla 2007).

Sulfate concentrations were $\log_{10}$-transformed for all statistical tests. A two-factor analysis of variance (ANOVA) was used to test the effect of mercury additions (factor 1) and the transformed sulfate concentration (factor 2) on MeHg accumulation (final – initial mass) in soil and water from site P-36. Because only a limited number of sulfate amendment levels for the DB-15 site soils were tested without Hg(II) amendments (**Table 3B-5**), the effects of sulfate and mercury were assessed separately. For both sites, the quantitative relationship between MeHg accumulation and the transformed sulfate concentration was described with simple linear regression. The effect of mercury additions for DB-15 was assessed with Mann-Whitney-Wilcoxon rank sum test within the two sulfate levels for which both Hg(II) conditions were tested. For all statistical tests, significance was assessed at $\alpha = 0.05$.

Table 3B-5. Experimental design for sulfate-amendment incubations. Sulfate amendment levels indicate nominal sulfate amounts added in addition to ambient sulfate concentrations (<0.2 and 0.5 mg/L for DB-15 and P-36 waters, respectively). Hg(II) was added at an initial concentration of 115 nanograms per liter (ng/L) (DB-15) or 132 ng/L (P-36). Red and blue dots indicate that the treatment combination was applied to DB-15 and P-36 soils, respectively. For the highest sulfate amendment, 20 mg/L was used for DB-15 soils and 26 mg/L was used for P-36 to complement another experiment that is not discussed in this document.

Sulfate Amendment Level	Hg(II) Not Added	Hg(II) Added
+ 0 mg/L (control)	● ●	● ●
+ 0.5 mg/L	●	● ●
+ 1.0 mg/L	●	● ●
+ 2.5 mg/L	●	● ●
+ 5.0 mg/L	●	● ●
+ 10 mg/L	● ●	● ●
+ 20 (●)/ +26 (●) mg/L	●	● ●

Results and Discussion

The rate of sulfate depletion, which can be assumed to be biological sulfate reduction (Lamers et al. 1998), was proportional to the initial sulfate concentration (**Figure 3B-13**). Dissolved MeHg and THg concentrations for the raw unamended DB-15 water were 0.048 and 0.550 ng/L, respectively, and higher for P-36 water (0.16 and 1.39 ng/L, respectively). Mixing with soil at the initiation of the experiment reduced the dissolved MeHg concentrations for both sites, so there was very little aqueous MeHg at the beginning of the experiment. Since the volumes for all vessels were uniform, the ending (time = Day 14) MeHg concentrations were statistically interchangeable with the mass accumulations. Both factors in the experimental design (sulfate and Hg(II)) significantly ($p < 0.001$) increased the accumulation of dissolved MeHg in the waters of the experimental vessels for both sites (**Figure 3B-14**), with interaction (for P-36 subset subjected to ANOVA, $F_{Sulf*Hg} = 9.58$, $p = 0.01$). Very little MeHg accumulated in the water when inorganic Hg(II) was not added, regardless of the amount of sulfate used. When inorganic Hg(II) was added, sulfate additions increased the amount of MeHg dissolved in the water (**Figure 3B-14**), at about 0.25 nanograms (ng) per log-unit sulfate. Accumulation of dissolved MeHg within Hg(II)-amendment groups was remarkably similar for the two sites across sulfate concentrations 0.3 to 20 mg/L (**Figure 3B-14**).

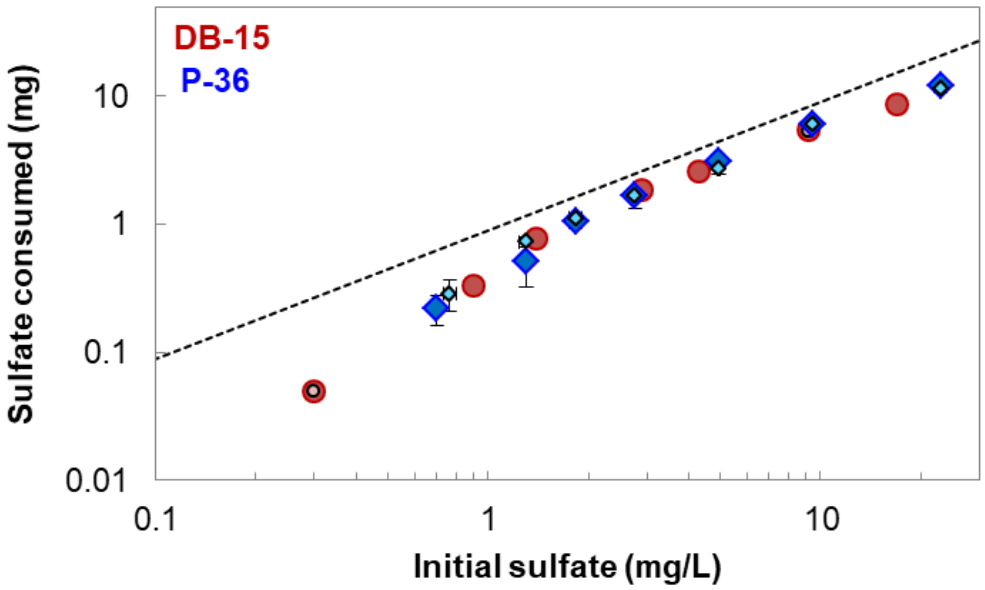

Figure 3B-13. Sulfate consumption in milligrams (mg) from water after 14 days in flasks of soil and water from sites DB-15 (WCA-3A) and P-36 (ENP) dosed with a range of sulfate concentrations. Larger, darker filled symbols represent treatment groups that received a spike of Hg(II). Smaller, lighter symbols represent treatment groups that did not receive a Hg(II) spike. Each point represents the mean of triplicate vessels. Error bars represent standard error. See **Table 3B-5** for the experimental design. The dashed line represents the total initial aqueous sulfate mass in the 900 mL of water. Both axes are shown in log scale.

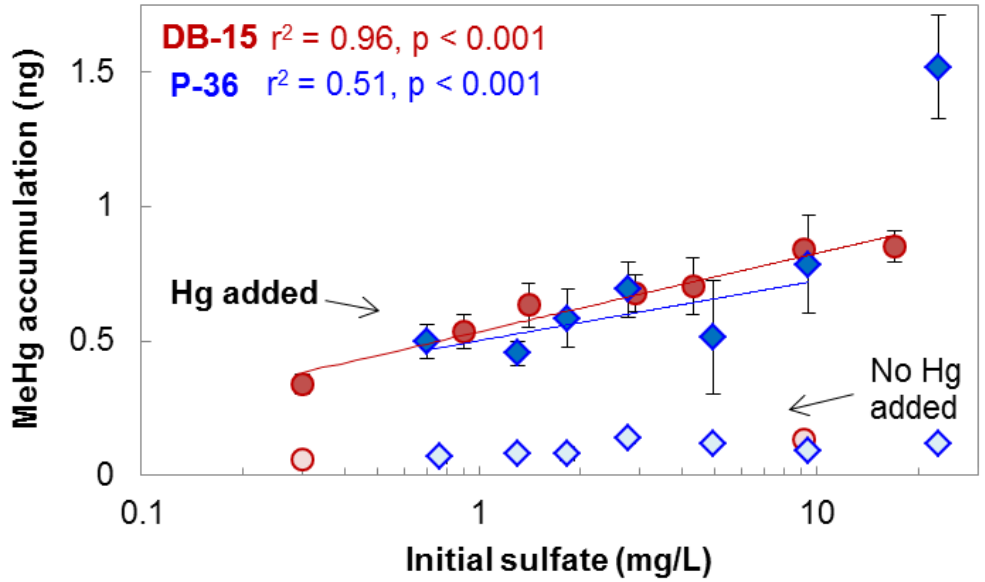

Figure 3B-14. MeHg mass accumulated in water after 14 days in flasks of soil and water from sites DB-15 (WCA-3A) and P-36 (ENP) dosed with a range of sulfate concentrations. Each point represents the mean of 3 triplicate vessels. Error bars represent standard error. See **Table 3B-5** for the experimental design. Linear regressions only include vessels amended with Hg(II); there was no statistical relationship with sulfate in the vessels that did not receive Hg(II) (not shown). The very high MeHg response by the highest-sulfate group for P-36 is not included in the regression shown. Note the log scale of the horizontal axis.

However, on a mass basis, the vast majority of the MeHg accumulated in the soil, rather than in the water (**Figure 3B-15**), because MeHg has a high affinity for organic molecules like those in these peat soils. Furthermore, MeHg associated with solids (i.e., carbon sources) is much more relevant to bioaccumulation in biota than aqueous MeHg (Scheidt and Kalla 2007, Liu et al. 2008, Lange 2011). Therefore, it is appropriate to consider the total amount of MeHg accumulated in the soils and water of the experimental vessels. The addition of inorganic mercury resulted in marked total MeHg (soil + water) buildup from both sites, regardless of the amount of sulfate (**Figure 3B-15**), even at the lowest sulfate levels (< 1 mg/L) that are thought to restrict methylation. Sulfate itself had no discernible effect on the total amount of MeHg produced (**Figure 3B-15**), even though microbial sulfate reduction was stimulated by the addition of sulfate (**Figure 3A-13**).

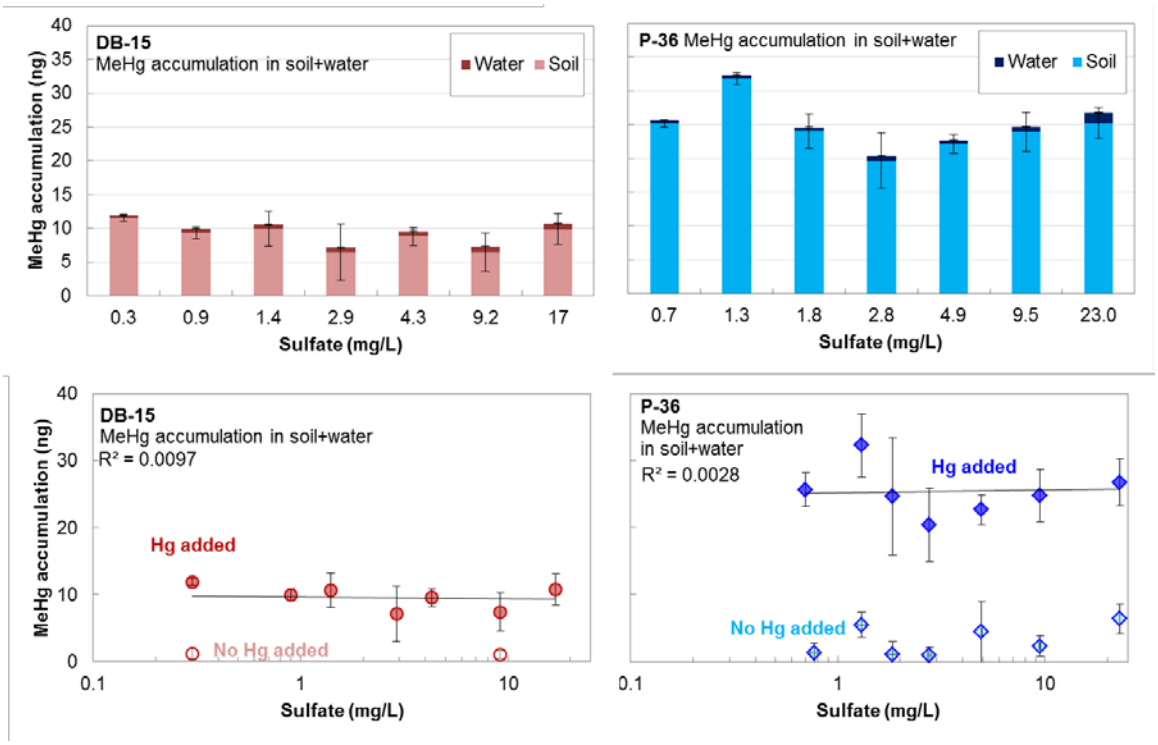

Figure 3B-15. (Top) MeHg mass accumulated in soil and water after 14 days in flasks of soil and water from sites DB-15 (WCA-3A) and P-36 (ENP) dosed with a range of sulfate concentrations and with inorganic Hg(II). (Bottom) Total mass of MeHg accumulated after 14 days in the soil and water. Note log scales of horizontal axes of bottom panels. See **Table 3B-5** for the experimental design.

When inorganic mercury was available (added), soils from each site produced MeHg at the same rate regardless of the absence, presence or concentration of sulfate (**Figure 3B-15**). Heretofore, the best-known methylating organisms were those that require sulfate to function (SRB), so sulfate was viewed as imperative for methylation. However, recent laboratory studies have now shown that other microbial consortia such as syntrophs and methanogens, which do not require sulfate, can methylate mercury at rates comparable to SRB (Gilmour et al. 2018, Yu et al. 2018). These new findings may have dramatic implications for the Everglades, where our earlier work showed that syntrophs were the most abundant microorganisms carrying the gene for methylation (Bae et al. 2014). The similar total MeHg production across all levels of sulfate availability in the experiments presented here (**Figure 3B-15**) appears to provide further evidence of the methylating action of non-sulfate-dependent microorganisms in the Everglades. However, an important caveat remains for Everglades restoration: longer-term sulfate exposure could potentially still exacerbate mercury methylation by stimulating the proliferation of SRB, which appear to

grow more quickly and abundantly than some of the other microbial groups (Gilmour et al. 2018, Yu et al. 2018). Our research team currently is establishing a large-scale mesocosm facility at DB-15 to address this possibility, and to characterize the effects of long-term sulfate dosing on mercury levels in biota.

Although sulfate did not appear to affect MeHg production in DB-15 and P-36 surficial soils, it did seem to increase the amount of MeHg dissolved in the water (**Figure 3B-15**). While intriguing, the best data currently available indicate that MeHg in water is not readily accumulated by biota (e.g., Liu et al. 2008). The mechanism by which sulfate mobilized MeHg to the water is under investigation, but may be related to sulfurization (abiotic incorporation of reduced sulfur compounds) of the naturally occurring DOM (e.g., Poulin et al. 2017). It is not yet clear what role DOM sulfurization might play in stimulating mercury methylation by enhancing the bioavailability of Hg(II) to methylating organisms (Graham et al. 2017, Poulin et al. 2017), but that effect is not evident from the results of total (soil + water) MeHg production in this experiment. Regardless, we recommend that future research on the biogeochemical controls of mercury methylation in the Everglades should only rely on MeHg responses in water with caution and should instead measure MeHg accumulation in components of the food web.

SULFUR SOURCES TO THE EVERGLADES

SULFATE WITHIN THE EPA

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The primary source of mercury to the Everglades is through global transport and atmospheric deposition, as previously noted. Once deposited, mercury can be converted to MeHg, primarily by reducing bacteria, particularly SRB which utilize sulfate to metabolize organic matter under anaerobic conditions. During this process, some SRB have been observed to methylate THg (Gilmour et al. 1998, 2013). The exact quantitative role that sulfate plays in the sulfur-mercury biogeochemical cycle in Everglades marshes is still not clear; biogeochemical cycling of mercury within the Everglades is confounded by many variables, particularly food web dynamics, water quality, and hydrological conditions (Julian 2013, Julian and Gu 2015). In spite of this complexity, research suggests that sulfate can potentially influence the mercury-MeHg cycle under some suite of ambient conditions. From an environmental management perspective, the mercury-related end products of these complexities must be predictable and quantified before an effective control or management strategy can be considered. Furthermore, various sulfate sources to the Everglades originate from both natural (i.e., oxidation of peat soil, groundwater, etc.) and anthropogenic sources (i.e., atmospheric deposition, fertilizer application, etc.). Within this context, this section provides an update to the status of sulfate within the EPA, although its role in the mercury problem remains uncertain.

Methods

A regional synoptic approach similar to that used for water quality evaluations in previous SFERs was applied to sulfate data for WY2018 to provide an overview of sulfate concentrations within the EPA. Consolidating regional water quality data provides the ability to analyze data over time across a limited spatial scale within each region.

Water Quality Sampling Stations in the EPA

To efficiently assess annual and long-term water quality trends, a network of water quality sampling sites has been identified (Figures 3A-1 through 3A-4 in Chapter 3A of this volume). These sites are part of the District's long-term monitoring network and are sampled for different purposes. These stations were

carefully selected to be representative of either the EPA boundary conditions (i.e., inflow or outflow) or ambient marsh conditions (i.e., interior). Sampling locations throughout the WCAs and ENP were categorized as inflow, interior, or outflow stations within each region based on their location and function. Furthermore, an effort has been made to utilize a consistent group of stations among previous annual reports to ensure consistent and comparable results. Every attempt is made to maintain the same sampling frequency for the network of monitoring sites to ensure a consistent number of samples across years. The data available for each year undergo the same careful quality assurance and quality control screening to assure accuracy. An overview of the water quality monitoring projects, including project descriptions and objectives with limited site-specific information, is available on the District's website at www.sfwmd.gov/environmentalmonitoring. The majority of the water quality data evaluated in this chapter were retrieved from the District's DBHYDRO database (www.sfwmd.gov/dbhydro). Additionally, water quality data from the nutrient gradient sampling stations monitored by the District were obtained from the District's Water Resources Division database.

Analysis Periods

This section summarizes sulfate concentrations within the EPA during WY2018 and describes trends or changes in these concentrations over time. To accomplish this objective, comparisons are made across discrete periods that correspond to major restoration activities occurring within the EPA. The four periods are the (1) Baseline period including WY1979–WY1993, (2) intermediate period, or Phase I, including WY1994–WY2004, (3) Phase II best management practices (BMP)/STA implementation period after WY2004 (WY2005–WY2017), and (4) the current water year (WY2018).

The Baseline period corresponds to the timeframe prior to implementation of the EAA BMP Program and the Everglades Construction Project, i.e., Everglades STAs. Phase I represents the period in which the EAA BMP Program was being implemented and all the initial STAs were constructed and became operational. The Phase II BMP/STA implementation period corresponds to when the performance of the BMPs and STAs were being optimized and enhanced. Additionally, during this period, various restoration projects were being implemented under the *Everglades Protection Area Tributary Basins Long-Term Plan for Achieving Water Quality Goals* (Burns and McDonnell 2003) and the Comprehensive Everglades Restoration Plan (CERP). Because optimization, enhancement, and other restoration activities are expected to continue for years, the Phase II period will continue to expand in future SFERs to incorporate additional years of sampling. In addition, data for the current water year (in this case, WY2018) will be used to make comparisons with the historical periods and will be analyzed independently as the fourth period. These periods of analysis are also used in Chapter 3A of this volume.

Data Screening and Handling

Water quality data were screened based on laboratory qualifier codes, consistent with the FDEP's Quality Assurance Rule (Chapter 62-160, F.A.C.). Any datum associated with a fatal qualifier (e.g., G, H, J, K, N, O, V, Q, Y, Z, or ?) indicating a potential data quality problem was removed from the analysis. Fatal qualifiers are standard data qualifiers used by both laboratories and field samplers to indicate that the quality or accuracy of the data may not be suitable for statistical analysis. As such data qualifiers can be used to indicate that a sample was not properly preserved (qualifier Y), sample was not analyzed within the acceptable window (qualifier Q), the analysis was flawed (qualifier G, J, K, N, O, V, and ?), or data was estimated with a lower accuracy method (qualifier H). Multiple samples collected at the same location on the same day were considered as one sample, with the arithmetic mean used to represent the sampling period. Additional considerations in the handling of water quality data are the accuracy and sensitivity of the laboratory method used. For purposes of summary statistics presented in this section, data reported as less than the MDL were assigned a value of one-half the MDL unless otherwise noted. All data in this chapter, including historical results, were handled consistently with regard to screening and MDL replacement.

Data Analyses

Unless otherwise noted, all inflow and outflow summary statistics (geometric mean, minimum, maximum, etc.) were performed using data collected on flow events only. All valid data (i.e., non-qualified data) were used to compute summary statistics for all other regions (i.e., interior and rim). Surface water sulfate concentrations were summarized for each period, region, and classification using basic descriptive statistics including arithmetic mean, standard deviation, sample size, minimum, maximum, and median. Typically, geometric mean concentrations were employed when reporting concentrations at a given sampling location. However, due to low sample size at each station, arithmetic mean concentrations were also employed for some monitoring locations. Trend analysis was performed on annual arithmetic mean sulfate concentration for inflow and interior regions of the EPA using the Kendall's τ correlation analysis (Base stats R package) and Sen's slope estimate (zyp R package). All statistical operations were performed with R© (Version 3.5.0, R Foundation for Statistical Computing, Vienna, Austria) and the critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Sulfate Concentrations

Sulfur is an essential plant macro-nutrient (Bellinger and Van Mooy 2012) and enters the Everglades ecosystem primarily as sulfate (Orem et al. 2011), but the role of organic sulfur in the total mass of sulfur entering the region remains undetermined. As stated above, sulfate is of concern due to its ability under some circumstances to influence biogeochemical processes that lead to mercury methylation and support the production of reduced sulfur compounds under anaerobic conditions. Sulfate monitoring results are presented in this section to provide an overview of current concentrations and evaluate temporal and spatial patterns. Sulfate summary statistics relative to the Baseline, Phase I, Phase II, and current year (WY2018) are shown in **Table 3B-6**.

Sulfate concentrations follow a general north-to-south gradient (**Figure 3B-16**) similar to other nutrients assessed elsewhere in this report. Annual mean sulfate concentrations observed at inflow regions to the EPA during WY2018 range from 57.6 mg/L (LNWR) to 7.8 mg/L (WCA-3). Inflows into WCA-2 and WCA-3 experienced a slight decrease in annual mean (arithmetic and geometric) and median sulfate concentrations relative to the Phase II period. Meanwhile, annual mean (arithmetic and geometric) and median inflow sulfate concentrations into LNWR have remained relatively constant since implementation of Phase I (**Table 3B-6**). For inflows to ENP, annual mean and median sulfate concentrations increased slightly in WY2018 over the Phase II period, but across the POR, annual average sulfate concentrations have significantly declined for LNWR, WCA-3, and ENP (**Table 3B-7**). Inflow concentrations to WCA-2 have not significantly changed along the entire POR. However, since the implementation of Phase II, sulfate concentrations have significantly declined for WCA-2 inflows (**Table 3B-7**).

707 **Table 3B-6**. Summary statistics of sulfate concentrations in mg/L for the Baseline (WY1979–
708 WY1993), Phase I (WY1994–WY2004), Phase II (WY2005–WY2017), and WY2018 periods.

Region	Class	Period	Sample Size	Arithmetic Mean	Standard Deviation	Geometric Mean	Geometric Standard Deviation	Median	Minimum	Maximum
LNWR	Inflow	1979–1993	64	101.6	80.1	84.0	1.8	82.3	28.8	455.8
		1994–2004	309	55.6	34.7	48.9	1.7	50.7	6.7	460.7
		2005–2017	591	51.1	20.7	46.7	1.6	48.6	10.0	132.0
		2018	72	57.6	20.8	53.8	1.5	56.7	23.1	117.0
	Interior	1979–1993	340	16.6	21.5	10.3	2.6	10.0	2.5	220.2
		1994–2004	1,205	5.0	11.5	1.0	5.8	1.0	0.1	110.0
		2005–2017	3,035	5.3	11.8	0.6	9.4	0.5	0.1	95.1
		2018	333	6.7	14.6	0.5	12.1	0.2	0.1	84.9
	Outflow	1979–1993	61	45.1	36.6	35.8	2.0	34.4	7.3	257.2
		1994–2004	70	50.5	50.8	38.8	2.1	40.6	4.2	418.9
		2005–2017	180	30.8	19.0	23.7	2.3	28.0	1.4	85.4
		2018	25	28.7	21.9	20.4	2.6	23.0	1.5	81.8
	Rim	1979–1993	66	42.2	37.1	25.2	3.2	34.4	2.5	139.8
		1994–2004	345	57.2	26.9	51.0	1.7	49.6	1.6	210.0
		2005–2017	333	49.6	21.7	44.7	1.7	46.0	3.5	185.0
		2018	48	45.4	20.5	38.2	2.4	43.4	0.2	96.4
WCA-2	Inflow	1979–1993	73	75.8	114.9	53.6	2.1	53.8	7.3	945.3
		1994–2004	127	55.3	38.9	48.2	1.7	52.3	7.8	418.9
		2005–2017	589	47.3	17.3	42.6	1.8	47.2	0.1	106.0
		2018	79	37.1	15.7	32.7	1.8	34.9	1.5	81.8
	Interior	1979–1993	633	42.9	37.1	32.9	2.2	37.3	2.5	344.3
		1994–2004	1,269	43.8	23.9	35.5	2.1	42.0	0.1	180.0
		2005–2017	1,763	44.2	18.0	39.1	1.8	45.3	0.1	128.0
		2018	179	38.4	12.9	35.9	1.5	38.0	6.8	68.1
	Outflow	1979–1993	103	41.2	21.0	36.4	1.7	38.7	7.6	131.7
		1994–2004	95	28.6	10.9	26.2	1.6	27.9	5.8	54.3
		2005–2017	440	28.6	15.6	23.9	1.9	26.8	3.9	74.7
		2018	51	22.8	8.8	21.1	1.5	21.5	8.5	44.6
WCA-3	Inflow	1979–1993	268	36.7	35.2	24.2	2.7	29.8	1.0	286.0
		1994–2004	182	20.6	16.6	13.3	2.9	16.3	0.5	62.9
		2005–2017	880	17.7	18.9	6.7	6.1	7.4	0.1	74.7
		2018	246	7.8	10.8	3.8	3.1	2.9	0.5	44.6
	Interior	1979–1993	450	14.9	17.3	10.5	2.3	10.7	2.0	261.5
		1994–2004	1,620	10.8	34.8	3.8	5.3	4.5	0.1	1,300.0
		2005–2017	1,354	12.8	15.5	3.2	9.0	4.5	0.1	126.0
		2018	85	12.4	9.9	6.6	4.3	11.0	0.1	36.7
	Outflow	1979–1993	137	15.9	16.7	10.3	2.6	12.4	1.0	107.6
		1994–2004	134	6.9	7.7	2.5	6.2	4.5	0.1	36.5
		2005–2017	170	8.9	8.9	2.7	8.5	7.2	0.1	39.3
		2018	53	9.4	4.5	5.8	5.0	10.3	0.1	19.1
ENP	Inflow	1979–1993	142	15.4	16.3	10.1	2.6	11.5	1.0	107.6
		1994–2004	134	7.4	7.2	3.7	4.6	6.0	0.1	36.5
		2005–2017	206	7.9	7.2	3.7	5.0	6.4	0.1	35.8
		2018	59	9.1	4.4	6.5	3.6	10.2	0.1	19.1
	Interior	1979–1993	572	9.0	19.5	4.3	2.9	4.3	0.8	205.5
		1994–2004	864	5.5	17.7	2.1	4.2	2.6	0.1	403.0
		2005–2017	857	5.0	19.9	0.9	6.5	1.2	0.1	242.0
		2018	70	3.9	9.8	1.0	6.4	1.6	0.1	70.4

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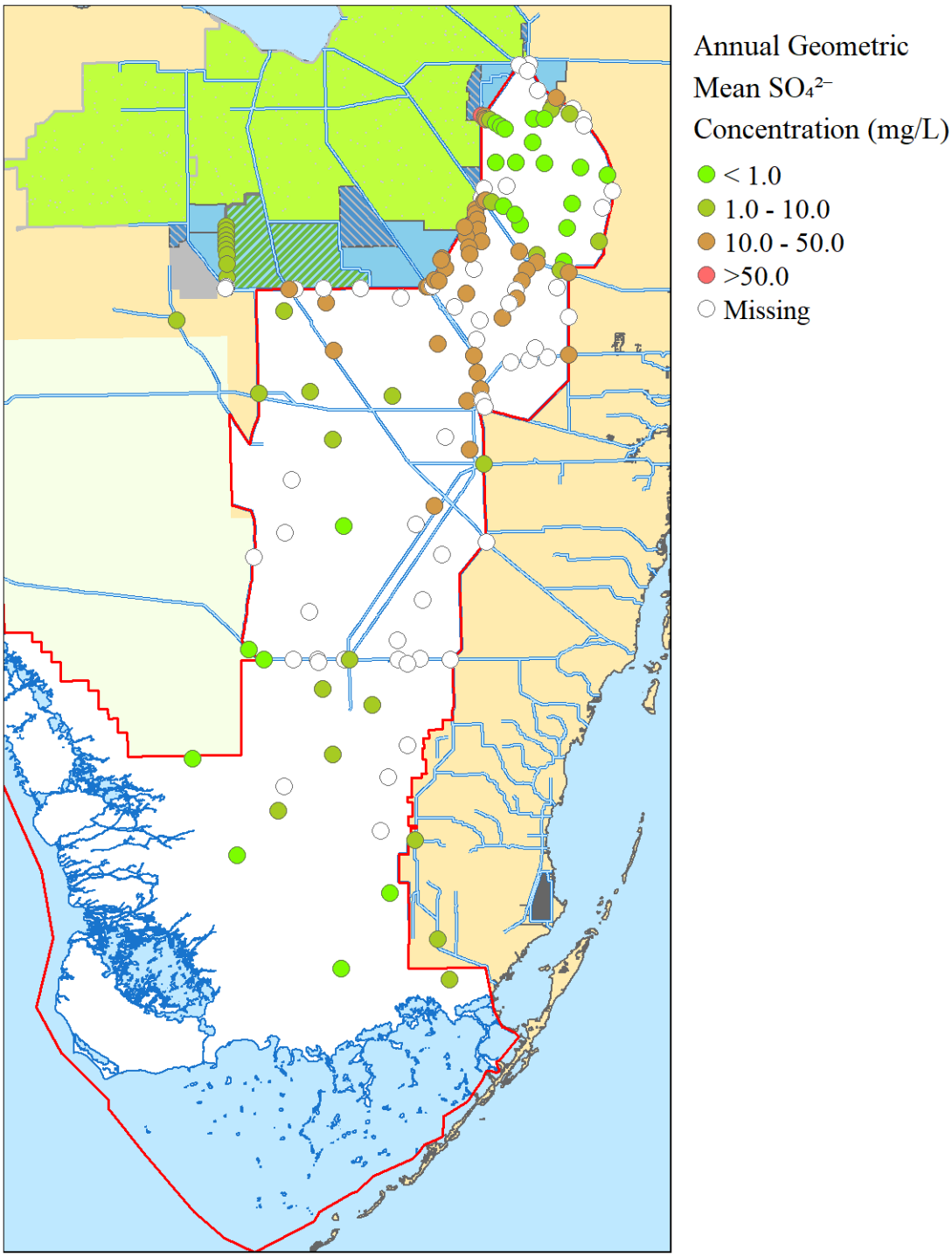

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711 **Figure 3B-16**. Annual geometric mean sulfate (SO_4^{2-}) concentrations
712 for all classifications at stations across the EPA in WY2018.

Table 3B-7. Kendall's τ annual arithmetic mean sulfate concentration trend analysis results for each region's inflow and interior classification within the EPA for the entire POR (WY1979–WY2018) and the period of WY2005 to present.

		POR (WY1979–WY2018)			Phase II & Current Water Year (WY2005–WY2018)		
Area	**Class**	**Kendall's τ**	**p-value**	**Sen's Slope Estimate [a]**	**Kendall's τ**	**p-value**	**Sen's Slope Estimate [a]**
LNWR	Inflow	-0.31	0.01	-0.73	0.10	0.67	0.46
	Interior	-0.36	<0.01	-0.22	0.10	0.67	0.18
WCA-2	Inflow	-0.23	0.05	-0.40	-0.63	<0.01	-1.16
	Interior	0.20	0.10	0.16	-0.27	0.19	-0.49
WCA-3	Inflow	-0.47	<0.01	-0.52	-0.50	0.01	-1.31
	Interior	-0.14	0.28	-0.06	-0.30	0.16	-0.27
ENP	Inflow	-0.29	0.01	-0.20	0.16	0.45	0.09
	Interior	-0.33	0.01	-0.14	-0.43	<0.05	-0.29

a. Expressed as microgram per liter (µg/L) per water year.

Some annual trends are more pronounced than others, as shown in **Figure 3B-17**. The trend in annual mean sulfate concentrations entering seems high even though throughout the POR, a significantly declining trend is apparent (**Table 3B-7**). This could be due to the natural conditions that exist in the eastern portion of the EAA and EPA. Historically, water quality within the surficial aquifer in this region is affected by saltwater intrusion and highly mineralized groundwater. Highly mineralized ground in this region is typically associated with ancient connate seawater, which was the result of the interglacial seas that inundated the area during the Pleistocene Epoch (Miller 1988). As noted in Axelrad et al. (2013), connate seawater could potentially be a relatively large source of sulfate, chloride, and dissolved solids (i.e., other minerals) to the EPA, more specifically to the LNWR. Another driving factor of interior trends are the biogeochemical processes associated with marsh dryout. During relatively dry periods, when water levels in the marsh recede below the soil surface, oxidation of organic matter occurs readily. Once the area is reflooded, a large upward flux of nutrients occurs including sulfate from the soil to the water column. This dryout and flux phenomena explains the relatively high annual concentrations experienced during the extremely dry period in the mid-1980s and the relatively dry period during the early to mid-2000s.

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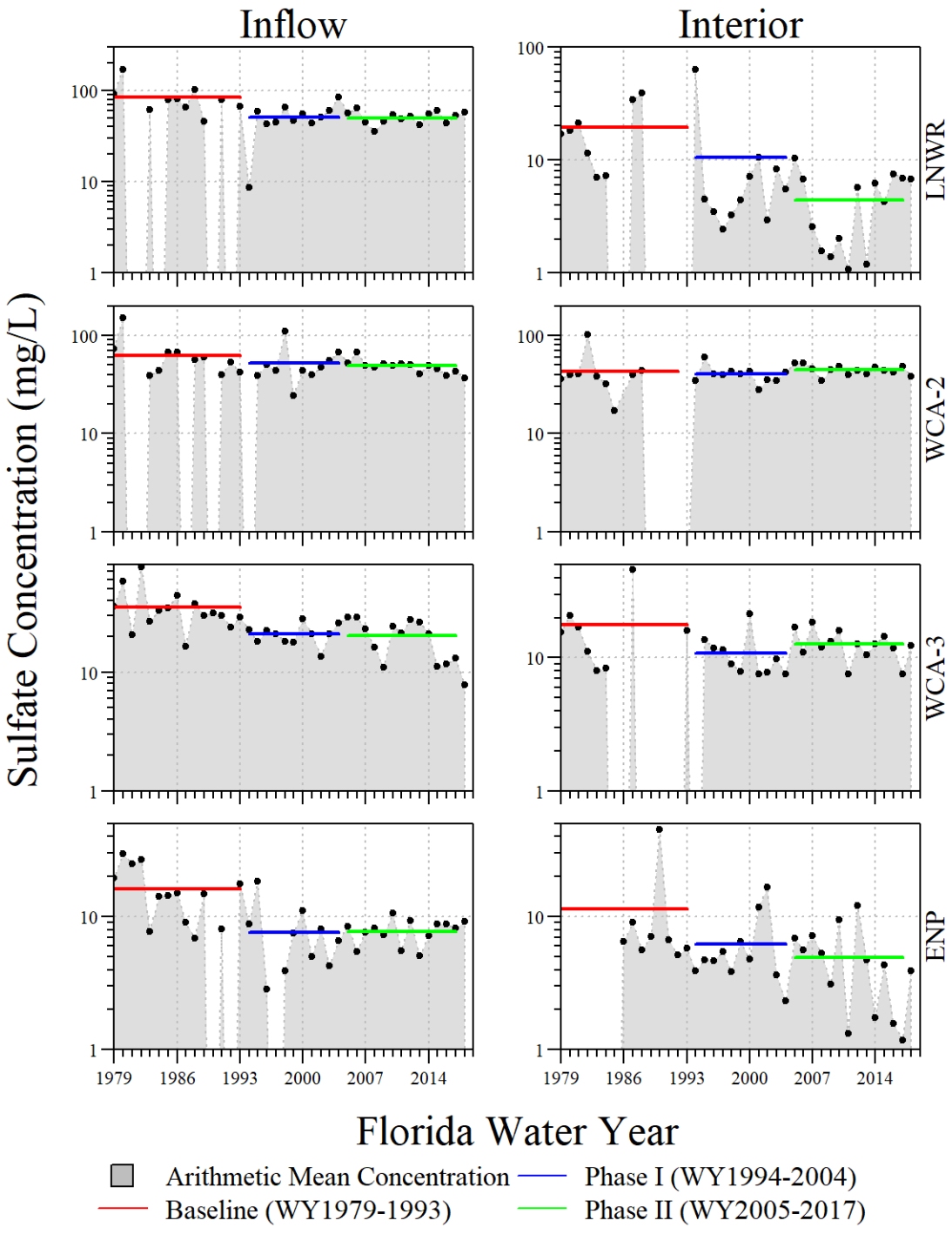

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733 **Figure 3B-17.** Annual arithmetic mean sulfate concentrations for inflow (left panel) and interior (right
734 panel) areas of the LNWR, WCA-2, WCA-3, and ENP during WY1979–WY2018. Bars indicate arithmetic
735 mean when flowing for inflow locations. The horizontal lines indicate the mean annual geometric mean
736 sulfate concentrations for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), and Phase II
737 (WY2005–WY2017) periods. (Note: Area with no gray indicates data gaps.)

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Much like other nutrients in the EPA (see Chapter 3A of this volume), the typical north-to-south gradient is disrupted slightly at interior monitoring stations within the EPA. During WY2018, WCA-2 interior had the highest annual mean sulfate concentration of 38.4 mg/L, followed by WCA-3 (12.4 mg/L), LNWR (6.7 mg/L), and ENP (3.9 mg/L). Across the POR (WY1979–WY2018), LNWR and ENP have experienced statistically significant decreases in annual arithmetic mean sulfate concentrations, with all other areas experiencing non-significant trends (**Table 3B-7**). During the shorter POR (WY2005–WY2018), ENP interior continued to experience significantly declining trends while all other areas have non-significant trends (**Table 3B-7**). Qualitative comparison of period-wide concentrations indicates sulfate concentrations within LNWR decreased between the Phase I and Phase II periods (**Figure 3B-17**), which could possibly be due to the construction and operation of STA-1 West and STA-1 East in combination with the rainfall-driven hydrology. However, it has been suggested that the Everglades STAs only reduce surface water sulfate concentrations and loads by a small portion, approximately 10% of the sulfate from the water column (SFWMD unpublished data). Other factors influencing this trend of decreasing sulfate concentrations with time could be changes in water management, establishing and managing BMPs within the EAA, and potential decreasing application of elemental sulfur as a soil amendment to agricultural fields in this region of the EAA. It should be noted that the BMPs implemented were not used to directly mitigate sulfate; but rather nutrient and sediment run-off. It is also important to note that sulfate concentrations within LNWR spiked after extended periods of dry conditions as observed between WY1985 and WY1994. This was not just isolated to water column sulfate, but phosphorus and nitrogen also spiked during these periods (Chapter 3A of this volume). The very low sulfate concentrations observed for the interior portion of LNWR indicate that either assimilation of sulfate is occurring and potentially could be in growth-limiting concentrations due to its low availability (Bellinger and Van Mooy 2012), or very little to no high sulfate water reached the interior portion (due to topography and hydrology) of LNWR and these low concentrations reflect deposition-driven water column concentrations. To further understand marsh sulfate dynamics, sulfur speciation and a more in-depth analysis of iron biogeochemistry is needed.

Annual mean sulfate concentrations within WCA-2 are approximately twice that of other regions within the EPA. This is due to historical EAA runoff containing both local and regional inputs of sulfate. The hydrology of WCA-2A spreads the canal inflow broadly, and WCA-2 soils have relatively high nutrient concentrations including sulfur. Samples collected between 2003 and 2004 at limited locations within each region indicate that WCA-2A soils had the greatest concentration of sulfur (14,025 ± 1,173 mg/kg; mean ± standard error), followed by WCA-3A (9,100 ± 576 mg/kg) and LNWR (8,825 ± 1,019 mg/kg; data source: District's DBHYDRO database). This restricted sampling effort did not take into account soil types or bulk density and was limited to two sampling locations per area. More recently, soil samples were collected along the impacted gradient within WCA-2A indicate that soil sulfur concentrations have not qualitatively changed (University of Florida unpublished data). This general trend is also apparent in a more spatially explicit data set (Everglades soil mapping data, Reddy et al. 2005). This larger effort showed high concentrations of soil sulfur in WCA-2 and around the periphery of WCA-1. These high concentrations of sulfur within the soils could result in enhanced internal sulfur loading, which explains why interior mean concentrations are higher than inflow mean concentrations. Due to these relatively high marsh concentrations within eutrophic/impacted portions of the WCA-2, it is reasonable to suggest that growth of biota within this region of the EPA is not sulfur or sulfate growth limited and corroborate results presented by Bellinger and Van Mooy (2012).

Feasibility of a Sulfate Criterion

Previous peer reviews of this SFER chapter (2013 and 2014 SFER – Volume 1, Appendix 1-2; SFWMD 2013, 2014) as well as peer reviewed literature (Corrales et al. 2011, Gabriel et al. 2014, Orem et al. 2011) have suggested the need to develop a site-specific water quality standard for sulfate in the EPA. As explained above, the sulfur-mercury biogeochemical cycle has proven to be altered by many environmental factors in the EPA. As a result, empirical evaluation of mercury and sulfate data provides little predictive

power to link water column concentrations or loads to environmental mercury levels. These factors together make development of a site-specific criterion impossible to defend at this time. It is uncertain based on the best available data that reduction of sulfur inputs can reduce mercury methylation at all or even shift methylation hot spots on the landscape or regional scale.

In an effort to provide more information on the role of sulfate in mercury cycling, FDEP is funding research to investigate mercury methylation potential at low sulfate concentrations in surface water. The results will provide evidence on the importance of background sulfate levels on methylation and will also help to assess if reduction of sulfur or sulfate will cause a positive or negative ecological response. So far this study has yielded interesting results that show relatively low sulfate additions (i.e., 0.5 to 1.0 mg/L) significantly increase water column MeHg concentrations indicating that non-abatable sources of sulfate could support meaningful MeHg production in the presence of bioavailable inorganic mercury (Dierberg et al. 2014, Jerauld et al. 2015).

The commonly referenced 1 mg/L sulfate CERP performance measure for the Greater Everglades was developed to indicate background marsh concentrations that would be consistent with sulfur limitation of mercury bioaccumulation. However, this performance measure was proposed without detailed technical support. While concerns have been raised that concentrations above this level could stimulate significant mercury methylation, the 1 mg/L sulfate goal is not consistently associated with any particular level of mercury in the Everglades (Julian et al. 2014, 2015a, b). In addition, this goal lacks empirical evidence on whether 1 mg/L is protective of flora and fauna or if higher concentrations are consistently associated with degraded water quality or ecological integrity. Furthermore, to date, no studies have justified either a numeric sulfate criterion of 1 mg/L, or a site-specific alternative criterion that incorporates other potential factors in the methylation process, for the protection of fish and wildlife in the EPA.

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