# 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 2019 (WY2019; May 1, 2018–April 30, 2019). The report fulfills the requirements of the Everglades Forever Act (EFA), Subparagraph 373.4592(4)(d)5, Florida Statutes. The information provided in this chapter is an update to Chapter 3B of the *2019 South Florida Environmental Report* (SFER) – *Volume I* (Julian et al. 2019).

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 WY2019 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:

- During WY2019, total mercury (THg) concentrations in mosquitofish (*Gambusia* spp.) ranged from 0.011 milligrams per kilogram (mg/kg) at site ROTENC to 0.079 mg/kg at site CA35ALT, with a mean value of 0.033 mg/kg. Mosquitofish THg concentrations during WY2019 exceeded the United States Environmental Protection Agency (USEPA) trophic level 3 (TL3) criterion (0.077 mg/kg) for protection of piscivorous wildlife at 1 of the 13 sites. Mosquitofish THg level in WY2019 decreased in 4 stations, increased in 1 station, and did not change in 8 stations.

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- During WY2019, the mean THg concentration in the TL3 sunfish (*Lepomis* spp.) species from 10 of the 13 active monitoring sites with data available was 0.134 mg/kg, with a range from 0.033 mg/kg at CA2NF to 0.268 mg/kg at CA33ALT. Mean sunfish THg concentrations in WY2019 exceeded the recommended USEPA protection of wildlife criterion (77 ng/g) at 7 stations. Mean THg concentrations in these species in WY2019 were highest in spotted sunfish (*Lepomis punctatus*; 0.184 mg/kg), followed by bluegill (*L. macrochirus*; 0.113 mg/kg), and redear sunfish (*Lepomis microlophus*; 0.111 mg/kg)
- During WY2019, THg concentrations in LMB were collected from 9 of the 13 locations within the EPA. THg concentration in LMB ranged from 0.187 mg/kg at site CA2NF (WCA-2) to 1.132 mg/kg at site L67F1 (ENP), with an overall WY2019 average of 0.503 mg/kg. Four locations exceeded the USEPA recommended criterion for the protection of human health (0.300 mg/kg).
- In assessing soil microbes, it was concluded that differing levels of sulfate, carbon and available nutrients contribute to the structure and abundance of microbial communities that have the ability to methylate Hg across environmental compartments (periphyton, floc, and soil).
- During WY2019, annual mean inflow sulfate concentrations ranged from 11.7 milligrams per liter (mg/L) for ENP to 59.3 mg/L for LNWR. The annual mean sulfate concentrations at interior marsh regions ranged from 1.1 mg/L for ENP to 45.3 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 methyl mercury (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 sulfur/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 - 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 Hg concentrations in fish and biota have been a concern for the Everglades regions since the 1970s (Ogden et al. 1973). Subsequently, elevated Hg 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 Hg deposition, the Everglades is considered sensitive to Hg methylation and subsequent bioaccumulation into piscivorous wildlife (Wiener et al. 2003). Methylation of inorganic mercury ($Hg^{2+}$) 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 Hg in native fish and wading birds from the Everglades region.

# SPATIAL AND TEMPORAL TRENDS IN MERCURY LEVELS IN EVERGLADES FISH

Binhe Gu

## Introduction

As discussed above, the Everglades ecosystem continues to experience elevated concentrations of mercury 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 mercury 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).

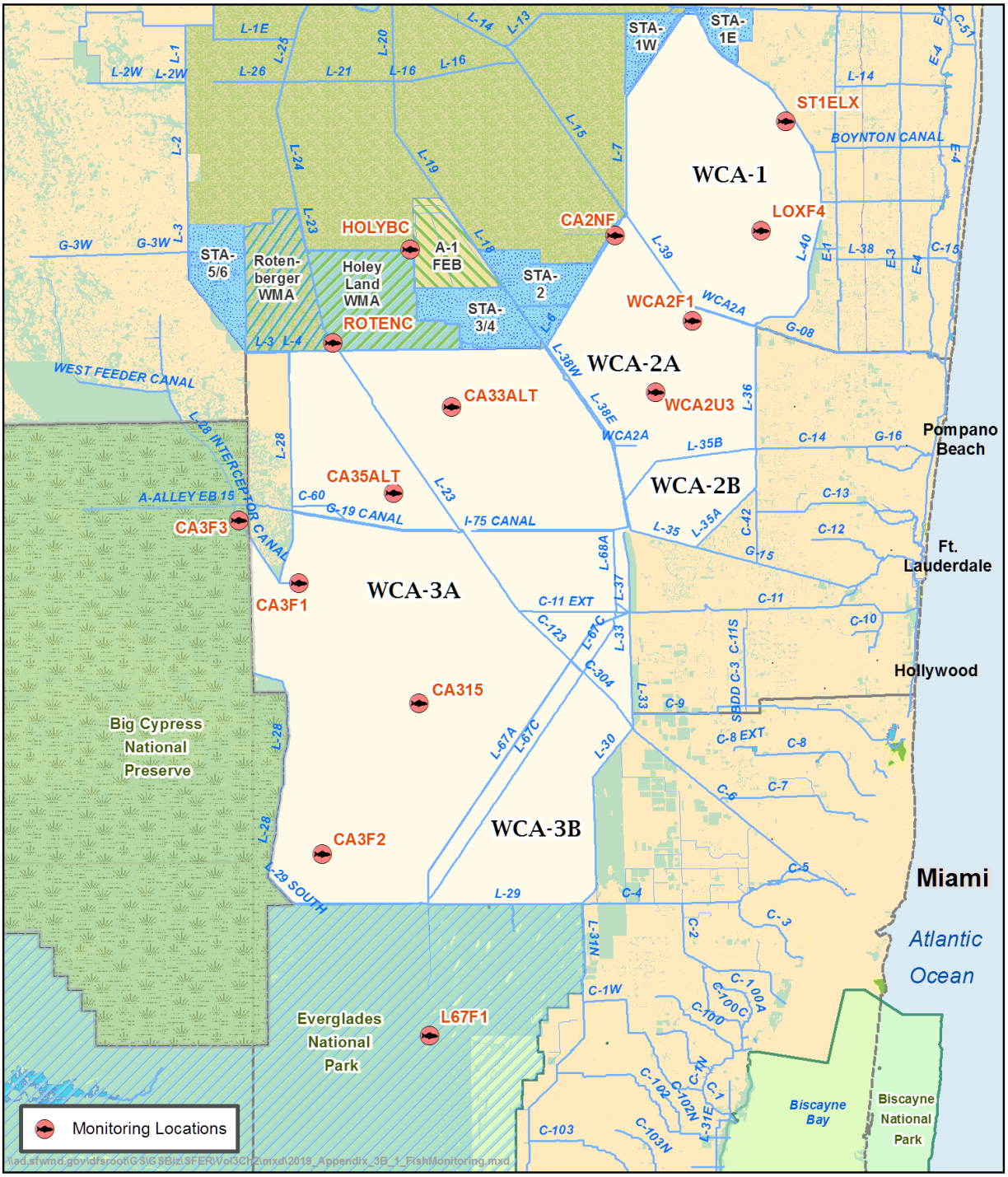


**Figure 3B-1**. Location of fish tissue monitoring locations within the EPA and Holey Land and Rotenberger WMAs. (Notes: Station CA3F1 is an inactive station with fish sampling activity suspended since October 2009. CA3F3 is used to replace CA3F1 since October 2010. STA – Stormwater Treatment Area.)

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 LMB 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 collection at each location. Similarly, 20 LMB 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 LMB were weighed, measured, sexed, and, for LMB only, the sagittal otoliths were removed for determination of age. Whole sunfish and whole axial muscle (fillet) samples of LMB 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 recommended 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 2019, and the entire period of record (POR; WY1999–WY2019; May 1, 1998–April 30, 2019). 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 ($\alpha$) set at 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 CA2F1 due to site access issue. In WY2019, no samples were collected from ST1ELX and CA315 due to the lack of fish presence. THg concentrations in mosquitofish ranged from 0.011 mg/kg at site ROTENC to 0.079 mg/kg at site CA35ALT, with a median value of 0.019 mg/kg (mean ± standard error [SE] = 0.033 ± 0.006 mg/kg; **Figure 3B-2**). Mosquitofish THg level in WY2019 decreased in 4 stations, increased in 1 station, and did not change in 8 stations.

Except for CA35ALT, no mosquitofish THg concentration in WY2019 exceeded the federal recommended criterion of 0.077 mg/kg MeHg for TL3 fish for wildlife protection (**Figure 3B-2**). For the POR, the median value of THg in mosquitofish is 0.049 mg/kg (mean ± SE = 0.057 ± 0.002 mg/kg) and 24% of the data exceeded the federal criterion. The highest value of mosquitofish THg throughout the POR was 0.373 mg/kg observed at WCA2U3 (WY2012), while the lowest observed value of 0.002 mg/kg was recorded at CA2NF. Site WCA2F1 (and its alternative sites) near the Hillsborough Canal had no exceedance for the entire monitoring period and displayed the lowest mosquitofish median THg value of 0.009 mg/kg. It is noteworthy that WCA2F1 and CA2NF are located in the northern portion of WCA-2A near the Stormwater Treatment Area (STA) 2 outflow and within the nutrient enriched areas of the marsh. Given the difference in trend direction (and statistical significance), it is possible the change over time at these sites are signaling a trophic dynamic shift driven by restoration efforts and changes to water quality. 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. Despite two consecutive decreases in THg, WCA2U3 had the highest median value (0.095 mg/kg) for the POR among the 13 monitoring stations.

**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–WY2019 (May 1, 1998–April 30, 2019).

| Area | Station | Kendall's τ | p-value | Sample Size | Trend Direction |
|---|---|---|---|---|---|
| WCA-1 | ST1ELX [a] | 0.05 | 0.84 | 12 | Not statistically significant |
| | LOXF4 | -0.51 | <0.01 | 20 | Decrease |
| WCA-2 | CA2NF | -0.38 | 0.06 | 14 | Not statistically significant |
| | WCA2F1 [b] | 0.45 | <0.05 | 18 | Increase |
| | WCA2U3 | -0.11 | 0.49 | 21 | Not statistically significant |
| WCA-3 | CA33ALT | -0.09 | 0.66 | 15 | Not statistically significant |
| | CA35ALT | 0.00 | 1.00 | 17 | Not statistically significant |
| | CA3F1/F3 | -0.08 | 0.61 | 21 | Not statistically significant |
| | CA315 [a] | -0.33 | <0.05 | 20 | Decrease |
| | CA3F2 | -0.33 | <0.05 | 21 | Decrease |
| ENP | L67F1 | -0.21 | 0.21 | 19 | Not statistically significant |
| WMAs | ROTENC | -0.06 | 0.75 | 16 | Not statistically significant |
| | HOLYBC | -0.20 | 0.21 | 21 | Not statistically significant |

a. No samples were collected due to lack of fish presence.

b. Since WY2017, site WCA2F1 is represented by two alternative sites: CA2F1ALT1 and CA2F1ALT2.

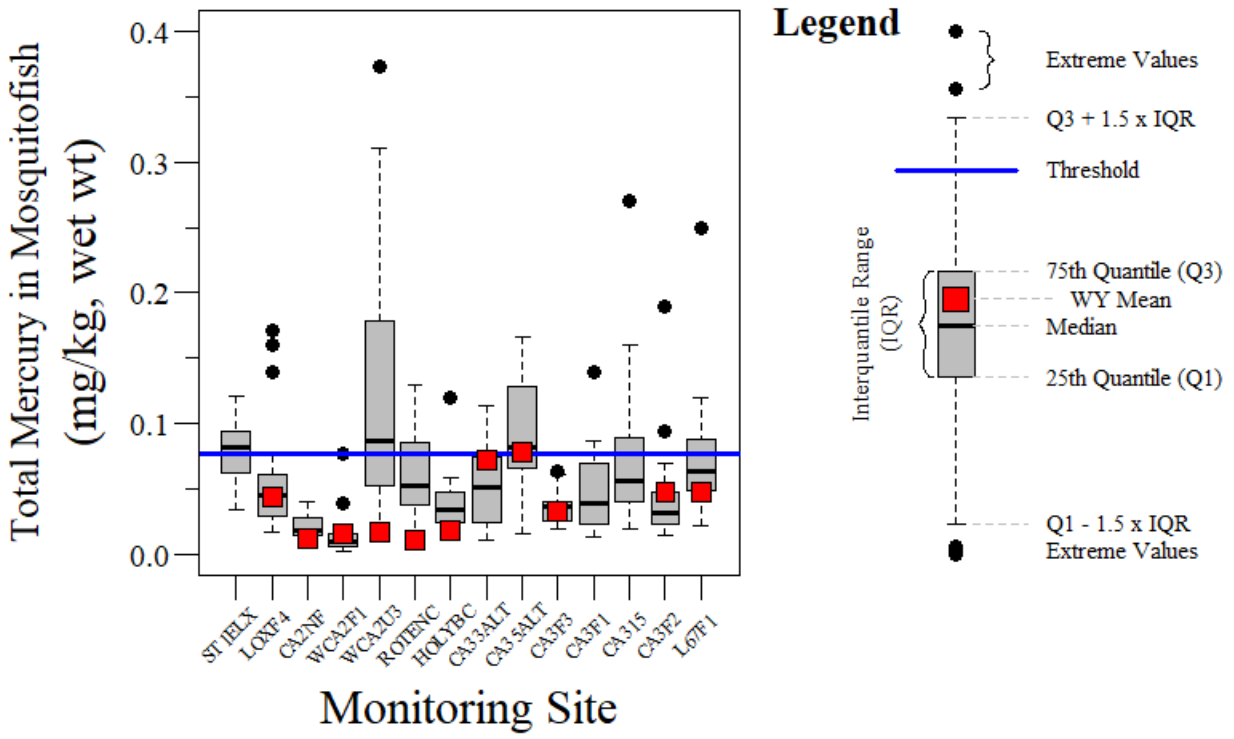


**Figure 3B-2.** Box plots of THg concentrations in mosquitofish in mg/kg, wet weight (wt), at each monitoring site in the EPA for WY1999–WY2019. Red boxes indicate WY2019 mean THg concentrations and the blue line denotes the 0.077 mg/kg USEPA MeHg recommended criterion for TL3 fish for protection of piscivorous wildlife. Site CA3F1 was replaced by CA3F3 in WY2011 and is no longer monitored. Sites ST1ELX and CA315 have no samples associated with WY2019 due to lack of fish presence.

Inter-annual and inter-site variations in mosquitofish THg concentrations between consecutive years remains high (**Figure 3B-3**). During WY2019, mosquitofish THg concentration was 0.018 mg/kg at WCA2U3. During WY2018, this site reported a THg concentration of 0.039 mg/kg. This change is over one-fold decrease in THg. Compared to WY2018, ROTENC in WY2019 displayed one-fold decrease in THg concentration. However, CA33ALT displayed a 64% increase over WY2018. To date, it is not clear what factor(s) control the dramatic intra-site temporal variations in mosquitofish THg concentration. It has been hypothesized that changes in inter-annual precipitation (i.e., wet years versus dry years) and site-specific biogeochemistry, including $SO_4^{2-}$, available $Hg^{2+}$, dissolved organic carbon (DOC), and reduction-oxidation (redox), have the ability to influence prey mercury concentrations.

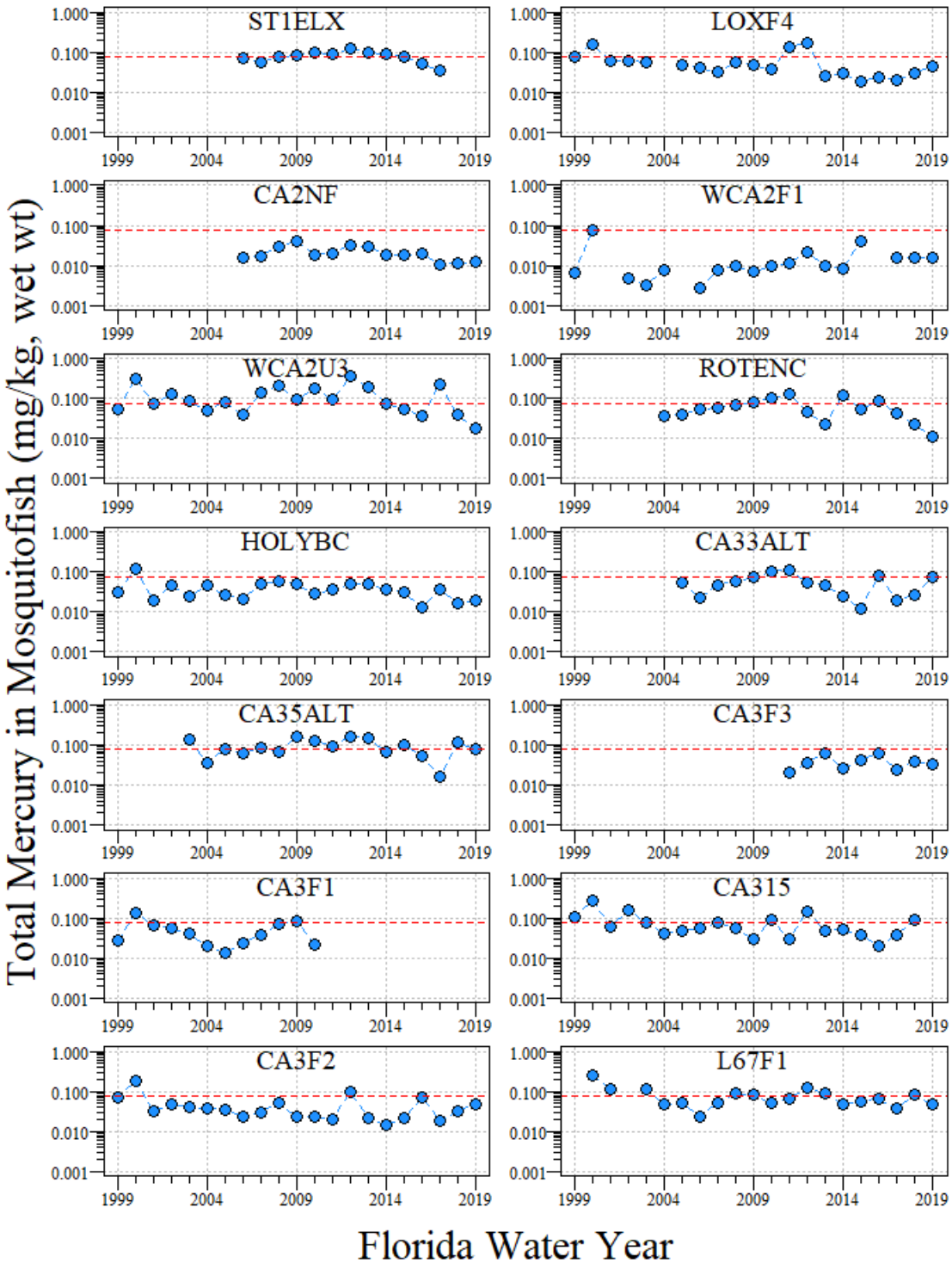


**Figure 3B-3.** Annual THg concentrations in mosquitofish composite samples at each monitoring site within the EPA for WY1999–WY2019. The red dashed lines indicate the 0.077 mg/kg USEPA MeHg recommended criterion for TL3 fish for protection of piscivorous wildlife.

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 (H = 0.91, degrees of freedom [df] = 1, probability factor [p] = 0.57). The median THg values for the wet (0.048 mg/kg, sample size (n) = 127) and dry season (0.051 mg/kg, n = 90) were similar. 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.

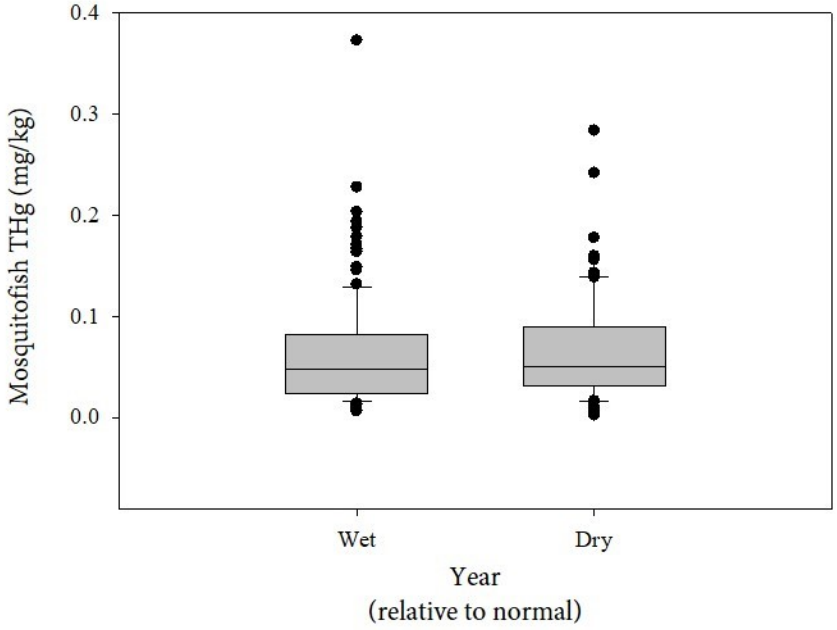


**Figure 3B-4**. Comparison of mosquitofish THg concentrations collected during wet and dry years for WY1999–WY2019 within the EPA.

Mosquitofish THg concentrations were compared by habitat with stations either characterizing canal or marsh habitat types. Overall variation in mosquitofish THg concentration was greater in marsh habitat (Variance = 0.00336) than canal habitat (Variance = 0.00135). This high degree of variation in marsh habitat could be due to relatively dynamic hydrology (i.e., drydown, dryout, 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 = 187) for marsh area and 0.047 mg/kg (n = 61) or canal and do not show statistical difference between habitat (H = 0.32, p = 0.57) (**Figure 3B-5**).

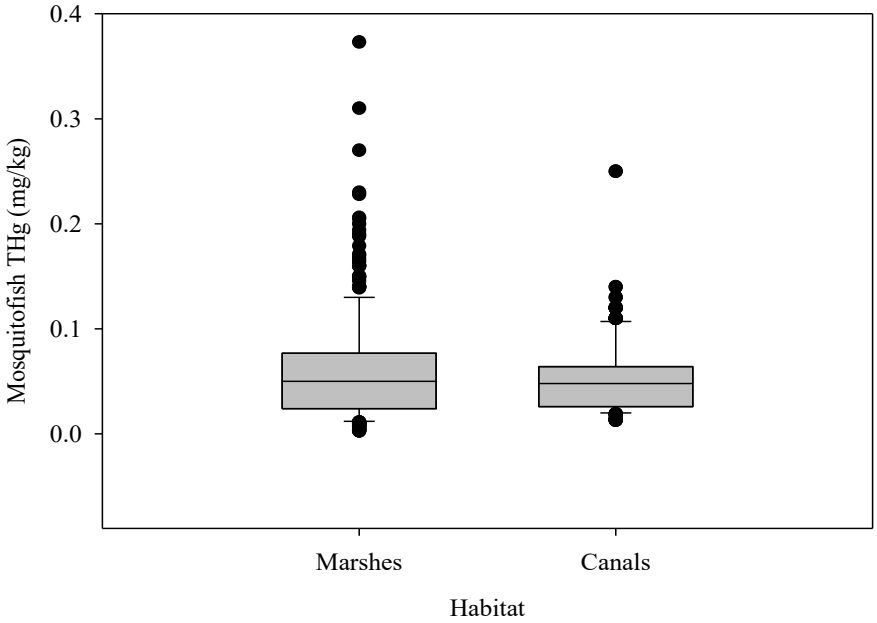


**Figure 3B-5**. Comparison of mosquitofish THg concentrations between marsh and canal habitats from data collected in the EPA for WY1999–WY2019.

During the entire POR, three sites (LOXF4, CA315, and CA3F2) experienced a significantly declining temporal trend in mosquitofish THg while one site (WCA2F1) showed a significant increasing trend (**Table 3B-1**). Considerable interannual variations of THg concentrations were observed within sites with low nutrient concentrations including interior stations (**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 0.166 ± 0.156 mg/kg (n = 3,931). Throughout the POR, 72% of annual mean sunfish THg concentrations exceeded the USEPA MeHg recommended criterion of 0.077 mg/kg for TL3 fish for protection of wildlife. The average ( ± SE) sunfish THg concentration from current monitoring sites (including alternative sites) was 0.175 ± 0.160 mg/kg (n = 3,476; median = 0.130 mg/kg). Except WCA2F1, all current monitoring stations observed annual mean sunfish THg concentrations above the USEPA MeHg criterion, with the nutrient enriched WCA2F1 experiencing the fewest number of exceedances (n = 1) during the POR.

The average THg concentration for the POR was highest (0.352 mg/kg) at the ENP site L67F1 and lowest (0.051 mg/kg) at a WCA-2A site (WCA2F1) near the Hillsborough Canal (**Figure 3B-1**). The THg concentration in sunfish tended to increase from north to south (**Figure 3B-6**). No monitoring station shows a significant increasing trend in annual median sunfish THg concentration while two sites, CA2NF and CA3F2, show a significant decrease trend throughout the POR (**Table 3B-2** and **Figure 3B-7**).

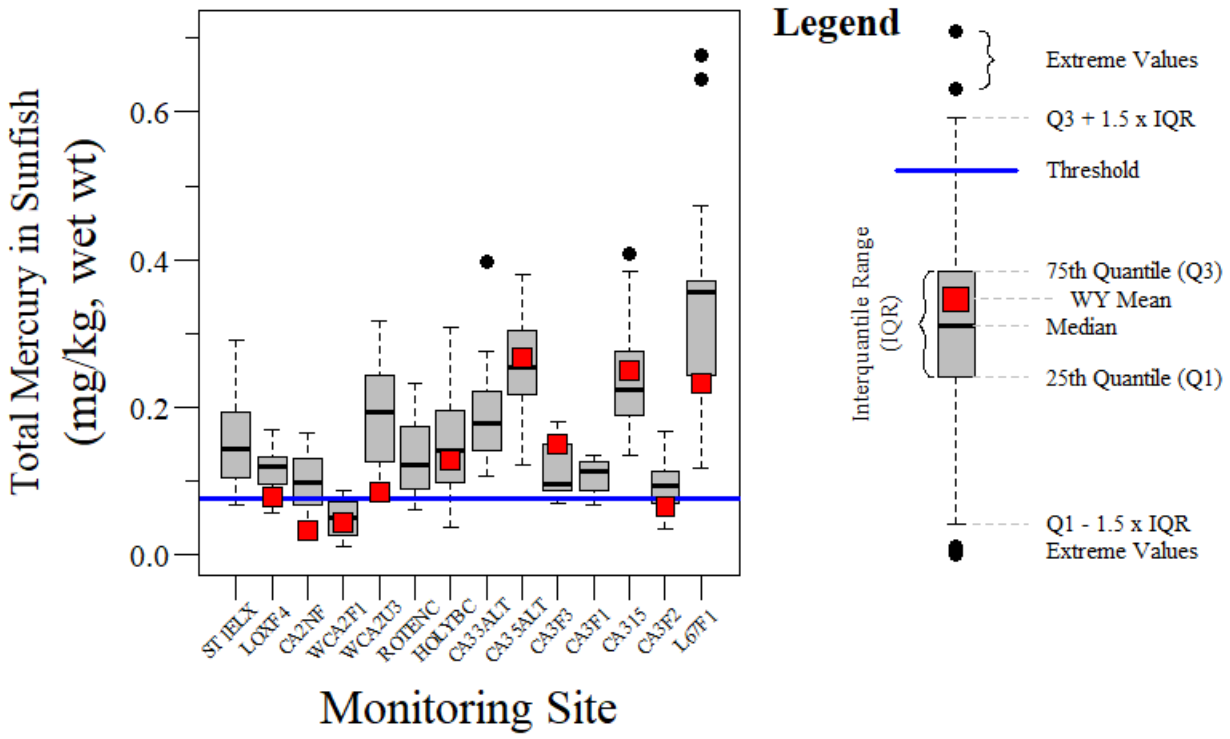


**Figure 3B-6.** Box plots of THg concentrations in mg/kg wet weight (wt) in TL3 sunfish at each monitoring site in the EPA for WY1999–WY2019. Red boxes indicate WY2019 mean THg concentrations and the blue line denotes the 0.077 mg/kg USEPA MeHg recommended criterion for TL3 fish for protection of piscivorous wildlife. Site CA3F1 was replaced by CA3F3 in WY2011 and is no longer monitored. Sites ST1ELX, CA33ALT and, ROTENC do not have samples for WY2019 due to lack of fish and access issues.

**Table 3B-2.** Kendall trend analysis of TL3 sunfish annual median THg concentration at the 13 active monitoring locations within the EPA for WY1999–WY2019.

| Area | Station | Kendall's τ | p–value | Sample Size | Trend Direction |
|---|---|---|---|---|---|
| WCA-1 | ST1ELX | -0.11 | 0.73 | 10 | Not statistically significant |
| | LOXF4 | -0.24 | 0.14 | 20 | Not statistically significant |
| WCA-2 | CA2NF | -0.42 | <0.05 | 14 | Decrease |
| | WCA2F1 [a] | 0.00 | 1.00 | 8 | Not statistically significant |
| | WCA2U3 | 0.05 | 0.74 | 21 | Not statistically significant |
| WCA-3 | CA33ALT [a] | 0.09 | 0.74 | 12 | Not statistically significant |
| | CA35ALT | 0.28 | 0.13 | 17 | Not statistically significant |
| | CA3F1/F3 | -0.07 | 0.70 | 21 | Not statistically significant |
| | CA315 | -0.13 | 0.41 | 21 | Not statistically significant |
| | CA3F2 | -0.35 | <0.05 | 21 | Decrease |
| ENP | L67F1 | -0.20 | 0.22 | 21 | Not statistically significant |
| WMAs | ROTENC | 0.00 | 1.00 | 12 | Not statistically significant |
| | HOLYBC | 0.06 | 0.72 | 20 | Not statistically significant |

a. No data for WY2015 and WY2016.

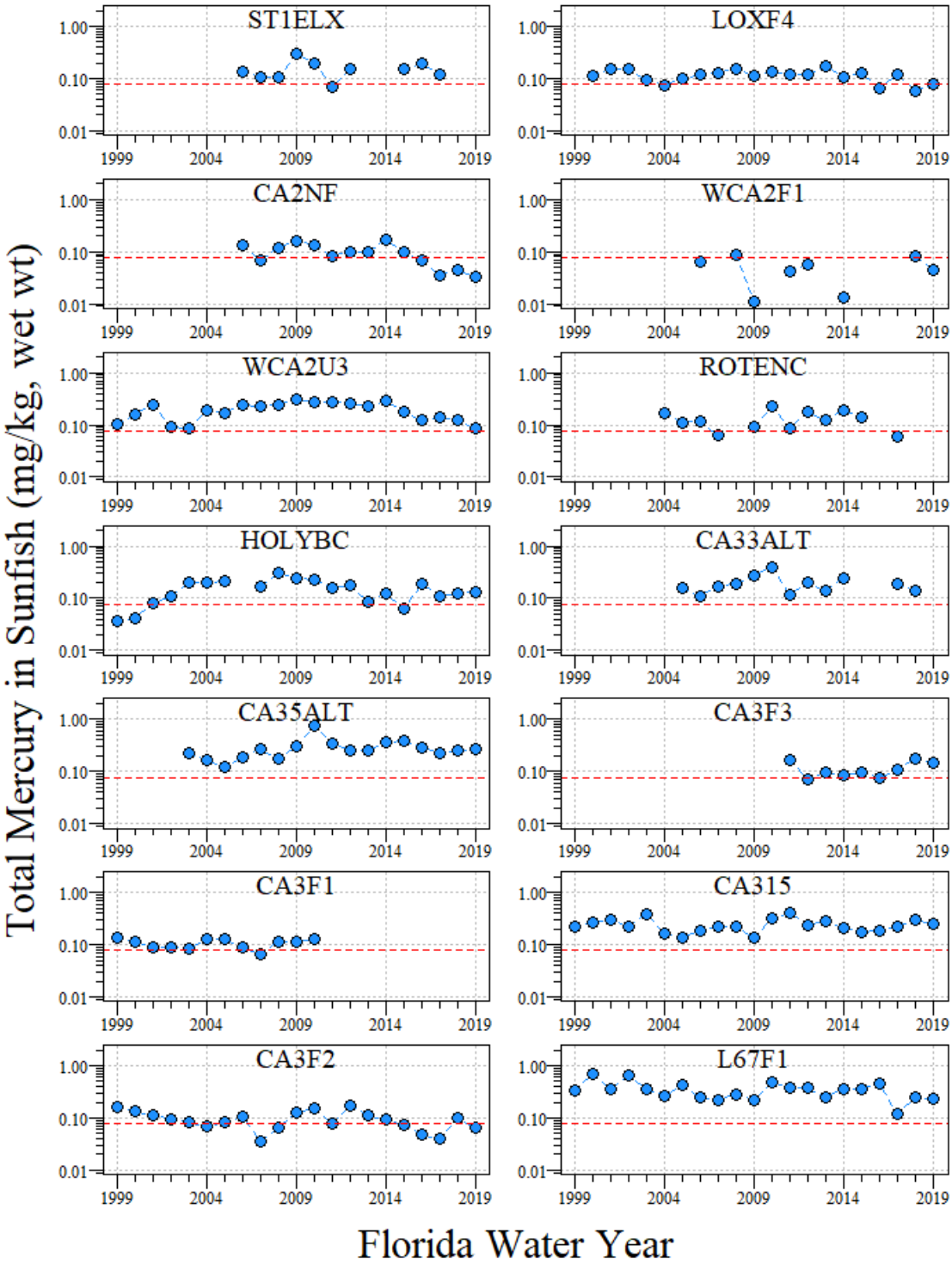


**Figure 3B-7.** Annual mean THg concentration in mg/Kg wet weight (wt) in TL3 sunfish whole body samples at each monitoring site in the EPA for WY1999–WY2019.

During WY2019, the mean THg concentration in the TL3 sunfish species from 10 of the 13 active monitoring sites with data available ranged from 0.033 mg/kg at CA2NF to 0.268 mg/kg at CA33ALT. The WY2019 overall average was 0.134 mg/kg, which represents a 10% decrease compared to WY2018 (0.150 mg/kg).

Median THg concentration varied significantly between sunfish species during WY2019 ($H = 10.3$, $df = 2$, $p = 0.006$). Throughout the entire POR all three species (annual mean) statistically differed in THg concentrations ($H = 36.35$, $df = 2$, $p < 0.001$) with the highest median THg concentration in spotted sunfish (0.236 mg/kg), followed by bluegill (0.193 mg/kg), and redear sunfish (0.116 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).

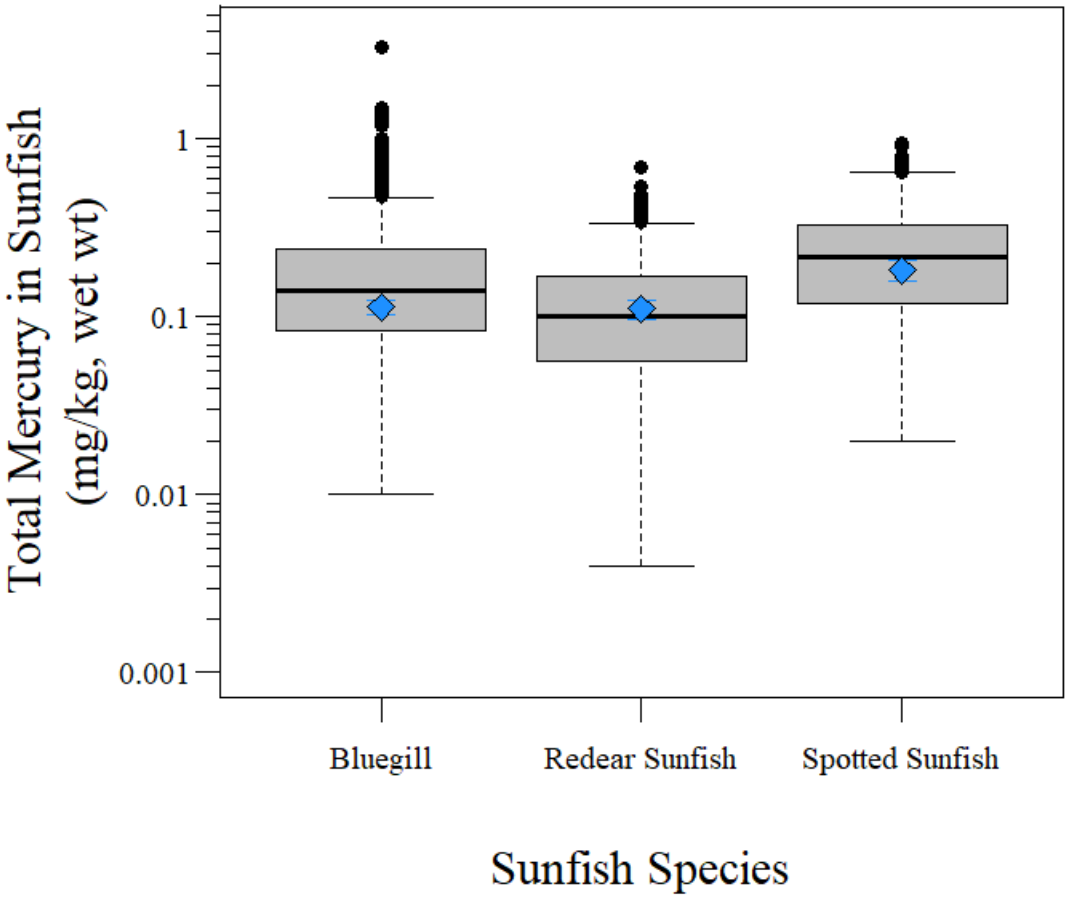


**Figure 3B-8**. Comparison of TL3 sunfish species collected in the EPA during WY1999–WY2019. Blue diamonds indicate WY2019 mean (± standard error) THg concentrations for each species.

### *Largemouth Bass*

Largemouth bass (LMB) axial tissue fillet samples have been collected across the EPA for THg analysis since WY1999. During WY2019, no fish were collected from 4 of the 13 monitoring stations. Average THg concentrations in LMB ranged from 0.187 mg/kg at site CA2NF (WCA-2) to 1.132 mg/kg at site L67F1 (ENP), with an overall WY2019 average ( ± SE) of 0.503 ± 0.103 mg/kg. This represents a 8% increase in the reported average for WY2018 (0.464 ± 0.073 mg/kg). Generally, LMB THg concentrations follow a strong north-to-south gradient with concentrations being lower in LNWR 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 by altering the Hg availability, biotic turnover rate, biotic uptake rate, food web dynamics, etc. (Julian and Gu 2015).

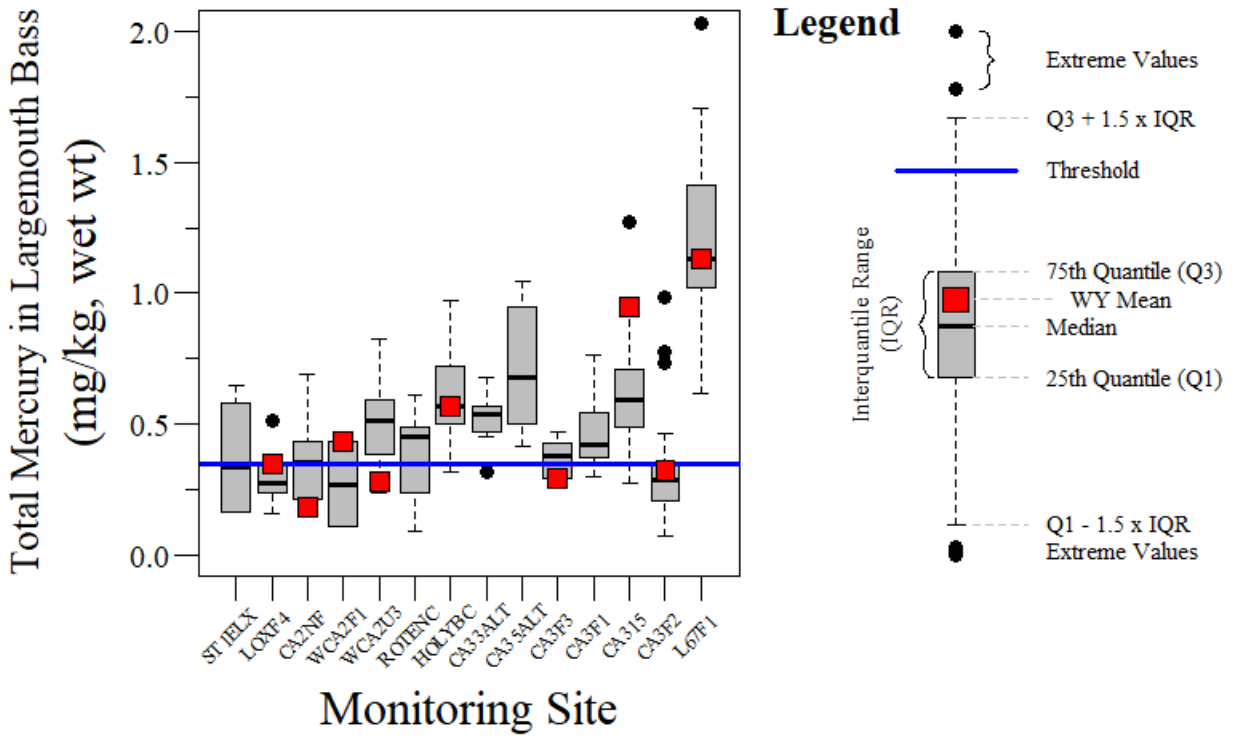


**Figure 3B-9.** Box plots of THg concentrations in mg/kg wet weight (wt) in LMB at each monitoring site during WY1999–WY2019. Red boxes indicate WY2019 mean THg concentrations and the blue line denotes the 0.300 mg/kg USEPA MeHg recommended criterion for protection of human health. Site CA3F1 was replaced by CA3F3 in WY2011 and is no longer monitored. Sites ST1ELX, CA33ALT, CA35ALT, and ROTENC were not sampled in WY2019 due to lack of fish and access issues.

During WY2019, 4 of the 9 monitoring stations with data in the region had average THg concentration above the USEPA recommended MeHg criterion for the protection of human health (0.3 mg/kg) (USEPA 2001) (**Figure 3B-9**). Exceedance rates of the recommended criterion in WY2019 is 63% which is 3% higher than the POR (60%) and 18% higher than WY2018 (**Figure 3B-10**). 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.

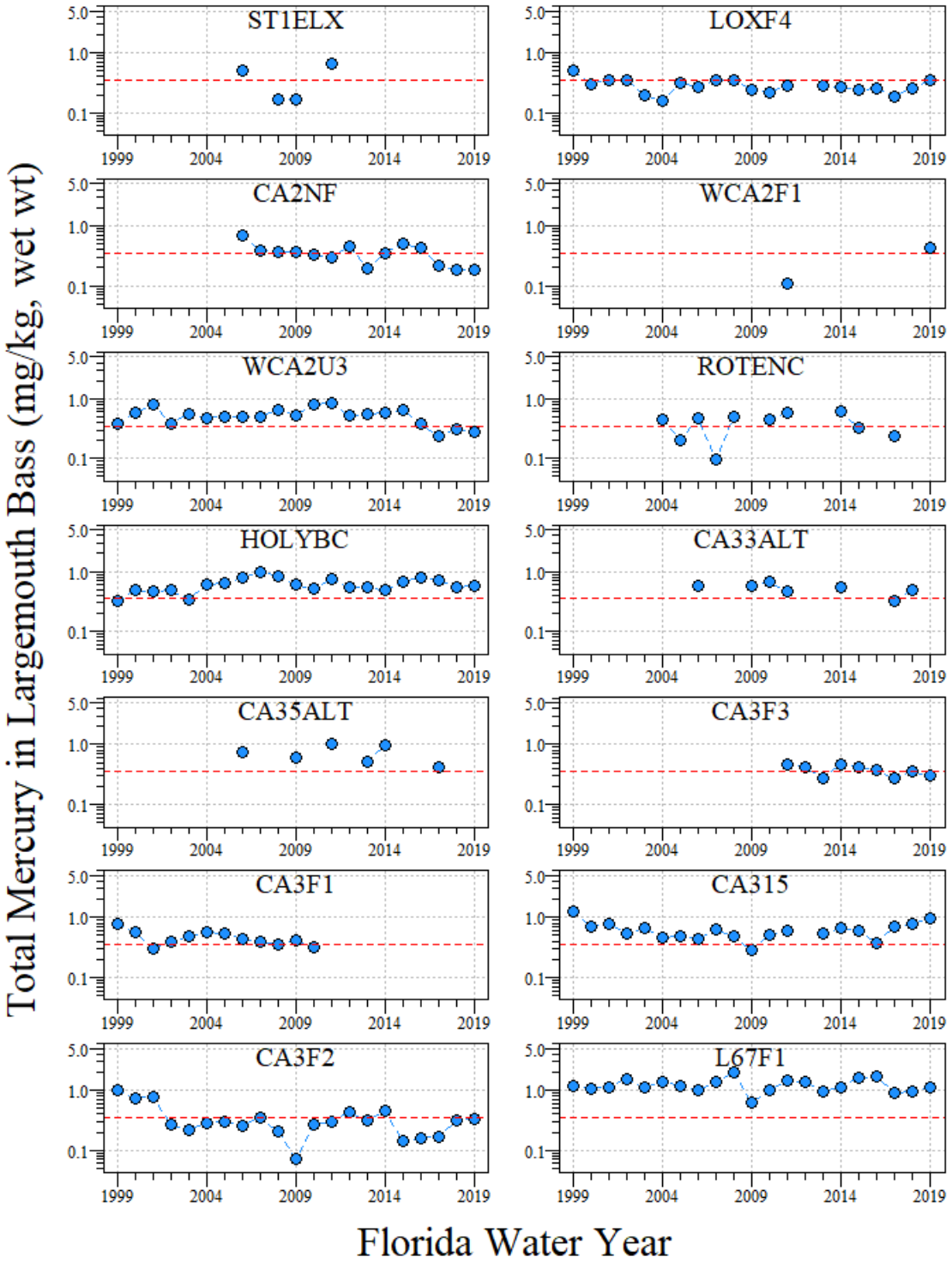


**Figure 3B-10.** Annual mean THg concentrations in mg/kg wet weight (wt) in LMB axial fillet samples at each monitoring site in the EPA for WY1999–WY2019. Red dashed line denotes the 0.300 mg/kg USEPA MeHg recommended criterion for protection of human health.

Throughout the POR (WY1999–WY2019), station HOLYBC maintained a significant increasing trend in annual median THg concentration. Meanwhile one station CA3F1/F3 (WCA-3) exhibit decreasing trends in LMB THg tissue concentration (**Table 3B-3**). 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).

**Table 3B-3.** Kendall trend analysis of LMB annual median THg concentration at the 13 active monitoring locations within the EPA for WY1999–WY2019.

| Area | Station | Kendall's τ | p-value | Sample Size | Trend Direction |
|---|---|---|---|---|---|
| WCA-1 | ST1ELX | 0.00 | 1.00 | 4 | Not statistically significant |
| | LOXF4 | -0.21 | 0.19 | 20 | Not statistically significant |
| WCA-2 | CA2NF | -0.39 | 0.06 | 14 | Not statistically significant |
| | WCA2F1 | --- | --- | --- | Not enough data |
| | WCA2U3 | -0.13 | 0.42 | 21 | 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 | -0.42 | <0.01 | 21 | Decrease |
| | CA315 | 0.12 | 0.50 | 20 | Not statistically significant |
| | CA3F2 | -0.18 | 0.25 | 21 | Not statistically significant |
| ENP | L67F1 | -0.03 | 0.83 | 21 | Not statistically significant |
| WMAs | ROTENC | 0.20 | 0.48 | 10 | Not statistically significant |
| | HOLYBC | 0.31 | <0.05 | 21 | Increase |

### *Summary*

Over the entire POR, the average mosquitofish THg concentration (0.066 mg/kg) has been below the federal recommended criterion for wildlife protection. For WY2019, mosquitofish THg concentrations exceeded the federal recommended criterion of 0.077 mg/kg MeHg for TL3 fish at 1 of the 13 active monitoring sites. POR THg concentration for TL3 sunfish (0.166 mg/kg) and LMB (0.549 mg/kg) has been above the wildlife and human health protection recommended criterion, respectively. During WY2019 sunfish THg concentrations exceeded the USEPA recommended criterion in 7 of the 10 sampled monitoring sites while LMB THg concentration exceeded the recommend USEPA criterion in 4 of the 9 (of the 13 active stations) sampled monitoring sites. Several sites experienced THg concentration declines in POR annual mosquitofish THg concentrations (sites LOXF4, CA315, and CA3F2), and two sites experienced significantly decreasing trends in THg concentrations throughout the POR for sunfish (CA2NF and CA3F2). For LMB, one site (CA3F1/F3) exhibited a statistically significant declining trend, while one site (HOLYBC) showed an increasing trend in annual median LMB THg concentration over the period of record.

Whether THg concentrations in fish are remaining constant over the past decade, as reported in previous SFERs, or whether there are recent increases or decreases in fish tissue THg concentrations, Hg bioaccumulation continues to be a significant water quality issue within the EPA. 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).

# MERCURY BIOGEOCHEMISTRY

## THE ABUNDANCE AND DIVERSITY OF MERCURY METHYLATING ASSEMBLAGES IN THE FLORIDA EVERGLADES PROTECTION AREA

Hee-Sung Bae[3], Andy Ogram[3], Forrest E. Dierberg[4], Mike Jerauld[4], and Thomas DeBusk[4]

### Introduction

The methylation of inorganic mercury ($Hg^{2+}$) to the potent neurotoxin MeHg ($CH_3Hg^+$) in the Everglades and other affected environments remains a challenge for research scientists and policy makers. Microorganisms are the prime agents responsible for mercury methylation. Recent studies using *hgcAB*, which are essential genes for mercury methylation (Parks et al. 2013), as a biomarker have revealed that the potential for mercury methylation is spread much more broadly across more diverse microbial taxa than previously thought (Bae et al. 2014, Liu et al. 2014, Schaefer et al. 2014, Christensen et al. 2016, Bravo et al. 2018).

Among ecological compartments in the Everglades, periphyton, floc, and the underlying soil (peat or muck) have been identified as important in producing MeHg (Cleckner et al. 1999, Liu et al. 2008, 2009, Li et al. 2012). Little is known about the temporal and spatial variability of potential mercury methylating microorganisms that dwell within these compartments in the Everglades. Given the differences in phosphorus (P), nitrogen (N), carbon (C), and sulfur (S) in both insoluble and soluble forms within these compartments, variation among microbial clades harboring *hgcAB* would be expected. To test this hypothesis, we explored the assemblage structures of organisms carrying *hgcAB* in the three compartments (periphyton, floc, and soil) within well characterized regions of the Everglades that represented different nutrient and sulfate statuses.

### Site Description and Methods

Four sites were selected (**Figure 3B-11**). See Bae et al. (2019) for sampling frequency and numbers for soil, floc, and periphyton within each of the four sites. Sites F2 and U3 are both sulfate-rich regions from the discharge of drainage waters from EAA into WCA-2A, while sites DB14 and DB15 are located in the interior of WCA-3A and represent low sulfate regions. Sites U3, DB14, and DB15 are in relatively P-unimpacted ridge and slough habitats. On the other hand, due to its proximity to a major canal that discharged EAA water into WCA-2A, site F2 is in a dense cattail monoculture and the floc, periphyton, and soil there contain higher concentrations of P than the other three sites (Bae et al. 2019).

Detailed descriptions of deoxyribonucleic acid (DNA) isolation, design of the primers for amplifying *hgcAB* genes, polymerase chain reaction (PCR), quantitative PCR (qPCR), and sequence analysis of *hgcAB*, as well as the analytical methods for MeHg, sulfate, sulfide, phosphorus, nitrogen, and total organic carbon, can be found in Bae et al. (2019).

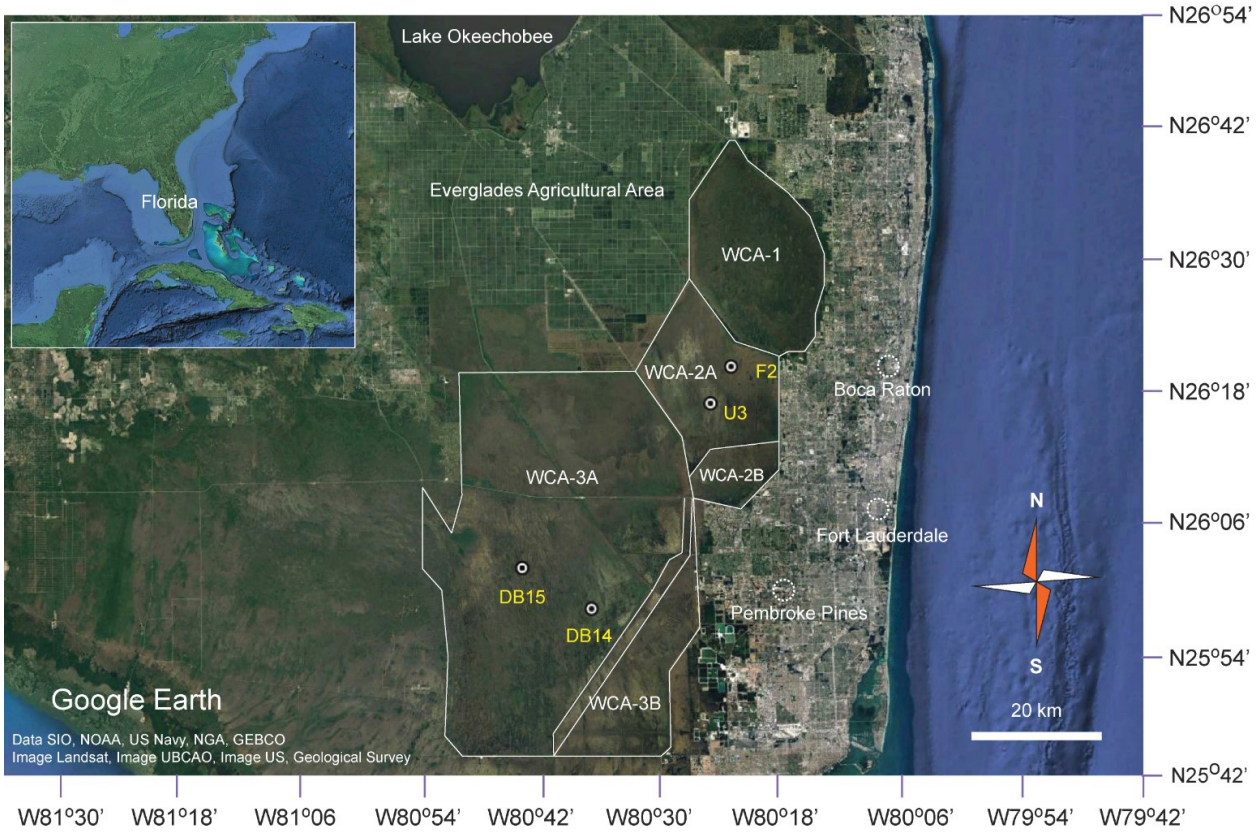


**Figure 3B-11.** Map presenting the study sites F2 and U3 within WCA-2A, and DB14 and DB15 within WCA-3A. Reprinted with permission from Bae et al. (2019).

## Results and Discussion

*hgcAB* was carried by a diverse assemblage of microbes (**Figure 3B-12**). In all three ecological compartments, copies of *hgcAB* were primarily recovered from methanogenic and syntrophic clades. Syntrophs can optionally metabolize sulfate, but they are also capable of establishing mutually beneficial relationships with other groups of microorganisms to grow without sulfate. Methanogens do not use sulfate. Interestingly, organisms from the family of "classic" mercury-methylators, obligate sulfate-reducing bacteria such as Desulfovibrionales, were essentially absent at these Everglades sites, accounting for no more than 4% of the total number of *hgcAB* copies.

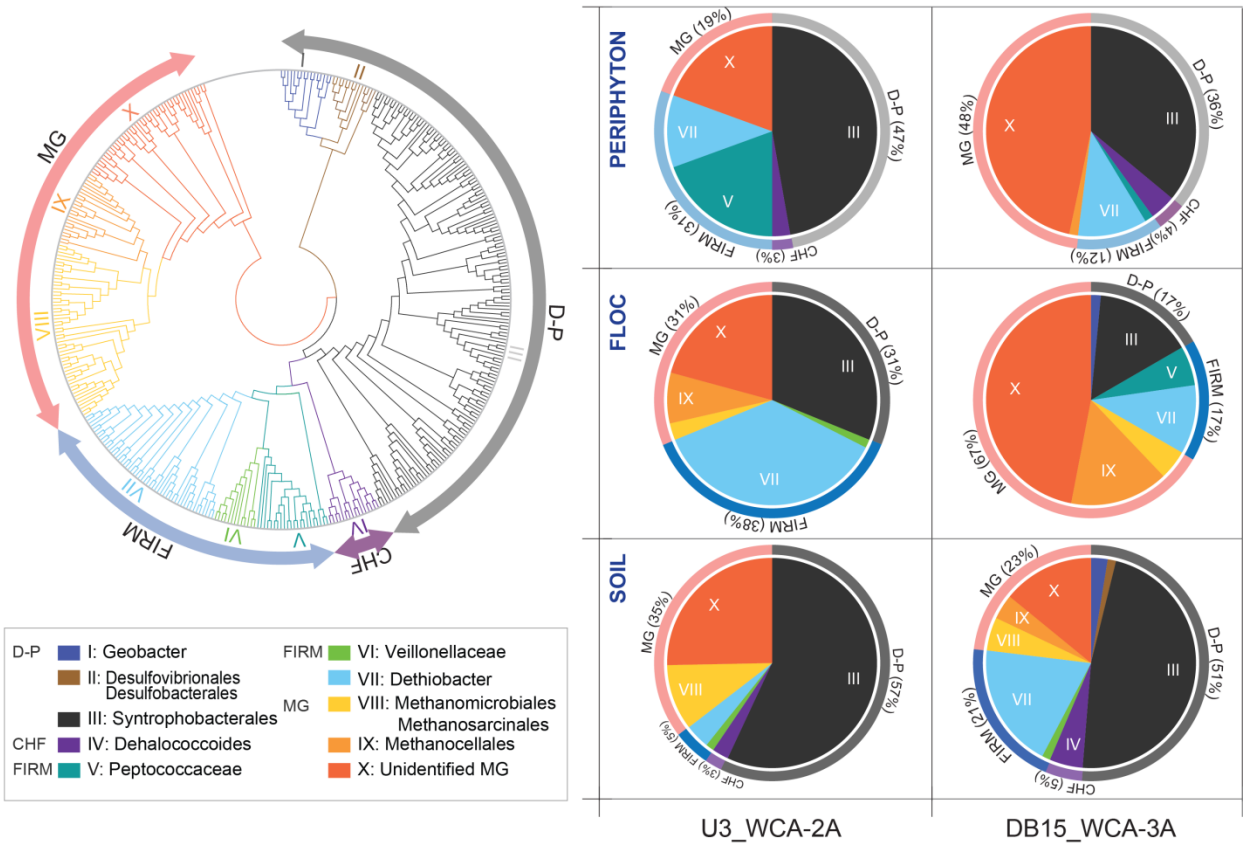


**Figure 3B-12.** Phylogeny of mercury methylators detected using *hgcAB* as a gene marker from periphyton, floc, and peat. The phylogeny was determined by maximum likelihood constructed using HgcAB protein sequences deduced from 774 *hgcAB* sequences collected in the Everglades samples through our previous (220 sequences) and current (554 sequences) studies (left panel). Pie graphs represent the composition of major taxa in the ecological compartments of the sulfate-enriched site U3 within WCA-2A and the sulfate-depleted site DB15 within WCA-3A (right panel). (Reprinted with permission from Bae et al. [2019]).

The abundances of mercury methylators (based on copy numbers of *hgcAB*) were highly variable among compartments and geographical locations at the four sites, although some trends were observed. Among the compartments, the lowest abundance occurred in the periphyton; floc and soil had similar abundances. Among sites, F2 had the highest abundance in floc and soil. The abundance of *hcaAB*-positive organisms of all dominant clades (denoted by *hgcAB_SYN*, *hgcAB_MGxa,b,*c, and *hgcAB_total* in **Figure 3B-13**) was strongly positively correlated with concentrations of carbon, nitrogen, and phosphorous (**Figure 3B-13**), suggesting that the concentrations of substrates and nutrients affect the quantity of *hgcAB* at these sites. However, these trends were not in agreement with the MeHg concentration, which tended to be higher in periphyton than in soil and to be lower at F2 than sites DB14 and DB15 within WCA-3A (**Figure 3B-14**). Since the numbers of *hgcAB* may not be correlated directly with mercury methylation activity or concentrations (Christensen et al. 2019), the final concentrations of MeHg were likely to result from a combination of complex abiotic factors (e.g., MeHg transport and bioavailability of $Hg^{2+}$) and biological (e.g., demethylation) processes in the mercury cycle, both of which have been shown to occur in the Everglades (Marvin-Dipasquale and Oremland 1998, Benoit et al. 2001, Drexel et al. 2002, Haitzer et al. 2002, Li et al. 2012, Poulin et al. 2017). We also note that the body of research on the *hgcAB*-carrying organisms identified here for the Everglades, while growing (e.g. Gilmour et al 2018, Yu et al 2018), is much smaller than compared to the better known, "classic" methylating sulfate-reducing prokaryote (e.g., Gilmour et al. 2011).

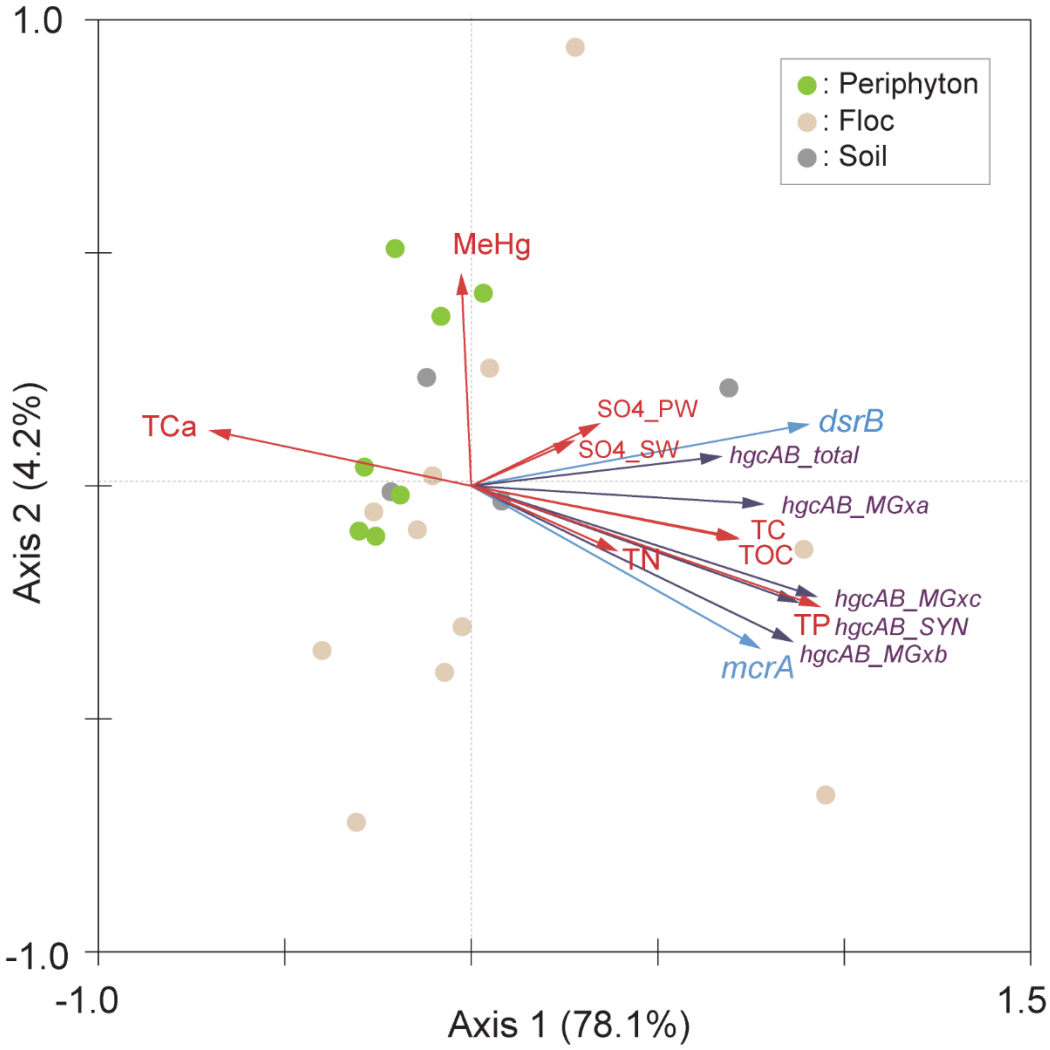


**Figure 3B-13.** Redundancy analysis (RDA) plot representing the relationship between gene copies of *hgcAB*, *dsrB*, and *mcrA*, geochemical parameters, and MeHg concentrations determined for all four sites. Arrows pointing in the same direction indicate positive correlations, and arrows pointing in the opposite direction indicate negative correlations. The arrow length corresponds to the variance explained by the environmental variable. The first two axes explain 82.3% of the total canonical eigenvalues with a significant Monte-Carlo test value ($p < 0.001$). (Note: SW – surface water; PW – pore water; $SO_4$ – sulfate; *hgcAB_SYN* – mercury methylators belonging to the *Syntrophobacteriales*; *hgcAB_MGxa,b,c* = mercury methylators within three groups (a,b, or c) of methanogens. (Reprinted with permission from Bae et al. [2019]).

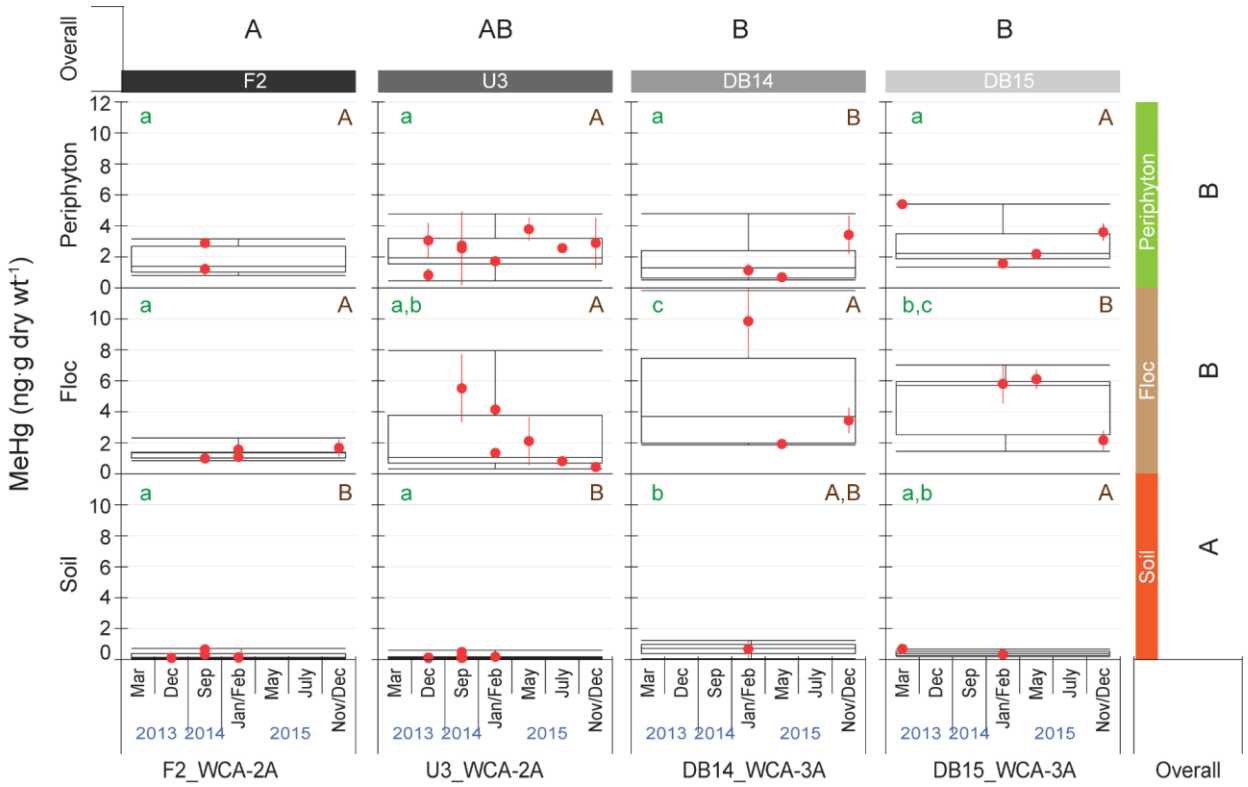


**Figure 3B-14.** MeHg concentrations in periphyton, floc, and soil across sites F2 and U3 within WCA-2A, and DB14 and DB15 within WCA-3A. Error bars in each data point (circle symbol) represent ±1 standard deviation (n ≥ 3). Box-and-whisker plots were generated from the pooled data obtained from each compartment at individual sites. Boxes show the medians (horizontal lines in the boxes) and the lower and upper quartiles (bottoms and tops of the boxes, respectively). The vertical bars (whiskers) on the box plots represent the maximum and minimum values, excluding outliers. Significant differences ($p \leq 0.05$ by the Tukey-Kramer honest significant difference [HSD] test) between compartments within each site and between sites within each compartment are denoted on top at right and left, respectively, on each plot with different letters. Significant differences between sites and between compartments are also denoted on the top and right sides of this figure, respectively. (Reprinted with permission from Bae et al. [2019]).

The numbers of *hgcAB* carried by the *Syntrophobacteriales*, and *hgcAB* carried by methanogen populations, were nearly equivalent to the numbers of *dsrB* and *mcrA*, respectively, which are gene markers for the total sulfate-reducing prokaryote and methanogen populations (each gene marker includes both methylating and non-methylating taxa). This indicates that *hgcAB* is widespread within each of these clades, and further, is therefore relatively common in the overall microbial assemblage since sulfate-reducing prokaryotes and methanogens can be dominant groups in much of the Everglades (Entry et al. 2015). Significantly, a previous study (Bae et al. 2014) indicated that most Everglades sulfate-reducing prokaryote belong to the syntrophs, which is consistent with the correspondence between the number of *hgcAB* copies carried by syntrophs and the total number of *dsrB* copies carried by the sulfate-reducing prokaryote population observed in the current study.

A compelling reason to identify the taxonomic affiliation of mercury methylators is to gain insight into the environmental controls on their activities, and hence on mercury methylation rates. For the last few decades, sulfate has been considered a key factor in mercury methylation (Gilmour et al. 1992) because sulfate-reducing prokaryotes have been thought to be primary mercury methylators in many environments (Compeau and Bartha 1985). Our analyses revealed that the *hgcAB* gene distributions were negatively correlated with sulfate concentrations when the sulfate- and P-enriched F2 site was excluded, even though

the relatively high-sulfate U3 site was included (**Figure 3B-15**). These results suggest that the impact of the sulfate on the *hgcAB* gene distribution would be site (or geographic location) specific and largely reliant on other environmental factors, such as carbon substrates and nutrients when sulfate concentrations are relatively low.

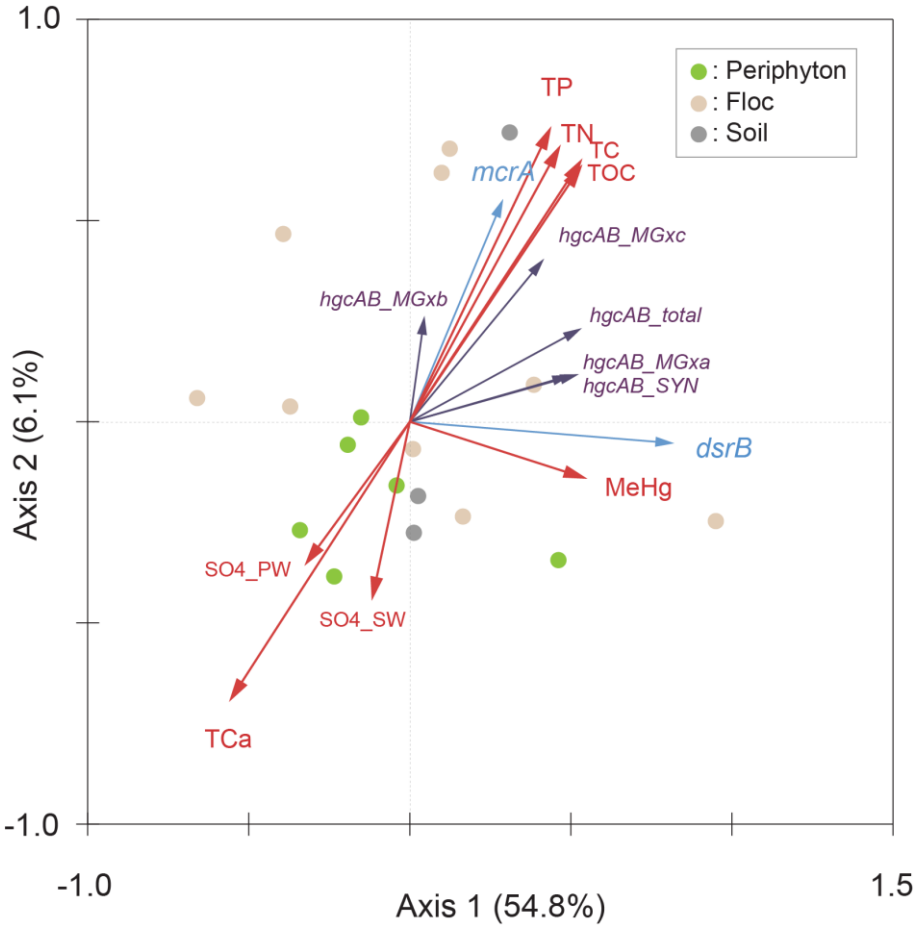


**Figure 3B-15.** Redundancy analysis (RDA) plot representing the relationship between gene copies of *hgcAB*, *dsrB* and *mcrA*, geochemical parameters, and methylmercury (MeHg) concentrations excluding F2 data. Arrows pointing in the same direction indicate positive correlations, and arrows pointing in opposite direction indicate negative correlations. The arrow length corresponds to the variance explained by the environmental variable. The first two axes explain 60.9% of the total canonical eigenvalues. (Note: SW – surface water; PW – pore water; $SO_4$ – sulfate; *hgcAB_SYN* – mercury methylators belonging to the *Syntrophobacteriales*; *hgcAB_MGxa,b,c* – mercury methylators within three groups (a,b,c) of methanogens.) (Reprinted with permission from Bae et al. [2019].)

A conclusion from this work is that different levels of sulfate, along with available C and nutrient concentrations, contribute to the structure and abundance of the assemblages of *hgcAB*-carriers within environmental compartments, which are widely spread across the Everglades ecosystem. The dominant *hgcAB*-positive organisms in periphyton, floc, and soil included methanogens and *Syntrophobacteriales*, which point to fermentative and syntrophic relationships and processes, and not solely to sulfate respiration by sulfate-reducing prokaryotes, controlling mercury methylation in the Everglades. Our finding for the Everglades, first reported by Bae et al. (2014), is consistent with more recent studies from other environments (Gilmour et al. 2018, Yu et al. 2018).

# SULFUR SOURCES TO THE EVERGLADES

## SULFATE WITHIN THE EVERGLADES PROTECTION AREA

Paul Julian II[1] and Alyssa Gilhooly[1]

### Introduction

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 sulfate-reducing bacteria (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). However, as described above and elsewhere, other microbial guilds have the potential to methylate Hg independent of sulfate concentrations. 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, soil microbial community 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 WY2019 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 Everglades Protection Area*

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 WY2019 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 (WY1979–WY1993), (2) intermediate period, or Phase I (WY1994–WY2004), (3) Phase II best management practices (BMP)/STA implementation period after WY2004 (WY2005–WY2018), and (4) the current water year (WY2019).

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 Long-Term Plan for Achieving Water Quality Goals in the Everglades Protection Area Tributary Basins (Burns and McDonnell 2003) and the Comprehensive Everglades Restoration Plan (CERP; USACE and SFWMD 1999). 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, WY2019) 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), a 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 $\tau$ 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 water year (WY2019) are shown in **Table 3B-6**.

Sulfate concentrations follow a general north-to-south gradient (**Table 3B-6** and **Figure 3B-16**) at inflow locations similar to other nutrients assessed elsewhere in this report. Annual mean sulfate concentrations observed at inflow regions to the EPA during WY2019 range from 59.3 mg/L (LNWR) to 11.7 mg/L (ENP). 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 the implementation of Phase I (**Table 3B-6**). For inflows to ENP, annual mean and median sulfate concentrations increased slightly in WY2019 over the Phase II period, but across the POR, annual average sulfate concentrations have significantly declined in inflows to all areas of the EPA (**Table 3B-7**). In interior locations, the north-to-south gradient is strongest between WCA-2 and ENP with LNWR generally having lower sulfate concentrations due to the soft water (low mineral) nature of this compartment. During WY2019, annual mean sulfate concentrations in interior regions ranged from 45.3 mg/L in WCA-2 to 1.1 mg/L in ENP (**Table 3B-6**).

**Table 3B-6**. Summary statistics of sulfate concentrations in mg/L for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), Phase II (WY2005–WY2018), and WY2019 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 |
| | | 2005-2018 | 663 | 51.8 | 20.8 | 47.4 | 1.6 | 49.3 | 10.0 | 132.0 |
| | | 2019 | 75 | 59.3 | 21.9 | 55.2 | 1.5 | 58.0 | 19.9 | 125.0 |
| | 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 |
| | | 2005-2018 | 3,368 | 5.5 | 12.1 | 0.6 | 9.7 | 0.5 | 0.1 | 95.1 |
| | | 2019 | 294 | 5.3 | 12.1 | 0.5 | 10.7 | 0.3 | 0.1 | 71.9 |
| | 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 |
| | | 2005-2018 | 205 | 30.5 | 19.3 | 23.2 | 2.3 | 26.6 | 1.4 | 85.4 |
| | | 2019 | 23 | 31.5 | 15.5 | 24.0 | 2.8 | 30.0 | 1.2 | 56.8 |
| | 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 |
| | | 2005-2018 | 381 | 49.1 | 21.6 | 43.8 | 1.8 | 45.3 | 0.2 | 185.0 |
| | | 2019 | 48 | 52.3 | 20.1 | 48.1 | 1.5 | 49.7 | 16.9 | 91.1 |
| 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 |
| | | 2005-2018 | 668 | 46.1 | 17.4 | 41.3 | 1.8 | 46.1 | 0.1 | 106.0 |
| | | 2019 | 78 | 42.9 | 16.6 | 37.9 | 1.9 | 39.0 | 1.2 | 94.3 |
| | 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 |
| | | 2005-2018 | 1,965 | 43.4 | 17.8 | 38.4 | 1.8 | 44.4 | 0.1 | 128.0 |
| | | 2019 | 169 | 45.3 | 13.8 | 42.8 | 1.4 | 47.3 | 9.3 | 89.6 |
| | 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 |
| | | 2005-2018 | 491 | 28.0 | 15.1 | 23.6 | 1.9 | 26.1 | 3.9 | 74.7 |
| | | 2019 | 44 | 25.6 | 10.3 | 23.4 | 1.6 | 26.5 | 8.9 | 49.3 |
| 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 |
| | | 2005-2018 | 1,126 | 15.6 | 17.9 | 5.9 | 5.4 | 5.3 | 0.1 | 74.7 |
| | | 2019 | 148 | 11.9 | 15.9 | 3.8 | 5.0 | 2.6 | 0.2 | 70.8 |
| | Interior | 1979-1993 | 450 | 14.9 | 17.3 | 10.5 | 2.3 | 10.7 | 2.0 | 261.5 |
| | | 1994-2004 | 1620 | 10.8 | 34.8 | 3.8 | 5.3 | 4.5 | 0.1 | 1,300.0 |
| | | 2005-2018 | 1439 | 12.8 | 15.3 | 3.3 | 8.7 | 5.0 | 0.1 | 126.0 |
| | | 2019 | 72 | 13.4 | 13.3 | 4.5 | 7.6 | 7.8 | 0.1 | 43.5 |
| | 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 |
| | | 2005-2018 | 223 | 9.0 | 8.0 | 3.3 | 7.7 | 8.8 | 0.1 | 39.3 |
| | | 2019 | 56 | 11.9 | 8.8 | 5.6 | 6.7 | 11.8 | 0.1 | 45.9 |
| 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 |
| | | 2005-2018 | 265 | 8.2 | 6.7 | 4.2 | 4.8 | 7.4 | 0.1 | 35.8 |
| | | 2019 | 60 | 11.7 | 8.5 | 6.7 | 4.6 | 11.8 | 0.1 | 45.9 |
| | 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 |
| | | 2005-2018 | 927 | 4.9 | 19.3 | 0.9 | 6.5 | 1.2 | 0.1 | 242.0 |
| | | 2019 | 53 | 1.1 | 1.2 | 0.5 | 4.5 | 0.7 | 0.1 | 4.4 |

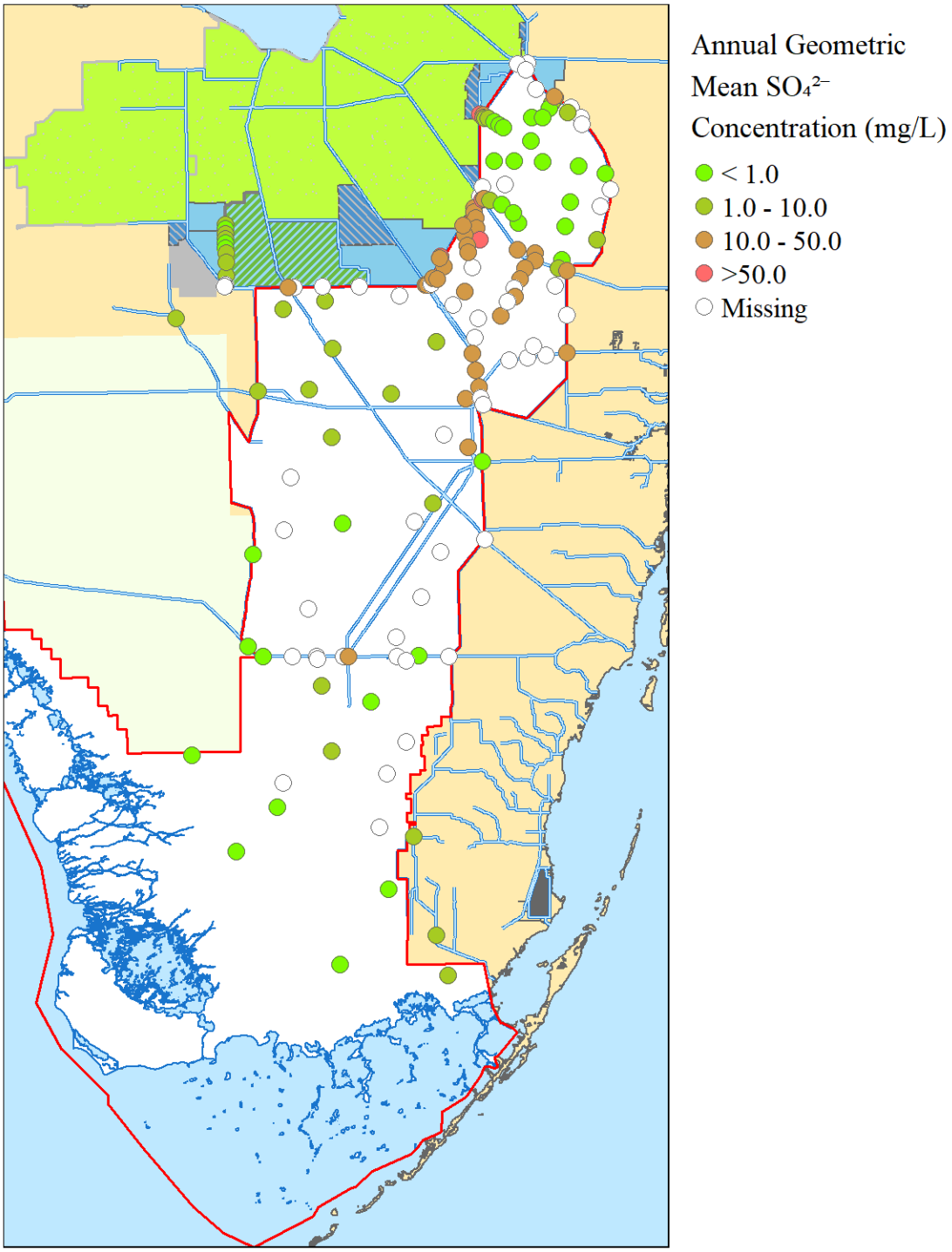


**Figure 3B-16**. Annual geometric mean sulfate ($SO_4^{2-}$) concentrations for all classifications at stations across the EPA in WY2019.

**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–WY2019) and the period of WY2005 to present.

| | | POR (WY1979–WY2019) | | | Phase II & Current Water Year (WY2005–WY2019) | | |
|---|---|---|---|---|---|---|---|
| **Area** | **Class** | **Kendall's τ** | **p-value** | **Sen's Slope Estimate [a]** | **Kendall's τ** | **p-value** | **Sen's Slope Estimate [a]** |
| LNWR | Inflow | -0.29 | <0.05 | -0.65 | 0.18 | 0.38 | 0.66 |
| | Interior | -0.35 | <0.01 | -0.22 | 0.09 | 0.70 | 0.18 |
| WCA-2 | Inflow | -0.24 | <0.05 | -0.40 | -0.61 | <0.01 | -1.07 |
| | Interior | 0.21 | 0.07 | 0.15 | -0.22 | 0.28 | -0.49 |
| WCA-3 | Inflow | -0.49 | <0.01 | -0.54 | -0.49 | <0.05 | -1.26 |
| | Interior | -0.11 | 0.38 | -0.04 | -0.20 | 0.33 | -0.21 |
| ENP | Inflow | -0.25 | <0.05 | -0.18 | 0.27 | 0.17 | 0.14 |
| | Interior | -0.37 | <0.01 | -0.14 | -0.50 | <0.05 | -0.35 |

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

Some annual trends are more pronounced than others, as shown in **Figure 3B-17**. The annual mean sulfate concentrations entering the LNWR appear elevated despite a significantly declining trend throughout the POR (**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.

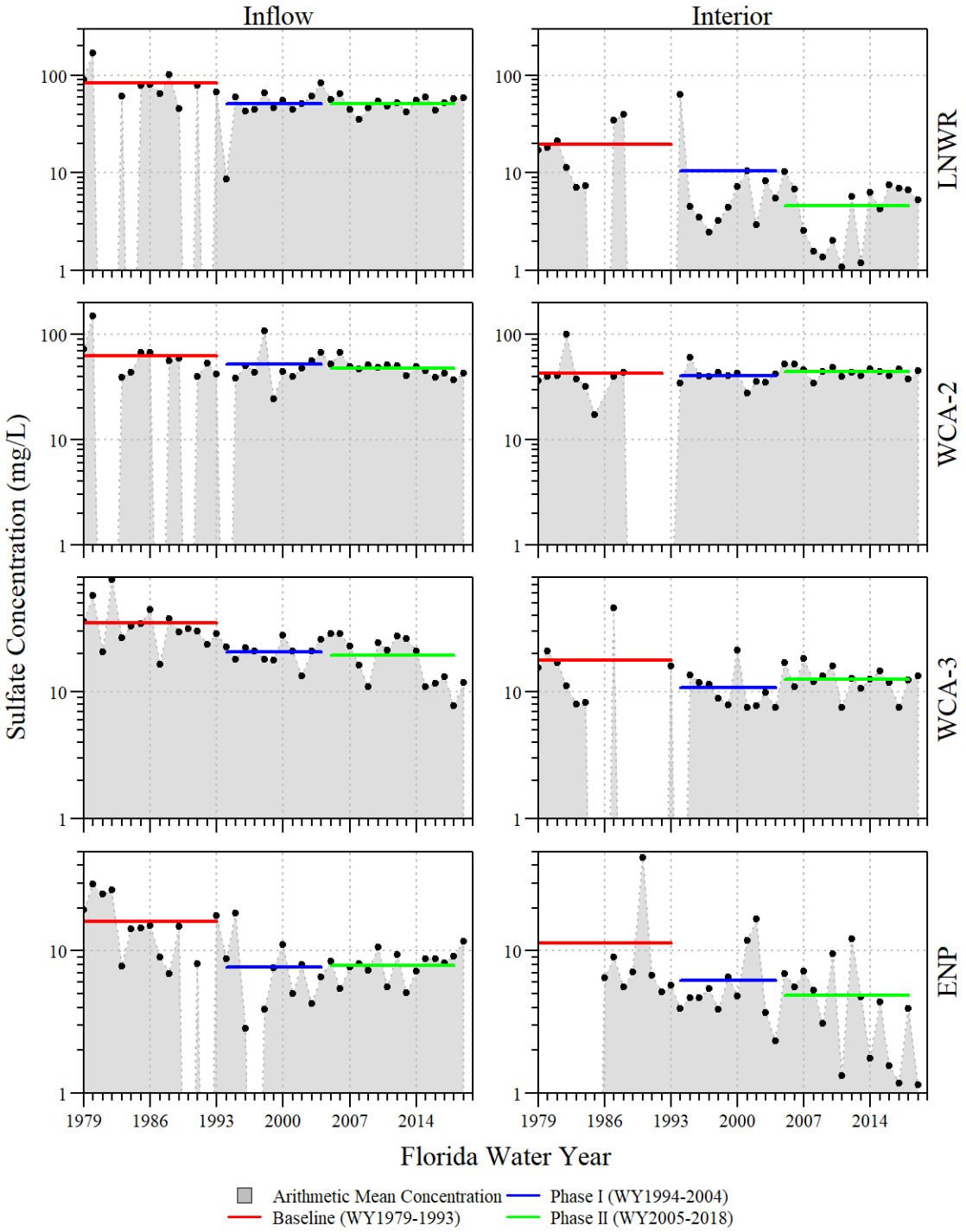


**Figure 3B-17.** Annual arithmetic mean sulfate concentrations for inflow (left panel) and interior (right panel) to areas of the LNWR, WCA-2, WCA-3, and ENP during WY1979–WY2018. Bars indicate arithmetic mean when flowing for inflow locations. The horizontal lines indicate the mean annual geometric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

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 WY2019, WCA-2 interior had the highest annual mean sulfate concentration of 45.3 mg/L, followed by WCA-3 (13.3 mg/L), LNWR (5.3 mg/L), and ENP (1.1 mg/L). Across the POR (WY1979–WY2019), LNWR and ENP interior 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–WY2019), 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 (Landing 2014). 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 (see 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 two to three times that of other regions within the EPA. Historical EAA runoff containing both local and regional inputs of sulfate and a prolonged legacy effect is suspected in causing these internal sulfate dynamics. The hydrology of WCA-2A spreads the canal inflow broadly, and WCA-2 soils have relatively high nutrient concentrations including sulfur. Soil samples from across the EPA confirm high sulfur concentrations in WCA-2A. Additional soil samples collected along the impacted gradient within WCA-2A indicate that soil sulfur concentrations have not qualitatively changed much over the last decade suggesting a long residence time pool of sulfur in the system (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, Orem et al. 2011, Gabriel et al. 2014) have suggested the need to develop a site-specific water quality criterion 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 scientifically support 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. It is therefore not possible at this time to define a numeric limit that will protect the designated uses of the EPA as required by the Clean Water Act to support defensible surface water quality criteria.

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 aquatic life and wildlife in the EPA.

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