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

Edited by Paul Julian II[1], Binhe Gu, Ken Weaver[2]

Contributions by Mike Jerauld[3], Forrest E. Dierberg[3], Thomas A. DeBusk[3], Janelle A.Potts[3], and Nichole R. Larson[3,4]

SUMMARY

This chapter provides an assessment of sulfur (S) and mercury (Hg) status within the Everglades Protection Area (EPA), Holey Land and Rotenberger wildlife management areas (WMAs) during the Water Year 2016 (WY2016) (May 1, 2015–April 30, 2016) reporting period. 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 *2016 South Florida Environmental Report* (SFER) – *Volume I* (Julian et al. 2016).

The analysis and summaries provide a synoptic view of Hg and S 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 Hg and S monitoring in the Everglades region and summarizes Hg concentrations in biota; Hg atmospheric deposition; and surface water sulfate (SO_4^{2-}) concentrations, loads, and atmospheric deposition to the EPA. Analytical data are reported for the following timeframes: WY2016 for wildlife—Hg in fish tissue for largemouth bass (LMB; *Micropterus salmoides*), sunfish (*Lepomis* spp.), and mosquitofish (*Gambusia* spp.)—and for surface water SO_4^{2-}; and WY2015 for atmospheric deposition of SO_4^{2-} (wet + dry) and Hg (wet).

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

- WY2016 total mercury (THg) concentrations in mosquitofish from 13 monitoring sites ranged from 0.013 milligram per kilogram (mg/kg) at site HOLYBC to 0.088 mg/kg at site ROTENC, with a median value of 0.036 mg/kg, which is 10 percent lower than the reported median value of WY2015. Mosquitofish THg concentrations during WY2016 exceeded the United States Environmental Protection Agency (USEPA) trophic level 3 (TL3) criterion (0.077 mg/kg) for protection of piscivorous wildlife at one of the 13 sites (ROTENC).

[1]Florida Department of Environmental Protection, Office of Ecosystems Projects, Fort Myers, FL

[2]Florida Department of Environmental Protection, Division of Ecosystem Assessment and Restoration, Tallahassee, FL

[3]DB Environmental, Rockledge, FL

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- In WY2016, THg concentrations in TL3 sunfish from 13 monitoring sites ranged from 0.049 mg/kg at CA3F2 to 0.455 mg/kg at L67F1. Ten stations were sampled for sunfish species; mean sunfish THg concentrations exceeded the USEPA protection of wildlife criterion at 6 stations. THg concentrations in these species from all sites were highest in bluegill (*Lepomis macrochirus*: 174 mg/kg), intermediate in spotted sunfish (*L. punctatus*; 0.172 mg/kg), and lowest in redear sunfish (*L. microlophus*; 0.114 mg/kg).
- During WY2016, THg concentrations in largemouth bass were determined from 8 of the 13 locations within the EPA. THg concentration from EPA sites ranged from 0.162 mg/kg at site CA3F2 (WCA-3A) to 1.700 mg/kg at site L67F1 (ENP), with a median value of 0.395 mg/kg. During WY2016, six locations exceeded the USEPA recommended criterion for the protection of human health (0.350 mg/kg).
- An analysis of mosquitofish data in combination with other matrices and parameters was completed to further explore Hg availability and bioaccumulation within the Everglades ecosystem.
- An analysis of historic Regional Environmental Monitoring and Assessment Program (REMAP) data was completed to expand on recent research in an effort to identify Hg biological hotspots within the EPA.
- Wet deposition is the dominant Hg source to the Everglades. During WY2015, wet deposition of THg accounted for 129.9 kg Hg to the EPA[5]. No statistically significant trend was apparent in annual Hg wet deposition.
- During WY2016, annual mean inflow SO_4^{2-} concentrations ranged from 8.8 milligrams per liter (mg/L) for ENP to 43.8 mg/L for LNWR. The annual mean SO_4^{2-} concentrations at interior marsh regions ranged from 1.6 mg/L for ENP to 40.1 mg/L for WCA-2.
- In WY2015, total SO_4^{2-} deposition (wet + dry) accounted for 5,214.6 metric tons (t) Hg to the EPA. Statistically significant decreasing trends were observed for both wet and dry atmospheric deposition and a significantly increasing trend in rainwater pH was observed throughout the entire period of record (WY1995–WY2015).
- A rigorous statistical analysis of historic REMAP data was completed in an effort to empirically quantify the hypothetical S-Hg relationship in the Everglades ecosystem.

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

[5] The complete data set for WY2015 is under quality assurance review and is expected to be reported in the 2017 SFER.

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 EVERGALDES

Over the past several decades, multiple research studies have been done regarding the factors that influence the formation of methyl Hg (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/SO_4^{2-} and MeHg production (Axelrad et al. 2008, 2013, Benoit et al. 1999a, 1999b, 2003, Gilmour et al. 1992, Orem et al. 2011). Since the development of the theoretical relationship between SO_4^{2-} and MeHg production, early S and Hg 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, Hg methylation is subject to large unexplained variations and appears to be controlled not only by SO_4^{2-} but a combination of many environmental factors (Gilmour 2011, Julian et al. 2014). Due to this complexity and variability, the SO_4^{2-}-Hg unimodal relationship is not spatially or temporally consistent within the Everglades (Julian et al. 2014, 2015b). The proof of an ecological concept lies in its predictive capability in nature and direct evidence of the SO_4^{2-} 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 a empirically rigorous relationships to explain MeHg formation and 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 Hg 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 Hg 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 and Paul Julian[1]

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 the relative condition of aquatic ecosystems, as various fish species exhibit different characteristics and they 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 along a trophic gradient of species and provides an opportunity to evaluate spatial and temporal trends in MeHg exposure levels for both wildlife and humans. Hg 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) 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 Hg 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. Hg concentrations in axial muscle tissue (fillets) from individual bass are used to assess human health risks associated with Hg exposure. Hg 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 Hg monitoring projects of the South Florida Water Management District (SFWMD or District) and the Florida Fish and Wildlife Conservation Commission (FWC).

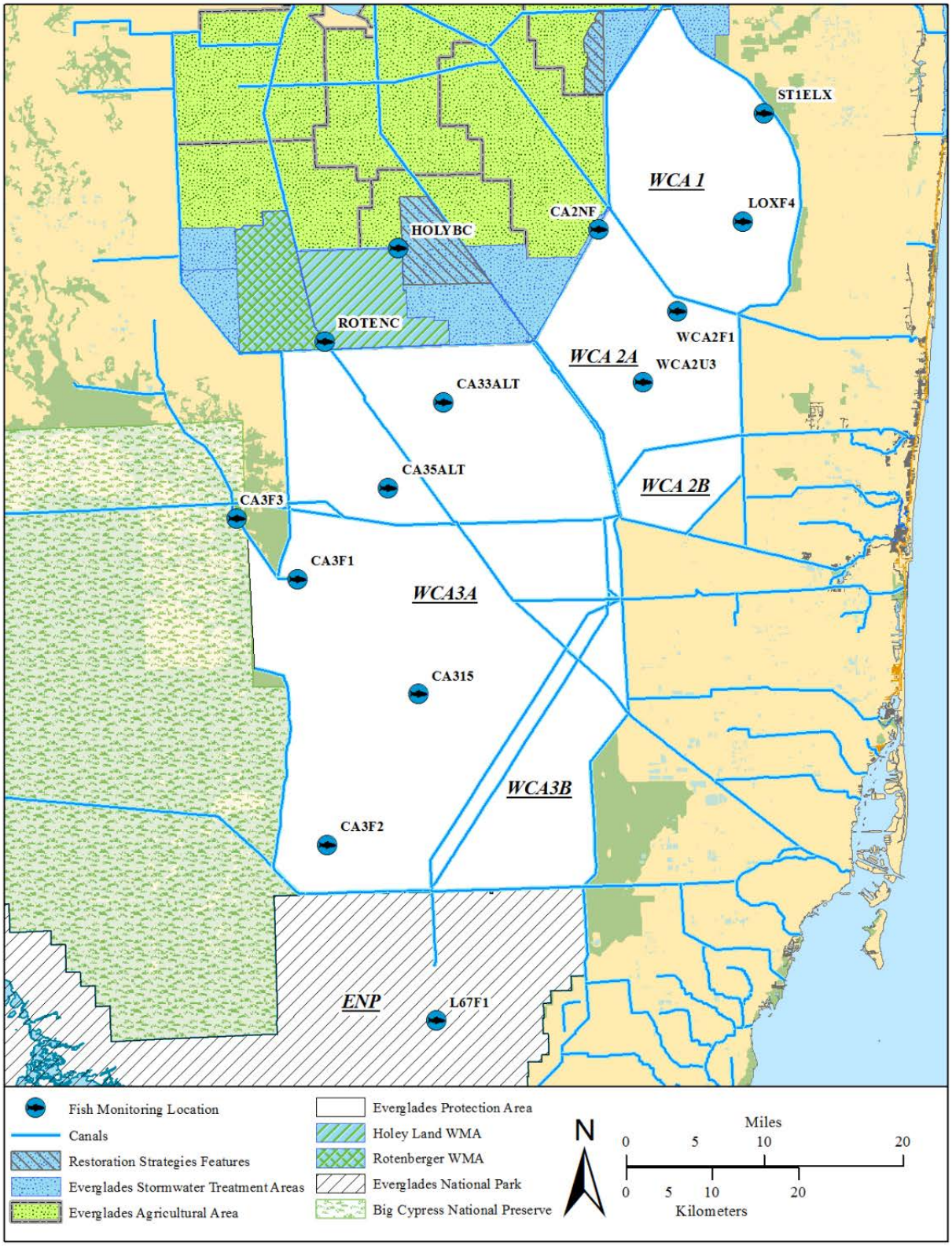

Figure 3B-1. Location of fish tissue monitoring locations within the EPA and Holey Land and Rotenberger WMAs. (Note: Station CA3F1 is an inactive station with fish sampling activity suspended since October 2009. CA3F3 is used to replace CA3F1 since October 2010.)

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 percent of the Hg 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 utilized to assessment human health risk from MeHg exposure. The USEPA-recommended MeHg criterion for the protection of human health (0.35 mg MeHg/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

Hg data evaluated in this section of the chapter were retrieved from the District's DBHYDRO database. 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 (FDEP's) Quality Assurance Rule (Chapter 62-160, Florida Administrative Code [F.A.C.]). Any datum associated with a fatal qualifier (e.g., H, J, K, N, O, V, Q, Y, or Z) 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, WY2016, and the entire period of record (POR; WY1999–WY2016; May 1, 1998–April 30, 2016). 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. All statistical operations were performed with SigmaPlot 17 and 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**). During WY2016, no sample was collected from CA2F1 due to a site access issue. THg concentrations in mosquitofish from 13 monitoring sites ranged from 0.013 mg/kg at site HOLYBC to 0.088 mg/kg at site ROTENC, with a median value of 0.036 mg/kg (**Figure 3B-2**). This value is 10 percent lower than the median value reported in WY2015. The lowest and the highest THg levels in mosquitofish were found at Rotenberger and Holey WMAs. Mosquitofish THg level in WY2016 decreased in 7 stations compared to WY2015. Mosquitofish THg in CA35ALT displayed the greatest decrease by 0.045 mg/kg while CA3F2 displayed the greatest increase by 0.049 mg/kg. In fact, 9 of 13 sites in WY2014 were below their POR median values (**Figure 3B-2**).

Table 3B-1. Temporal trend analysis of mosquitofish THg concentration at the 13 active monitoring locations within the EPA during WY1999–WY2016. (Note: NS – not statistically significant.)

Area	Station	Spearman's ρ	ρ-value	Sample Size	Trend Direction
WCA-1	ST1ELX	0.22	0.52	11	NS
	LOXF4	-0.63	*<0.01*	17	Decreasing
WCA-2	CA2NF	0.20	0.56	11	NS
	WCA2F1	0.50	0.06	16	NS
	WCA2U3	-0.02	0.93	18	NS
WCA-3	CA33ALT	-0.01	0.97	12	NS
	CA35ALT	0.15	0.61	14	NS
	CA3F3	0.54	0.30	6	NS
	CA3F1[a]	-0.15	0.64	12	NS
	CA315	-0.60	*<0.05*	18	Decreasing
	CA3F2	-0.17	*0.05*	18	Decreasing
ENP	L67F1	-0.17	0.52	16	NS
WMAs	ROTENC	0.38	0.20	13	NS
	HOLYBC	0.005	0.98	13	NS

a. Inactive monitoring station. No data has been collected since October 2009.

Mosquitofish THg concentrations in WY2016 exceeded the federal criterion of 0.077 mg/kg MeHg for TL3 fish at one of the 13 active monitoring sites (ROTENC) (**Figure 3B-2**). For the POR, the median value of THg in mosquitofish is 0.052 mg/kg and 28 percent of the data exceeded the federal criterion. The highest median value of mosquitofish THg throughout the POR was 0.373 mg/kg observed at WCA2U3, while the lowest observed value of 0.002 mg/kg was recorded at CA2NF. Site CA2NF near the L-6 canal and site WCA2F1 near the Hillsborough Canal had no exceedance for the entire monitoring period and displayed the lowest mosquitofish median THg value of 0.018 and 0.008 mg/kg, respectively. It is noteworthy that CA2NF and WCA2F1 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. 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.

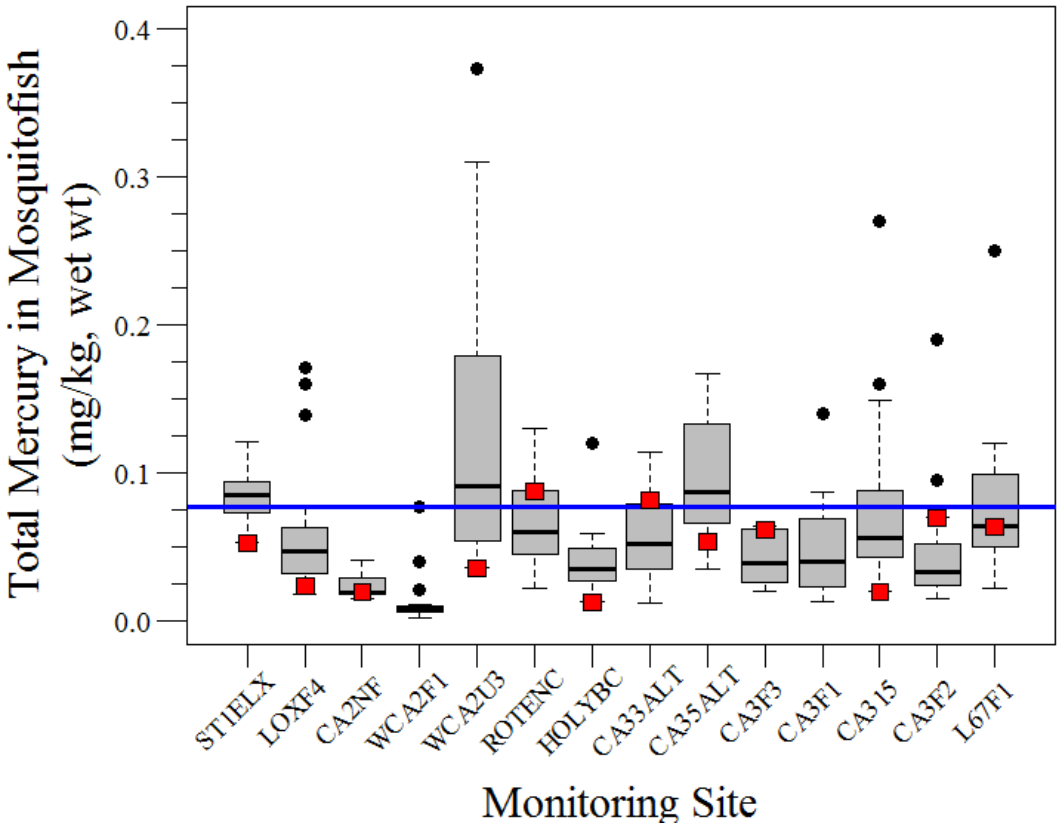

Figure 3B-2. Box plots of THg concentrations in mosquitofish at each monitoring site in the EPA during WY1999–WY2016. Red boxes indicate WY2016 mean THg concentrations and the blue line denotes the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of piscivorous wildlife.

Inter-annual and inter-site variations in mosquitofish THg concentrations between consecutive years remains high (**Figure 3B-3**). During WY2016, the highest observed mosquitofish THg concentration occurred at CA33ALT with a concentration of 0.54 mg/kg. During WY2015, this site reported a THg concentration of just 0.012 mg/kg. This one-year change is over three-fold increase in THg at this site. By contrast, CA2NF and HOLYBC experienced a 50 to 60 percent decline in mosquitofish THg concentrations between WY2015 and WY2016. To date, it is not clear what factor(s) control the dramatic intra-site temporal variations in mosquitofish THg concentration. It hypothesized that changes in inter-annual precipitation (i.e., wet years versus dry years) and site specific biogeochemistry including SO_4^{2-}, available inorganic mercury (Hg^{2+}), dissolved organic carbon (DOC), and reduction-oxidation (redox) have the ability to influence prey Hg 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, $H = 0.037$, $df = 1$, $p = 0.847$). 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 (see the Investigations on Biogeochemical and Food Web Controls on Methylmercury Accumulation in the Everglades Protection Area section below).

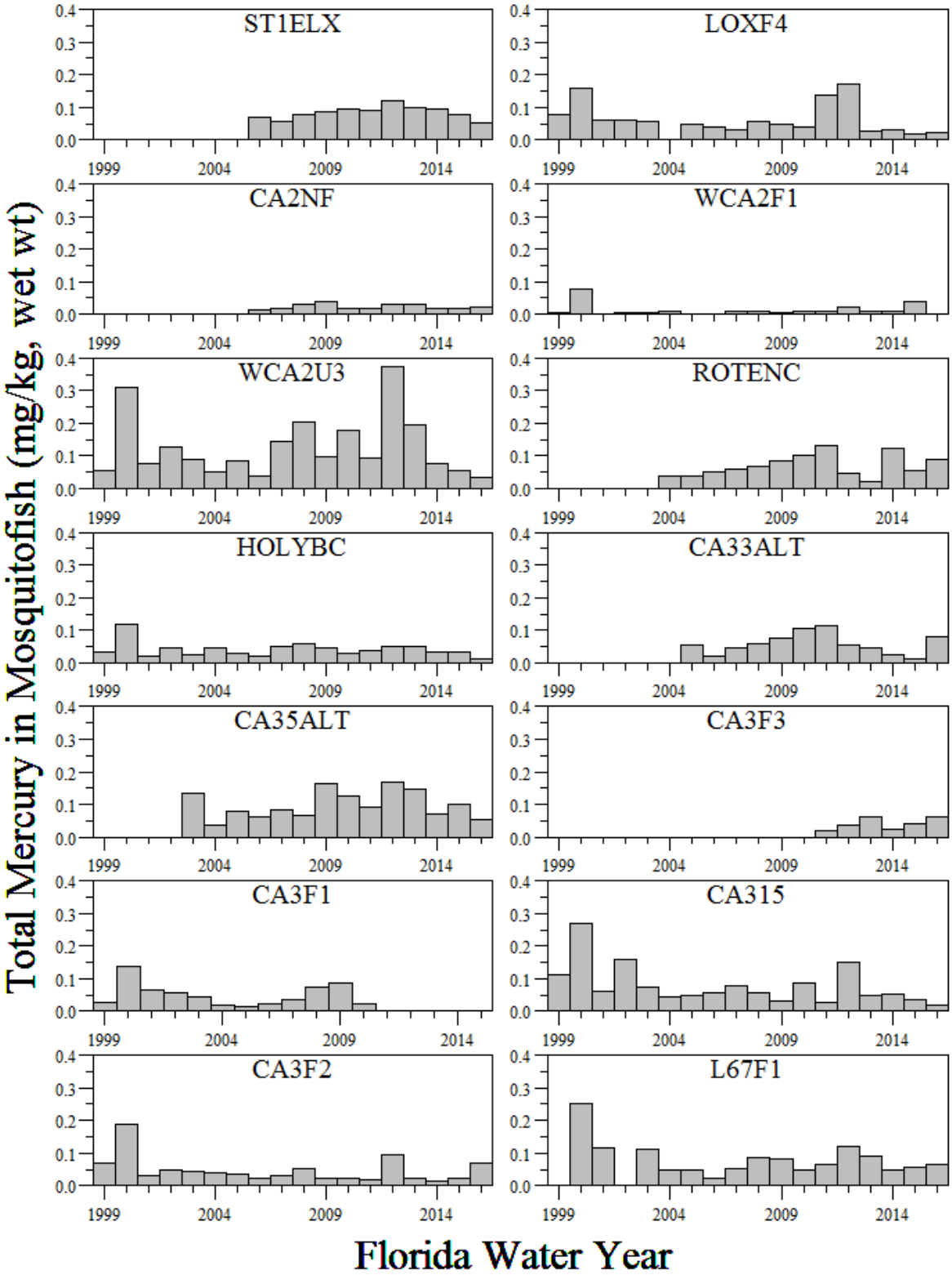

Figure 3B-3. Annual THg concentrations in mosquitofish composite samples at each monitoring site with the EPA during WY1999–WY2016.

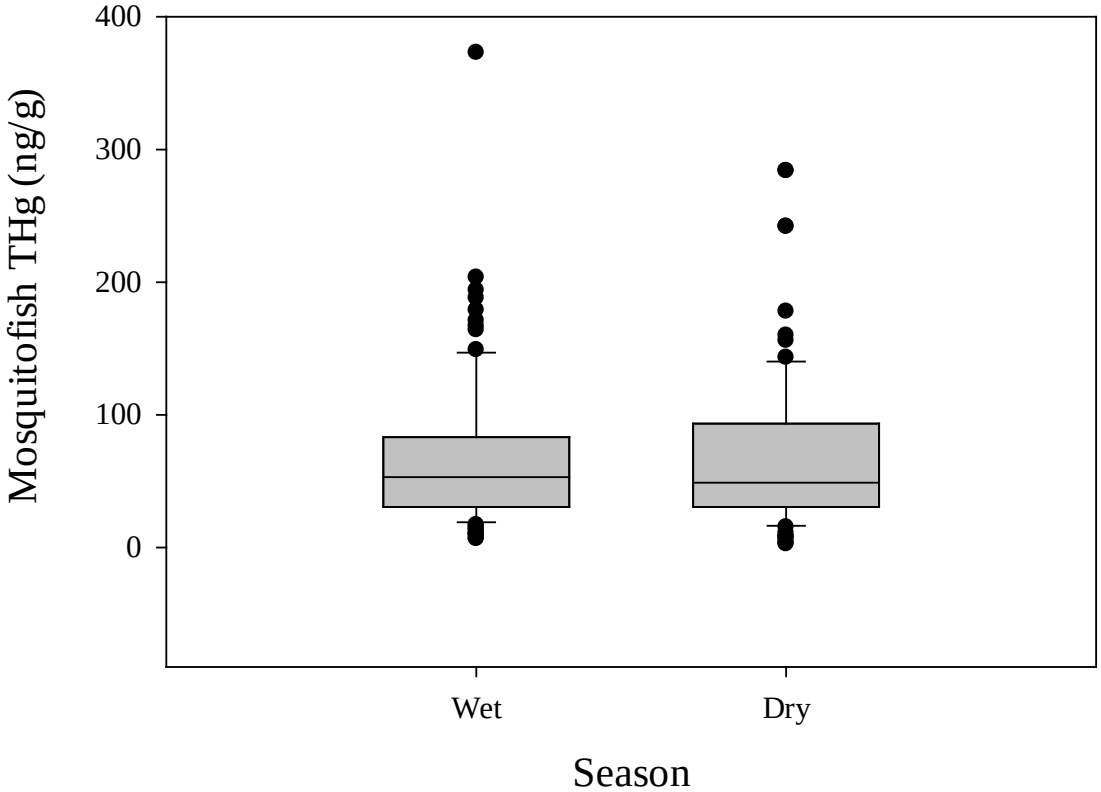

Figure 3B-4. Comparison of mosquitofish THg concentrations collected during wet and dry years during WY1999–WY2016 within the EPA.

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 = 0.00168) than canal habitat (Variance = 0.00352). 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 0.0665 mg/kg for marsh area (sample size (n) = 386) and 0.0250 mg/kg for canal (n = 231) and statistically differ between habitat (Kruskal-Wallis Analysis, Q=7.86, $p<0.001$) (**Figure 3B-5**).

During the entire POR, three sites (LOXF4, CA315, and CA3F2) experienced a significantly declining temporal trend in mosquitofish THg while one site (CA2F1) 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 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 wading bird feeding which has the potential to alter trophic structure, internal marsh sources of THg or MeHg from tree islands (Zhu et al. 2014), or other factors.

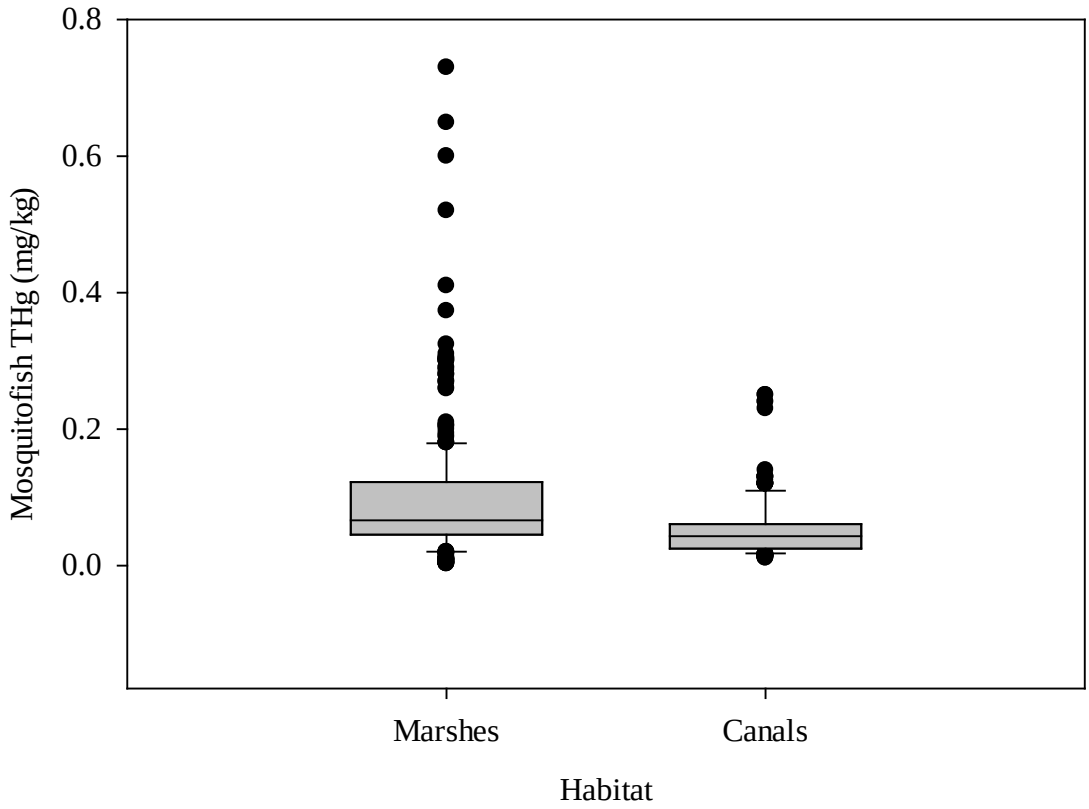

Figure 3B-5. Comparison of mosquitofish THg concentrations between marsh and canal habitats from data collected in the EPA during WY1999–WY2016.

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 0.172 ± 0.162 mg/kg (n = 3,359). Throughout the POR, 74 percent 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 (average = 0.042 mg/kg), all current monitored stations observed annual mean sunfish THg concentration above the USEPA MeHg criterion, with the enriched WCA2F1 experiencing the least number of exceedances (n = 3) during the POR.

The average THg concentration for the POR was highest at the ENP site L67F1 (0.375 mg/kg) and lowest (0.042 mg/kg) at a WCA-2A site (WCA2F1) near the Hillsborough Canal (**Figure 3B-1**). This pattern of THg concentrations in sunfish is consistent with THg concentrations observed in mosquitofish within the EPA. 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**). Only two monitoring stations showed a significant increasing temporal trend in mean sunfish THg concentration throughout the POR, while all other stations had no significant temporal trend (**Table 3B-2** and **Figure 3B-7**) and showed little pattern consistency across stations.

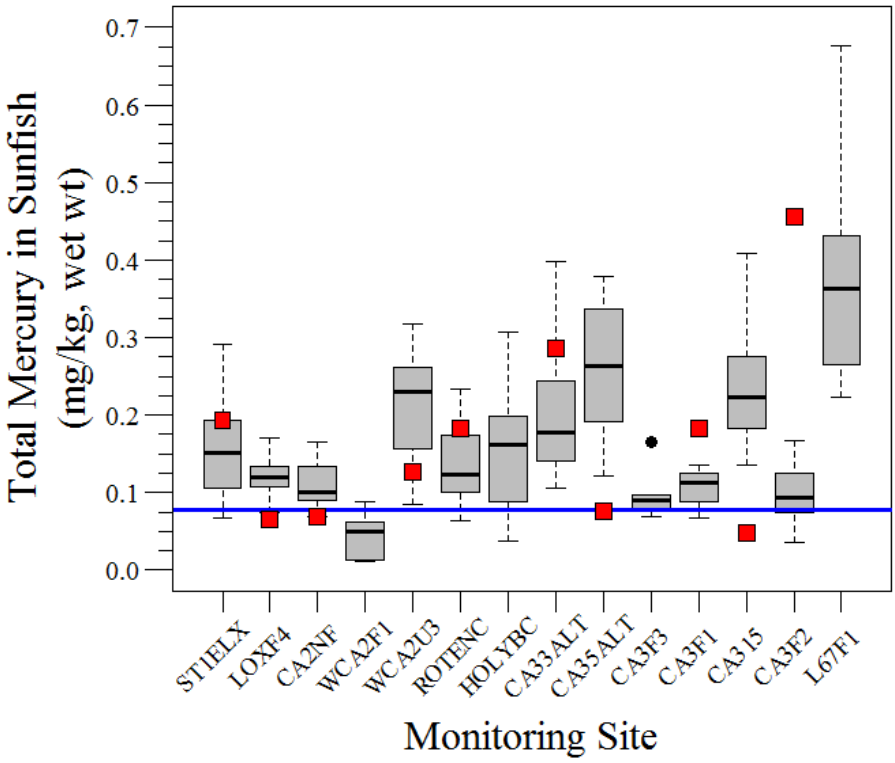

Figure 3B-6. Box plots of THg concentrations in sunfish at each monitoring site in the EPA during WY1999–WY2016. Red boxes indicate WY2016 mean THg concentrations and the blue line denotes the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of piscivorous wildlife.

Table 3B-2. Temporal trend analysis of mean sunfish THg concentrations at the 13 active monitoring locations within EPA during WY1999–WY2016. [Note: NS=not statistically significant].

Area	Station	Spearman's ρ	ρ-value	Sample Size	Trend Direction
WCA-1	ST1ELX	0.33	0.39	9	NS
	LOXF4	-0.06	0.81	17	NS
WCA-2	CA2NF	-0.21	0.54	11	NS
	WCA2F1	-0.54	0.30	6	NS
	WCA2U3	0.48	*<0.05*	18	Increasing
WCA-3	CA33ALT	0.29	0.41	10	NS
	CA35ALT	0.68	*<0.05*	14	Increasing
	CA3F3	-0.37	0.50	6	NS
	CA3F1[a]	-0.06	0.85	12	NS
	CA315	-0.16	0.51	18	NS
	CA3F2	-0.23	0.36	18	NS
ENP	L67F1	-0.07	0.80	18	NS
WMAs	ROTENC	0.34	0.31	11	NS
	HOLYBC	0.21	0.42	18	NS

a. Inactive monitoring station. No data has been collected since October 2009.

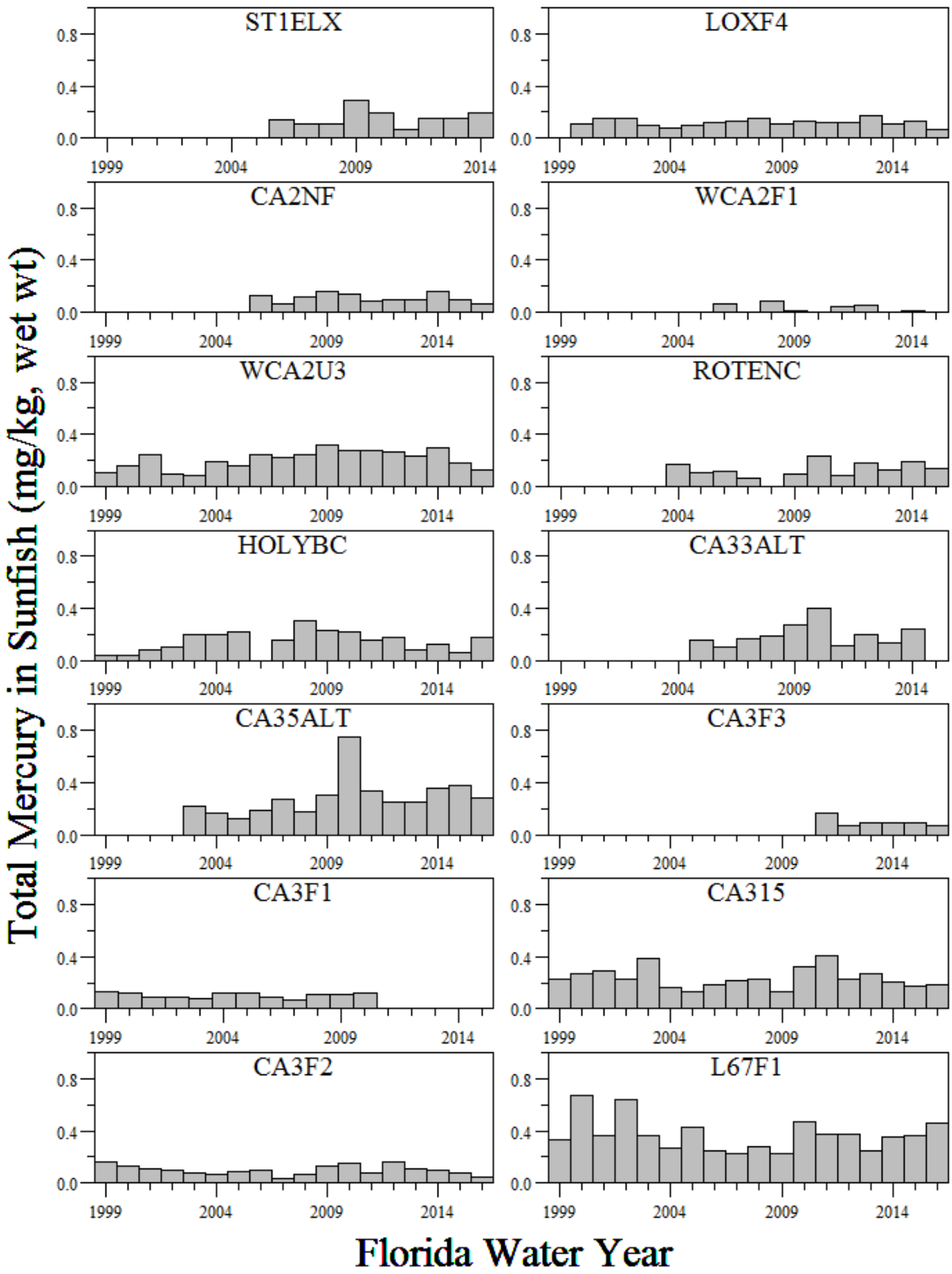

Figure 3B-7. Annual THg in TL3 sunfish whole body samples at each monitoirng site in the EPA during WY1999–WY2016.

During WY2016, the mean THg concentration in the TL3 sunfish species from 10 of the 13 active monitoring sites was 0.158 mg/kg (n = 166), with a range from 0.049 mg/kg at CA3F2 to 0.455 mg/kg at L67F1. Compared to WY2015 (0.184 mg/kg), an 18 percent decrease in mean sunfish THg concentrations occurred. THg concentrations in these species from all sites were highest in bluegill (174 mg/kg), intermediate in spotted sunfish (0.172 mg/kg), and lowest in redear sunfish (0.114 mg/kg).

Unlike WY2015, median THg concentration varied between sunfish species during WY2016 (Kruskal-Wallis Analysis; H = 4.38, df = 2, p = 0.112, **Figure 3B-8**). Throughout the entire POR all three species (annual mean) statistically differed in THg concentrations (Kruskal-Wallis Analysis**,** H = 34.4, df = 2, p < 0.001) with the highest THg concentration in spotted sunfish (0.248 mg/kg), followed by bluegill (0.197 mg/kg), and redear sunfish (0.125 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).

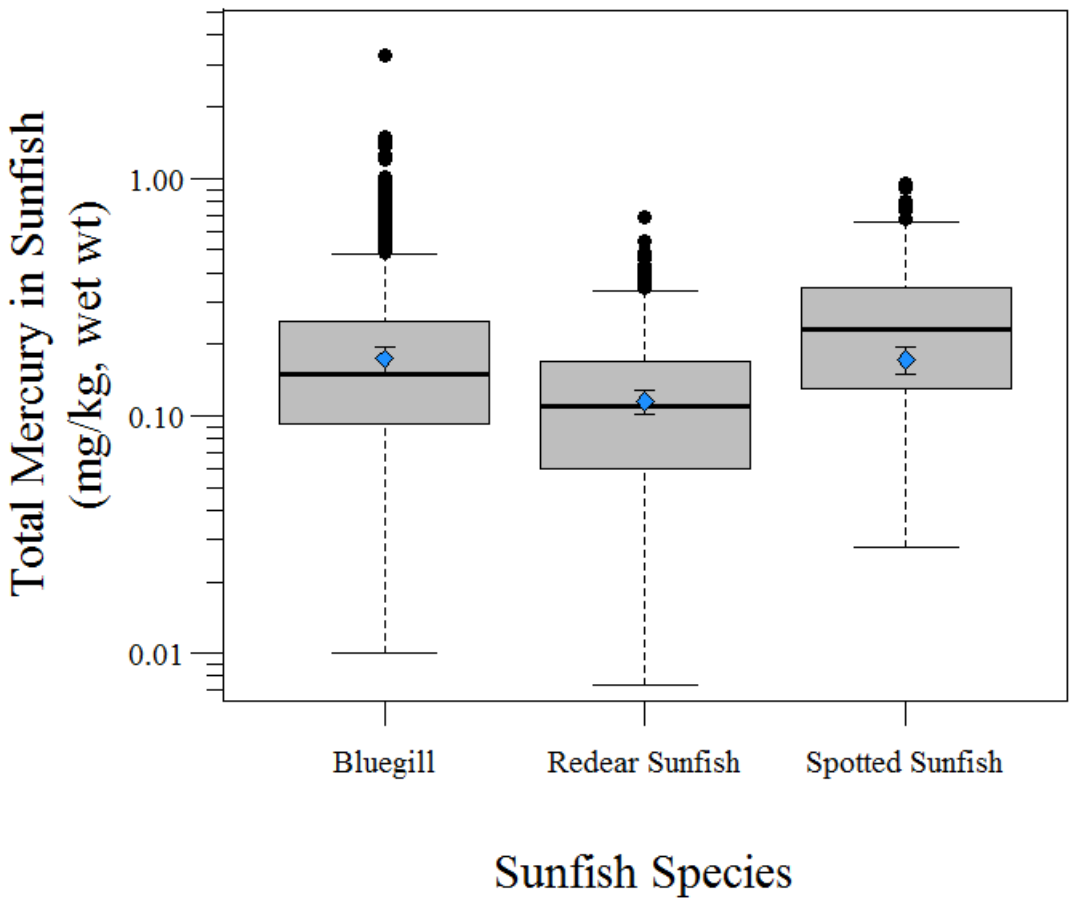

Figure 3B-8. Comparison of TL3 sunfish species collected in the EPA during WY1999–WY2016. Blue diamonds indicate WY2016 mean (±standard error) THg concentrations for each species.

Largemouth Bass

LMB axial tissue fillet samples have been collected across the EPA for THg analysis since WY1999. During WY2016, no fish was collected from five of the monitoring stations. Average THg concentrations in LMB ranged from 0.162 mg/kg at site CA3F2 (WCA-3A) to 1.700 mg/kg at site L67F1 (ENP), with an overall WY2016 median value of 0.395 mg/kg. This represents a 9 percent decrease in the reported median value for WY2015 (0.433 mg/kg). The lowest and highest THg concentration in LMB during WY2016 were observed at CA2NF (0.069 mg/kg) and L67F1 (3.110 mg/kg), respectively. 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 2014).

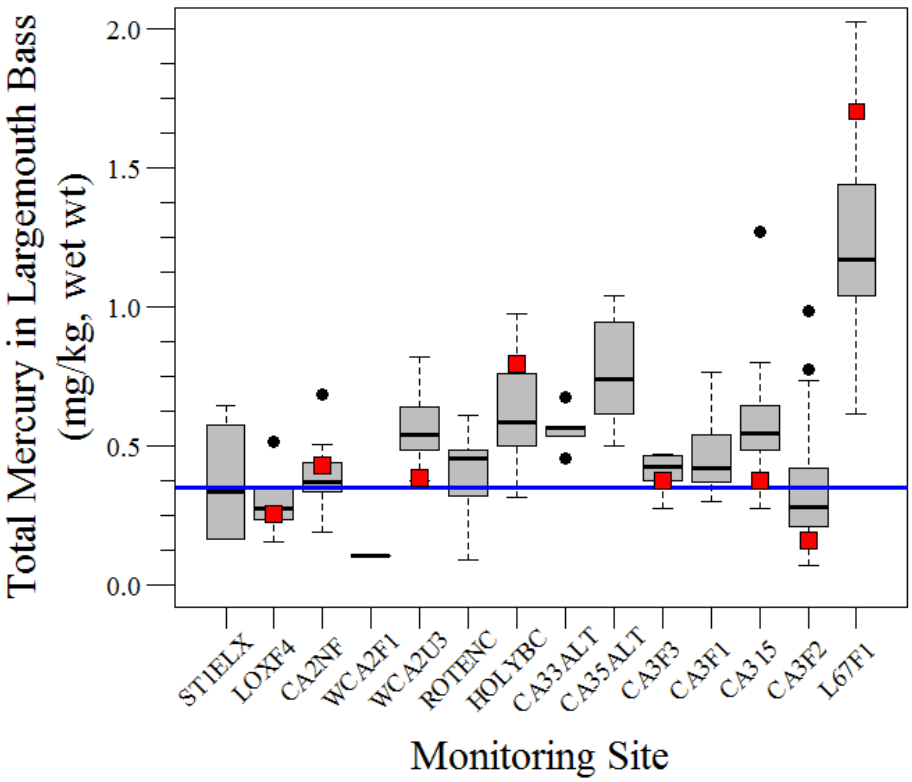

Figure 3B-9. Box plots of THg concentrations in LMB at each monitoring site during WY1999–WY2016. Red boxes indicate WY2016 mean THg concentrations and the blue line denotes the 0.350 mg/kg USEPA MeHg criterion for protection of human health.

During WY2016, 56 percent of the monitoring locations 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**). This rate decreased from 64 percent of the monitoring locations observed in WY2015. Overall, exceedance rates of the recommended criterion has improved from the POR start (WY1999) from exceedance rates of 86 percent for several years followed by several years with variable exceedance rates and after WY2003–WY2004 some years achieved approximately half of the stations exceeding the recommend criterion (**Figure 3B-10**). These exceedances of the recommended criterion could potentially be driven by hydrologic factors (i.e., wet versus dry years) and/or changes in water quality, quantity, and timing.

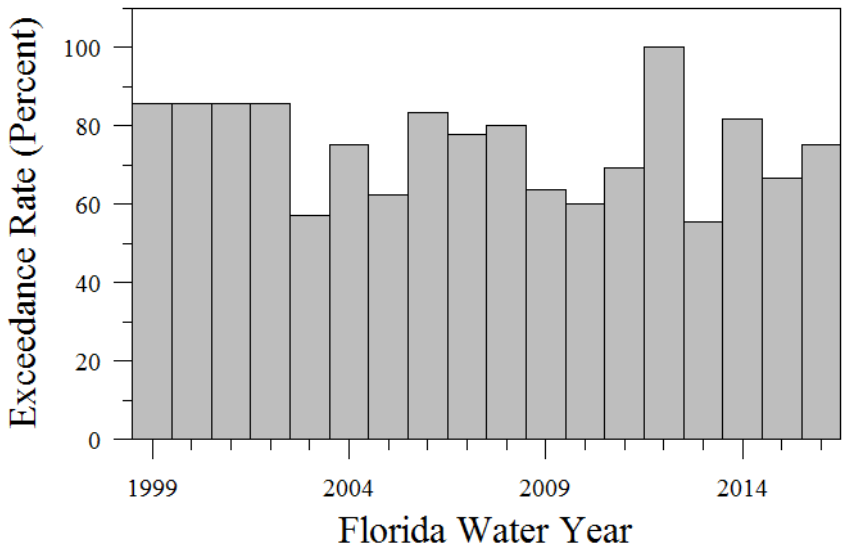

Figure 3B-10. Annual exceedance rate of LMB mean THg concentrations relative to the USEPA MeHg criterion for protection of human health.

Throughout the POR (WY1999–WY2016), except for station HOLYBC, no statistically significant temporal trends in LMB THg tissue concentration were evident (**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 (i.e. ,WCA2U3) or too much variation (i.e., ROTENC) in interannual concentrations at some sites, which could be driven by unexplained underlying conditions (discussed above) (**Figure 3B-11**).

Table 3B-3. Temporal trend analysis of annual mean LMB THg concentration at the 13 active monitoring locations within the EPA during WY1999–WY2016. [Note: NS=not statistically significant.]

Area	Station	Spearman's ρ	ρ-value	Sample Size	Trend Direction
WCA-1	ST1ELX	0.20	0.92	4	NS
	LOXF4	-0.44	0.08	17	NS
WCA-2	CA2NF	-0.09	0.80	11	NS
	WCA2F1	---	---	---	Not Enough Data
	WCA2U3	0.26	0.28	18	NS
WCA-3	CA33ALT	-0.50	0.45	5	NS
	CA35ALT	0.10	0.95	5	NS
	CA3F3	-0.37	0.50	6	NS
	CA3F1[a]	-0.46	0.13	12	NS
	CA315	-0.40	0.11	17	NS
	CA3F2	-0.42	0.09	18	NS
ENP	L67F1	-0.20	0.44	18	NS
WMAs	ROTENC	0.42	0.27	9	NS
	HOLYBC	0.51	<0.05	18	Increasing

a. Inactive monitoring station. No data has been collected since October 2009.

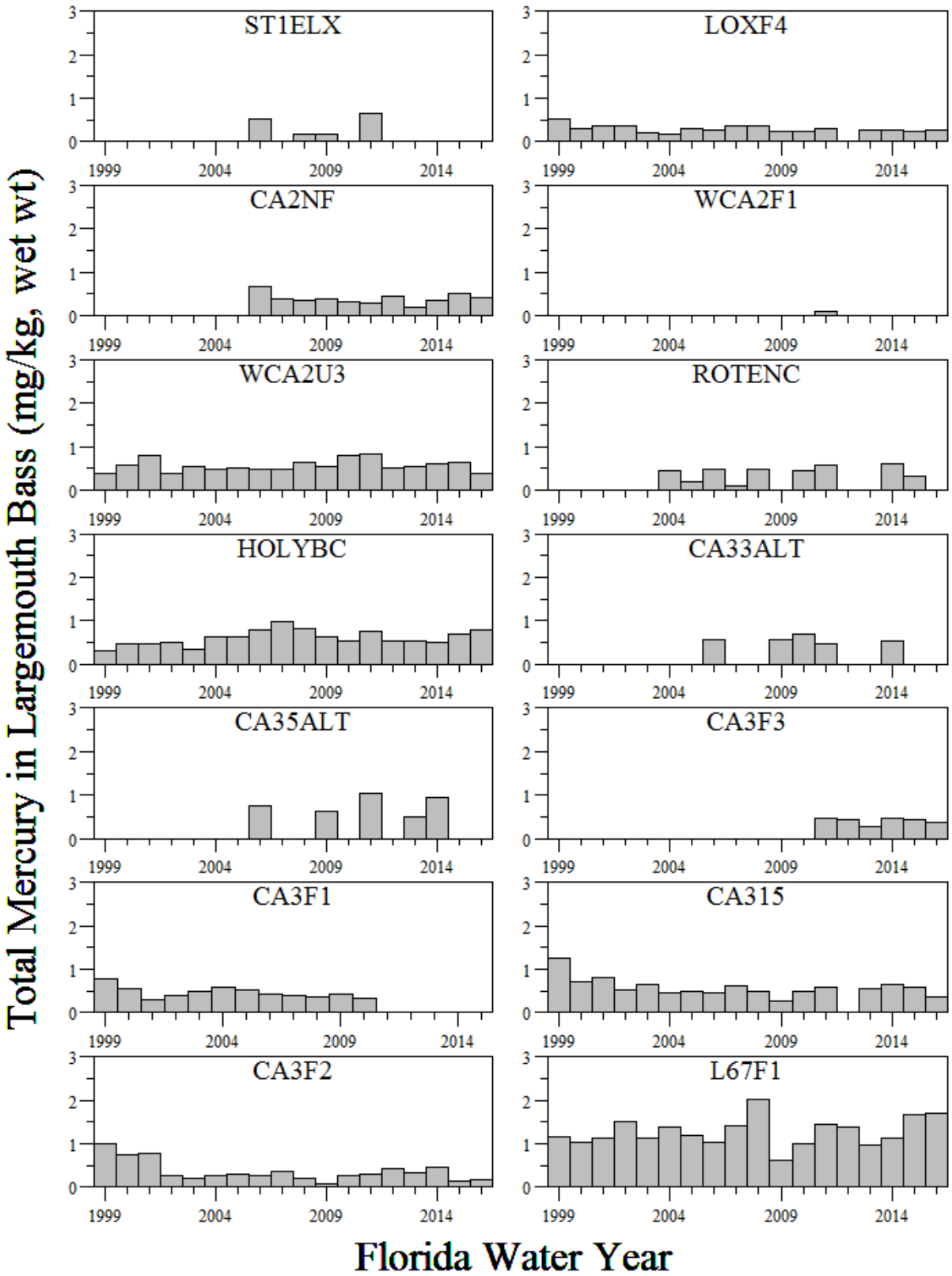

Figure 3B-11. Annual THg concentrations in LMB axial fillet samples at each monitoring site in the EPA during WY1999–WY2016.

Over the entire POR, the median mosquitofish THg concentration (0.050 mg/kg) has been below the federal criterion for wildlife protection. Meanwhile, POR THg concentration for sunfish (0.183 mg/kg) and LMB (0.583 mg/kg) has been above the wildlife and human health protection criterion, respectively. During WY2016, mosquitofish THg concentrations exceeded the USEPA criterion in one of the 13 sampled monitoring sites. By contrast, during WY2016, sunfish THg concentrations exceeded the USEPA criterion in three of the 9 (of the 13 active stations) sampled monitoring sites. Furthermore, during WY2016, LMB THg concentration exceeded the recommended USEPA criterion in one of the eight (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), while inclining trends in POR annual mosquitofish (CA2F1) and sunfish THg concentrations (WCA2U3 and CA35ALT). One of 13 sites (HOLYBC) exhibited a statistically significant temporal trend in annual LMB THg concentration throughout the 18-year POR.

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, Hg 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 Hg emissions and atmospheric deposition are highly uncertain (Krabbenhoft and Sunderland 2013), the response of fisheries to Hg 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 Hg issue beyond the state's total maximum daily load (FDEP 2013).

INVESTIGATIONS ON BIOGEOCHEMICAL AND FOOD WEB CONTROLS ON METHYLMERCURY ACCUMULATION IN THE EVERGLADES PROTECTION AREA

Mike Jerauld[3], Forrest E. Dierberg[3], Thomas A. DeBusk[3],
Janelle A. Potts[3], and Nichole R. Larson[3]

Introduction

Persistent spatial variability in Eastern mosquitofish (*Gambusia holbrooki*) tissue Hg concentrations across the Everglades has been recognized since at least 1996 (Scheidt and Kalla 2007, Stober et al. 2001). These patterns have been attributed to the distribution of net Hg methylation rates, as determined by the intersection of the distributions of sulfate (promotes Hg methylation by the key microbial group, sulfate-reducing bacteria [SRB]) and sulfide (inhibits Hg methylation by reducing the availability of inorganic Hg to methylating organisms) levels (Benoit et al. 1999a, Gilmour et al. 1998, Orem et al. 2011). In particular, analyses of broad-level ecosystem monitoring data have suggested a unimodal relationship exists between fish tissue Hg and surface water sulfate concentrations across the Everglades (**Figure 3B-12**; Axelrad et al. 2013, Gabriel et al. 2014, Pollman 2012). It has also been speculated that fluctuations in sulfate availability contributed to observed temporal variability in mosquitofish Hg concentrations (Axelrad et al. 2005). However, it has been recognized that, despite the appearance of said trend, there exists a great deal of unexplained variability in the fish Hg-surface water sulfate relationship, which must be explained if an effective MeHg mitigation policy is to be devised (Axelrad et al. 2013).

Importantly, fish tissue Hg exhibits unimodal graphical relationships, similar to that with sulfate, with a wide variety of physical and biogeochemical factors in the Everglades (**Figure 3B-13**). Thus, it is difficult to attribute spatial patterns in fish Hg levels to any one of these variables, including sulfate. Focused

examination of relationships within specific sites may control some of the geographic covariance in the many factors that potentially affect Hg methylation and accumulation in the Everglades (e.g., sulfate, sulfide, DOC; Scheidt and Kalla 2007). In addition, methylation and accumulation of Hg may be affected by ecological variables such as food web length and character, and microbial community composition, not previously studied by Everglades Hg researchers.

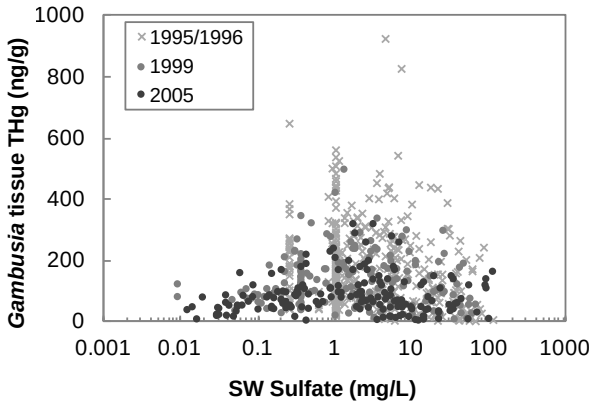

Figure 3B-12. Cross plot of *Gambusia* tissue THg concentration with surface water (SW) sulfate.

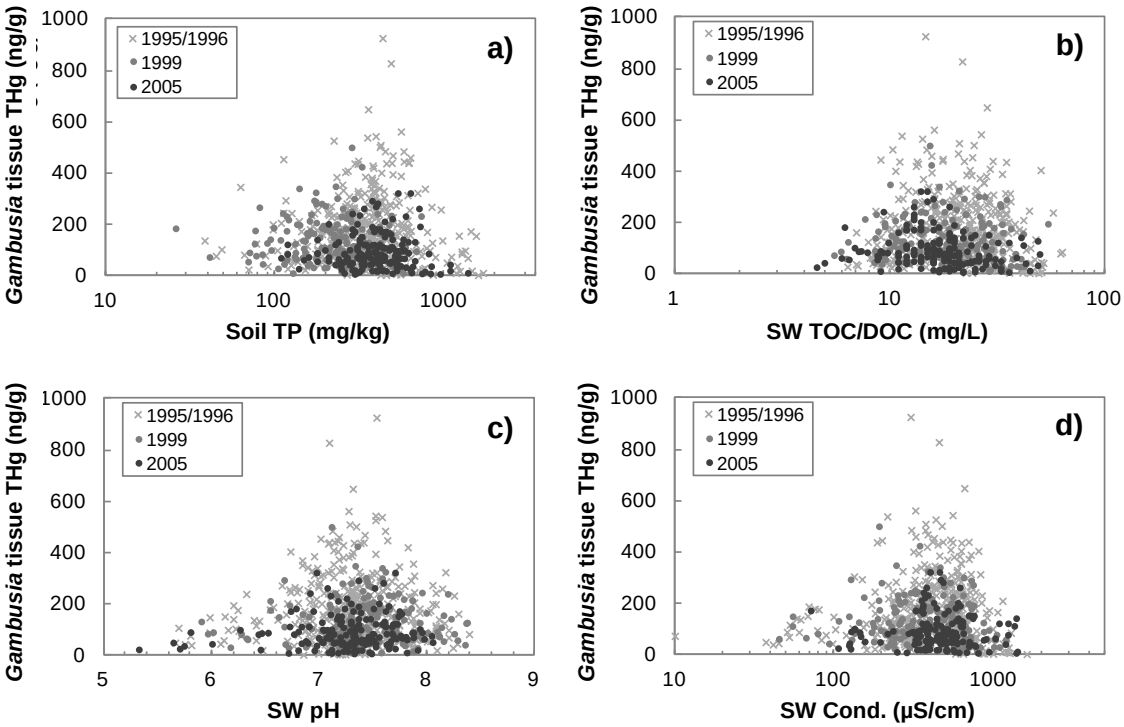

Figure 3B-13. Cross plots of *Gambusia* tissue THg concentration with (a) soil total phosphorus (TP), (b) surface water (SW) organic carbon*, (c) SW pH, and (d) SW conductivity. *During the 1995/1996 and 1999 events, SW total organic carbon (TOC) was measured; in 2005, SW DOC was measured.

Several recent studies have been conducted in areas of persistently high and low mosquitofish Hg in an effort to resolve the suite of controlling factors. Both U3, a high sulfate slough site (in WCA-2A), and 3A-15, a low sulfate slough site (in WCA-3A), historically have exhibited Hg-enriched *Gambusia*, and therefore are considered Hg hot spots. These sites, fairly similar in habitat, lie at opposite ends (3A-15 at the low end, and U3 at the high end) of the bell shaped unimodal curve (**Figure 3B-12**). However, examination of historical data suggests no increase in *Gambusia* Hg as a function of increased sulfate at WCA315, or of a decrease in sulfate at WCA2U3 (**Figure 3B-14**).

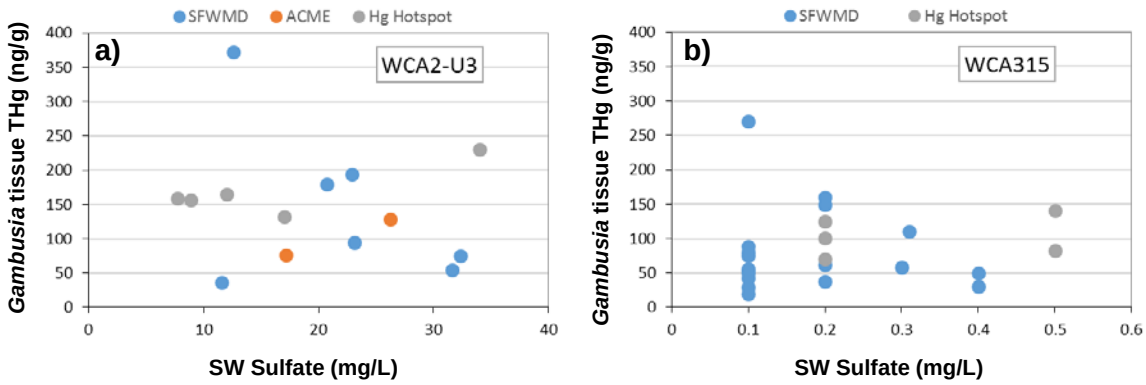

Figure 3B-14. Cross plots of *Gambusia* tissue THg concentrations against surface water sulfate concentrations at long-term monitoring sites U3 in WCA-2A and 3A-15 in WCA-3A. Data retrieved from SFWMD, Aquatic Cycling of Mercury in the Everglades project (ACME), and Everglades Hg Hot Spot project databases. Data presented includes only values when contemporaneous (i.e., within two weeks) fish Hg and water sulfate data were available. Collection dates span 1999 through 2013.

Methods

As part of an ongoing research program, in late 2013, DB Environmental, Inc. (DBE) began monitoring mosquitofish tissue Hg concentrations (alongside a suite of other matrices and parameters) at several locations in WCA-2A, WCA-3A, and ENP (**Figure 3B-15**). A broad suite of surface water and porewater chemical parameters are being characterized at these sites to facilitate comparisons among MeHg levels in soils, water and periphyton, as well as with total Hg levels in fish tissues. The research team also is sampling omnivorous fish (such as *Gambusia*), herbivorous fish and assorted invertebrates at selected locations to characterize potential spatial and temporal differences in food webs and Hg accumulation pathways. In addition to Hg analyses of these organisms, stable carbon and nitrogen isotope analyses are being performed to help pinpoint the trophic status of the various organisms among the northern, central, and southern Everglades hydrologic units; the length and character of the food web is an important contributor to biological Hg accumulation (Loftus 2000) and is known to vary across space and time in the Everglades (Abbey-Lee et al. 2013).

DBE has focused recent sampling efforts on established sites in both these WCAs, as well as in ENP, to better define the biogeochemical and/or food web factors that lead to Hg-enriched *Gambusia*. Selected findings from 2015 are described below.

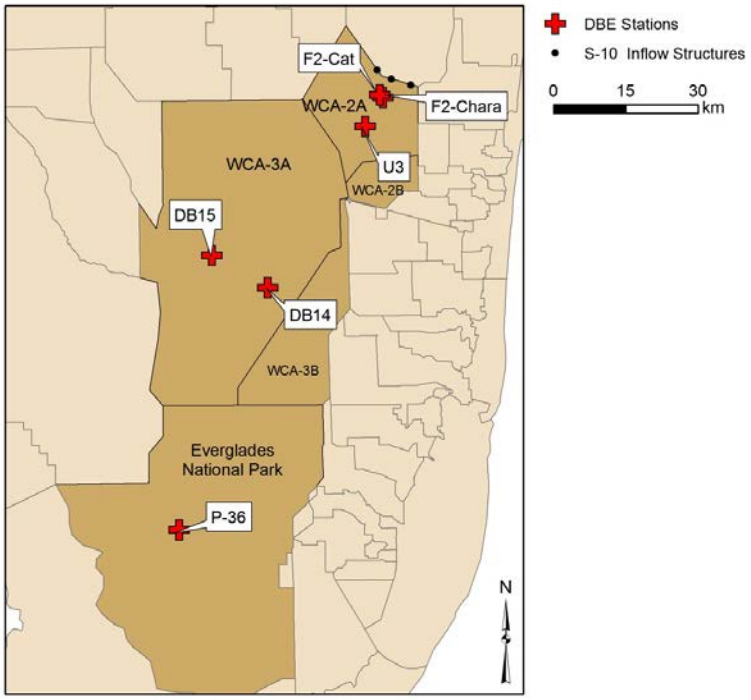

Figure 3B-15. Map showing the locations of the six sites where DBE investigations were performed in 2015.

Results

Site Characteristics and General Spatial Patterns

Several biogeochemical factors measured at sites displayed north-to-south gradients. There were several sharp differences that distinguished sites in WCA-2A from those in downstream compartments, WCA-3A, and ENP. The well studied gradient of surface water total phosphorus (TP) in WCA-2A was evident, decreasing from northern (F2) to central reaches (U3; **Figure 3B-16a**). At sites further downstream, in WCA-3A and ENP, surface water TP was typically extremely low. Surface water sulfate concentrations were dichotomous; sites in WCA-2A had high sulfate (~20 mg/L), while sites in WCA-3A and ENP had extremely low sulfate (typically < 1 mg/L). As with TP and sulfate, DOC concentrations displayed a north-to-south gradient, with the highest values in northern WCA-2A. Finally, dissolved iron was very low in surface waters of WCA-2A compared to WCA-3A and ENP, due to differences in native underlying soils.

Across our four sampling events in 2015, surface water MeHg varied with respect to space and time. There were no distinct spatial patterns, although concentrations tended to be slightly higher in WCA-2A than in WCA-3A or ENP within each event (**Figure 3B-17a**). Extremely high surface water MeHg was observed at U3 and DB-15 in May, at a time when water levels were low. Unlike MeHg in surface waters, total Hg in *Gambusia* was consistently higher in WCA-3A and ENP than in WCA-2A (**Figure 3B-17b**). This generally agrees with past observations (Scheidt and Kalla 2007), although historically *Gambusia* Hg has been quite elevated at U3 as well (Julian et al. in press). The highest *Gambusia* Hg value was observed at DB-15 in February, notably at a time when surface water sulfate was below the detection limit (0.2 mg/L). MeHg was measured in soils only once (January–February), but was generally higher in WCA-3A (3 to 6 nanograms per gram [ng/g] dry weight) than WCA-2A (~2 ng/g dry weight; **Figure 3B-17c**).

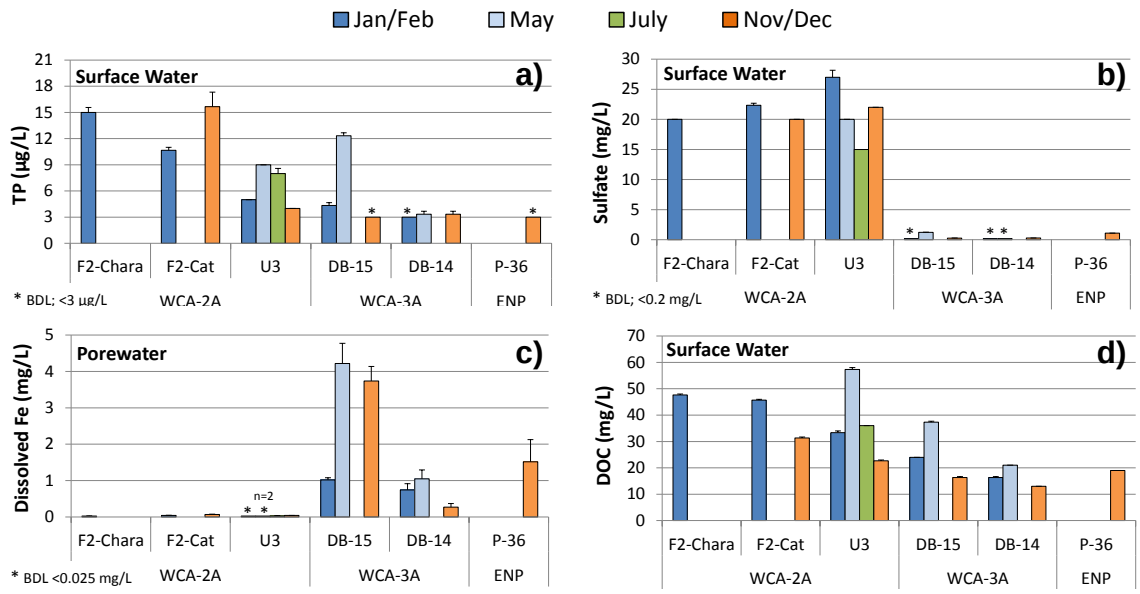

Figure 3B-16. Average (n = 3) ± 1 standard error (SE) concentrations of (a) surface water TP, (b) surface water sulfate, (c) porewater dissolved iron (Fe), and (d) surface water DOC at six field sites across WCA-2A, WCA-3A, and ENP sampled by DBE in 2015.

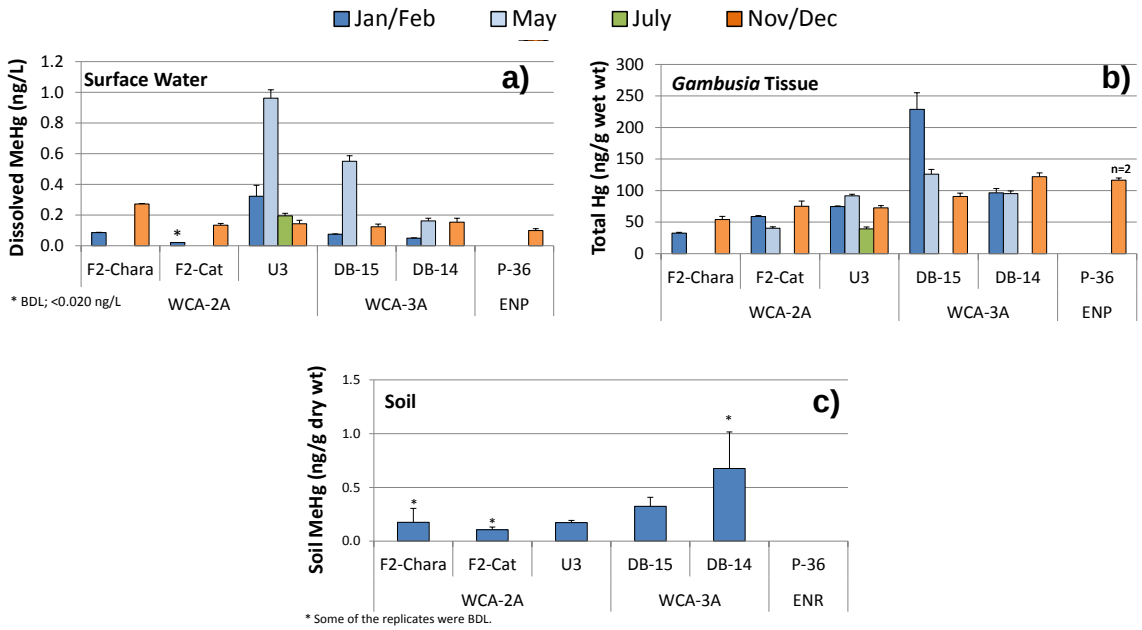

Figure 3B-17. Average (n = 3) ± 1 SE concentrations of (a) surface water dissolved MeHg, (b) *Gambusia* tissue THg, and (c) soil (top 0–4 centimeters) MeHg at six field sites across WCA-2A, WCA-3A, and ENP sampled by DBE in 2015.

Key Relationships among Biogeochemical Parameters

Surface water MeHg has occasionally been used as a response variable indicative of net methylation rates or MeHg contamination (Gilmour et al. 2007, Krabbenhoft and Fink 2001). However, within and among the sites we studied in 2015, there was no relationship between surface water MeHg and surface water sulfate (**Figure 3B-18a**). Further, there was no relationship between *Gambusia* THg and surface water MeHg within or among the same sites (**Figure 3B-18b**). Notwithstanding the lack of sulfate data between 2 and 15 mg/L, fish Hg appeared to be inversely related to sulfate (**Figure 3B-18c**), perhaps due to sulfide inhibition of bioavailable Hg (Benoit et al. 1999b). Higher *Gambusia* tissue Hg was found at low sulfate sites (that is, WCA-3A and ENP) compared to the high sulfate WCA-2A sites. Importantly, we have not detected any statistical relationship between fish Hg and surface water sulfate within any site, in agreement with the longer-term data sets provided by SFWMD and Everglades Hg Hot Spot project (**Figure 3B-14**). *Gambusia* tissue Hg was also inversely related to DOC concentration, which was observed both among and within sites (**Figure 3B-18d**). Similar relationships have been reported previously (Liu et al. 2008), and may be related to effects of DOC on MeHg solid/aqueous partitioning and availability for uptake.

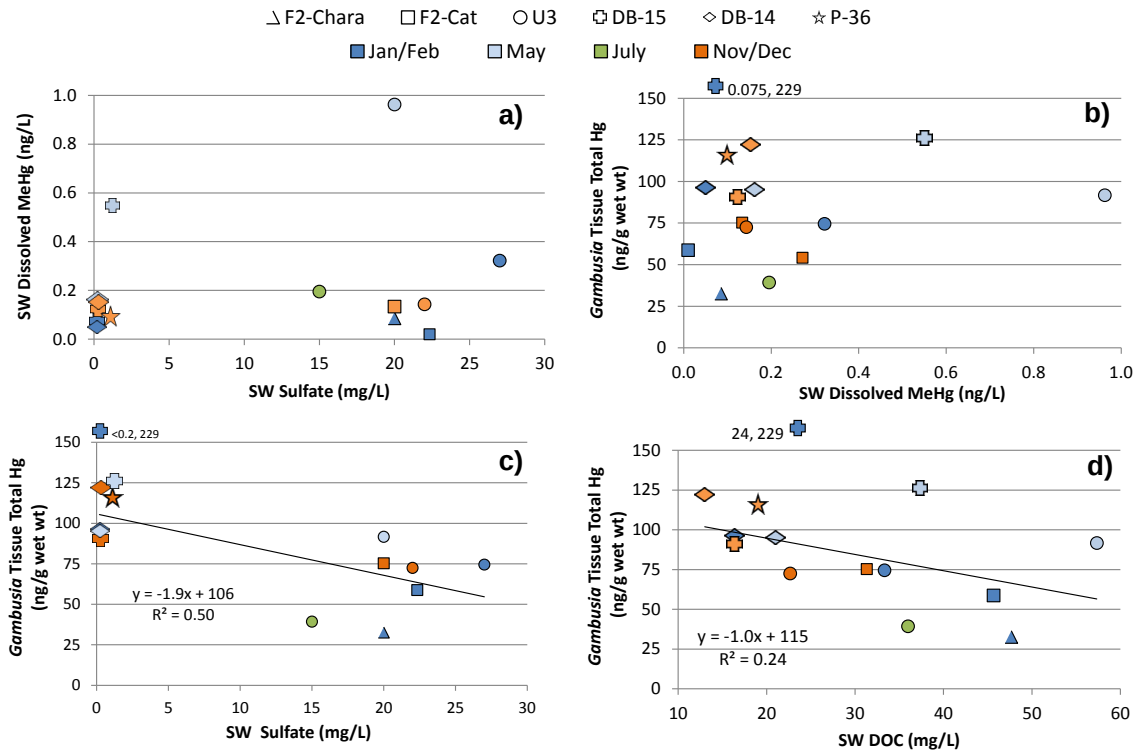

Figure 3B-18. Cross plots of (a) surface water MeHg against surface water sulfate, and *Gambusia* tissue THg against (b) surface water MeHg, (c) surface water sulfate and (d) surface water DOC at six field sites across WCA-2A, WCA-3A, and ENP sampled by DBE in 2015. Note that the regression lines for panels c and d do not include the single outlier shown in each panel.

Food Web Investigations

Biogeochemical factors have not been found to readily explain the spatial and temporal variability in *Gambusia* Hg levels. However, as would be expected, *Gambusia* tissue Hg is closely related to the MeHg in the gut contents, implying that the spatial patterns in *Gambusia* Hg enrichment also exist in (and likely originate from) *Gambusia* prey (**Figure 3B-19**). Therefore, we have begun to characterize MeHg concentrations and stable carbon and nitrogen isotopes in primary producers and other consumers to identify pathways of MeHg uptake and transfer.

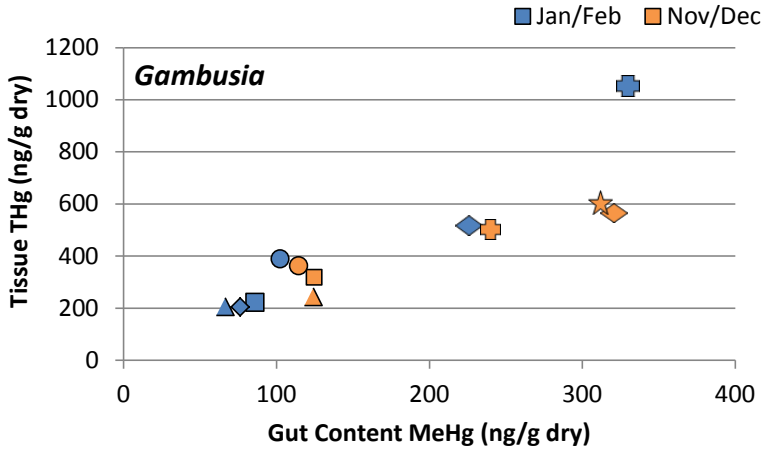

Figure 3B-19. Cross plot of *Gambusia* tissue THg against *Gambusia* gut-content MeHg.

Periphyton and detritus are likely two key basal food resources for Everglades food webs. Periphyton is thought to contribute to *Gambusia* Hg enrichment, based on correlations observed in the REMAP data sets (Liu et al. 2009, Scheidt and Kalla 2007). At our sites, periphyton MeHg was variable over time, and displayed changing spatial patterns (**Figure 3B-20a**). For example, in May, there were marked differences among each of the sites sampled, but in the January–February and November–December events, concentrations were remarkably similar across all visited sites. *Gambusia* Hg concentrations did not appear to reflect these changes to periphyton MeHg (**Figure 3B-21a**). On the other hand, detritus MeHg, while quite variable over time, was generally higher at WCA-3A and ENP sites than at WCA-2A sites (**Figure 3B-20b**). Further, we found relatively good correspondence between *Gambusia* Hg and detritus MeHg across sites, and within most sites (**Figure 3B-21b**).

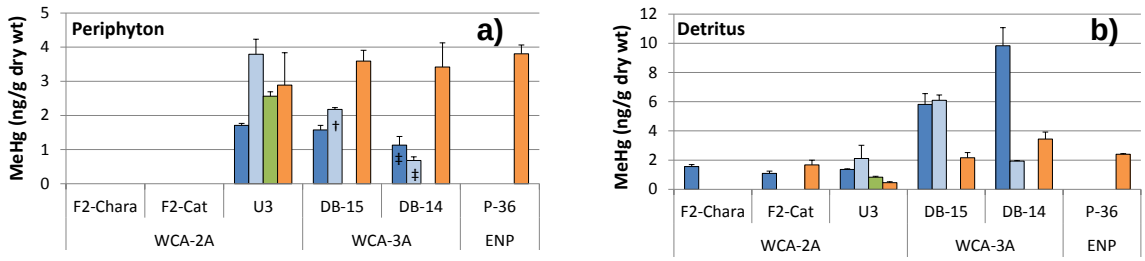

Figure 3B-20. Average (n = 3) ± 1 SE concentrations of (a) periphyton MeHg and (b) detritus MeHg at six field sites across WCA-2A, WCA-3A, and ENP sampled by DBE in 2015. All periphyton was epiphytic unless otherwise noted: † denotes benthic periphtyon; ‡ denotes floating periphyton.

Aquatic invertebrates are significant components of Everglades food webs, and several species are ubiquitous across the ecosystem. We measured MeHg concentrations in *Hyalella* spp, an amphipod, and *Palaemonetes paludosus*, the eastern grass shrimp. Both *Hyalella* and *Palaemonetes* exhibited spatial trends similar to *Gambusia*, with higher levels in WCA-3A and ENP compared to WCA-2A (**Figure 3B-22**), suggesting that MeHg enrichment occurs throughout the food web, down to the lowest consumers exemplified by *Hyalella*. Indeed, we found that the rate of biomagnification was similar across sites (**Figure 3B-23**), so relative differences in, say, *Gambusia* tissue Hg levels were reflected in and presumably primed by differences in MeHg concentrations of primary consumers, such as *Hyalella*. It is noteworthy that *Gambusia* tissue Hg was apparently related to both detritus MeHg and MeHg in *Hyalella*, a purported detritivore (Williams and Trexler 2006). This may point to the detrital food web as a key pathway of MeHg introduction into the Everglades biota, and will be a focal point of this continuing investigation.

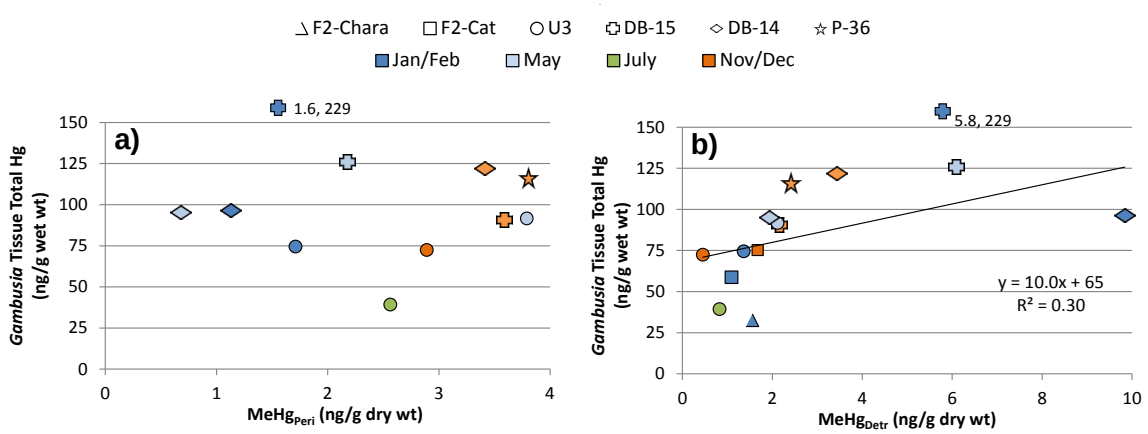

Figure 3B-21. Cross plot of *Gambusia* tissue THg against MeHg concentrations in (a) periphyton and (b) detritus. The regression line in panel (b) does not include the outlier.

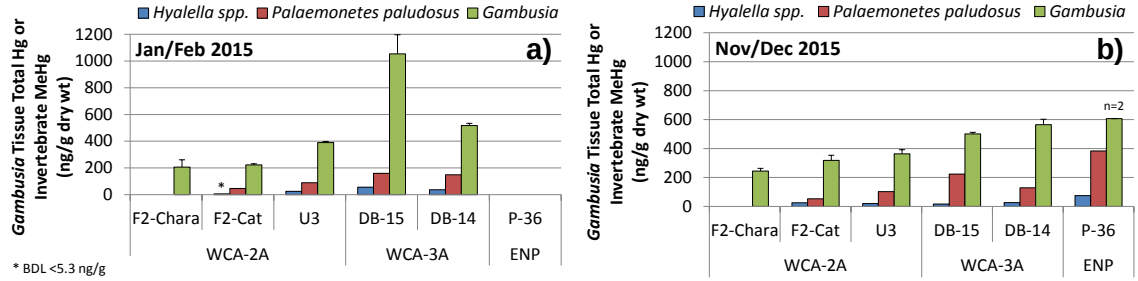

Figure 3B-22. Average (n = 3 for *Gambusia*, and n = 1 for invertebrates) THg (*Gambusia*) or MeHg (invertebrates) in *Gambusia*, the amphipod *Hyalella* spp., and the grass shrimp *Palaemonetes paludosus* at six field sites across WCA-2A, WCA-3A and ENP sampled by DBE in (a) January–February 2015 and (b) November and December 2015.

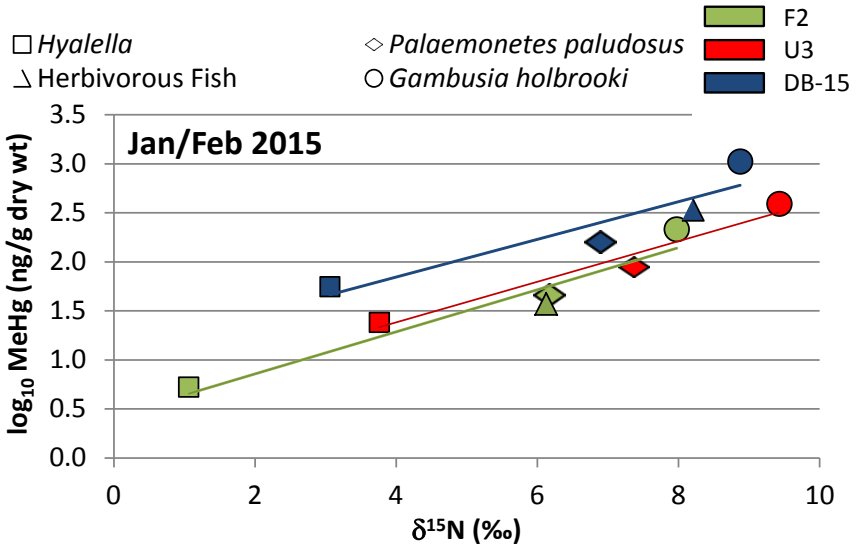

Figure 3B-23. Cross plot of the logarithm of tissue MeHg* against tissue $\delta^{15}N$ values for several Everglades aquatic organisms including the amphipod *Hyalella* spp, the eastern grass shrimp *Palaemonetes paludosus*, herbivorous fish including sailfin molly (*Poecilia latipinna*) and Florida flagfish (*Jordanella floridae*), and *Gambusia holbrooki.* *For *Gambusia*, total Hg was measured, and is reported here. $\delta^{15}N$, a nitrogen isotope, estimates the trophic level of an organism and the number of food chain steps in a food web. It is non-conservative throughout the food chain (increases with each trophic step), so higher values indicate greater trophic position.

MERCURY BIOLOGICAL HOTSPOT IDENTIFICATION

Paul Julian II[1]

The term "Hg hotspot" has been used to characterize areas of high Hg accumulation to either biota, soil or other ecosystem compartments. However, the specific criteria to identify and delineate hotspots has been somewhat vague and used inconsistently in the literature. Some studies in lake ecosystems have used wildlife tissue thresholds and criteria to define biological Hg hotspots (Evers et al. 2007, Hutcheson et al. 2008). There are important considerations in identifying and differentiating biological Hg hotspots especially when source of Hg contamination is not easily identified across ecosystems and factors that influence Hg transport, translocation, conversion, and accumulation in biota may vary greatly in importance.

Hotspots can be empirically derived using a variety of geostatistical methods including the Getis-Ord spatial statistic similar to methods used by Julian (2013) to identify soil Hg hotspots within WCA-3. Alternatively, a multi-prong assessment can be used to parse areas into "hotspot", "non-hotspot", and "suspect" based on soil and biotic Hg concentrations by using sediment quality guidelines (SQG; MacDonald et al. 2000) and wildlife protection criterion (USEPA 1997) presented above.

Soil nutrient stoichiometric relationships could also lend themselves to informing the location and extent of Hg hotspots (Julian and Wright 2016). It should be noted that all these methods are data intensive and require large-scale spatial sampling from several different ecosystem compartments. This section further expands upon the framework presented by Julian and Wright (2016) to a larger-scale spatially explicit data collection across the Everglades system.

Methods

Mosquitofish Hg and soil nutrient and Hg data collected during 2005 as part of USEPA Phase 3 REMAP was retrieved from USEPA (https://www.epa.gov/everglades/environmental-monitoring-everglades). Phase 1 and 2 (i.e. 1994–1995 and 1999) were not included in this analysis because Phase 3 data was the only data set with soil total carbon (TC), total nitrogen (TN), TP, and THg concentration data. More recent data has been collected as part of Everglades REMAP during 2013–2014, but these data have not been released by USEPA. Soil nutrient concentrations were converted from mass (mg/kg) to molar concentrations (millimole per kilogram [mmol/kg]). Molar ratios were calculated for soil TC and TN, TC and TP, and TN and TP.

Soil THg data were assessed against the 174 ng/g Hg SQG threshold effect level (TEL) for freshwater sediments as suggested by (MacDonald et al. 2000). The TEL is operationally defined as the concentration below which adverse biological effects rarely occur. Fish tissue concentrations were assessed against the USEPA criterion of 0.077 mg/kg for the protection of piscivorous wildlife (discussed above; USEPA 1997). Stations were placed into three categories, "hotspot", "non-hotspot", and "suspect" based on soil and mosquitofish THg concentrations. If a station exceeded both the soil SQG and the wildlife criteria it was considered a "hotspot", if a station exceeded only one of these limits it was considered "suspect" station, if a station did not exceed either of these metrics it was considered a "non-hotspot". Only stations with both mosquitofish and soil THg data were considered in this analysis. Soil THg concentrations, carbon (C):nitrogen (N), C:phosphorus (P) and N:P molar concentration were compared between station categories using the Kruskal-Wallis rank sum test and Dunn's test of multiple comparisons. Dunn's test was performed using the "dunn.test" r-package (Dinno 2015) All statistical operations were performed with R© (Version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria) and the critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Between the 2005 wet and dry seasons 170 stations that were sampled as part of Phase 3 USEPA REMAP had both mosquitofish and sediment THg data. Based on the hotspot identification criteria used in this study, 27 stations were identified as hotspots, 68 as non-hotspots and 75 identified as suspect with most of the hotspots occurring in the wet season (**Figure 3B-24**). During the wet season, sampling areas identified as hotspots occurred in all portions of the EPA with most occurring in central WCA-3A (**Figure 3B-24**). During the dry season several of the locations did not have enough data to assess their Hg biological hotspot potential. This lack of data is expected, during the dry season water levels are generally low resulting in reduced suitable mosquitofish habitat and limitation sampling; as a result, at some sites no mosquitofish were collected. Stations identified as suspect predominately (~76 percent of suspect stations) exceeded the fish tissue criteria but not the sediment SQG limit, suggesting a temporal disconnect as mosquitofish can accumulate Hg faster than sediment or soils. Due to the differences between sediment or soil and mosquitofish metrics, areas identified as suspect should continue to be monitored in an effort to track and accurately assess the ecological hazard associated with Hg exposure in these areas. More research is needed to further understand Hg accumulation dynamics, translocation, and transport in both sediment and lower trophic level organisms to clarify areas considered suspect Hg biological hotspots. Based on the distribution of hotspot and non-hotspot stations (**Figure 3B-24**), it appears a potential hydrologic component is a hotspot driver.

Soil quality guidelines allow for the evaluation of ambient site conditions over a period of time. Furthermore, utilizing the SQG TEL provides a representative assessment of ecological health and linked to a biological response of indicator species. Additionally, assessing fish tissue concentration against a protection criterion represents an ecological hazard to higher trophic level wildlife.

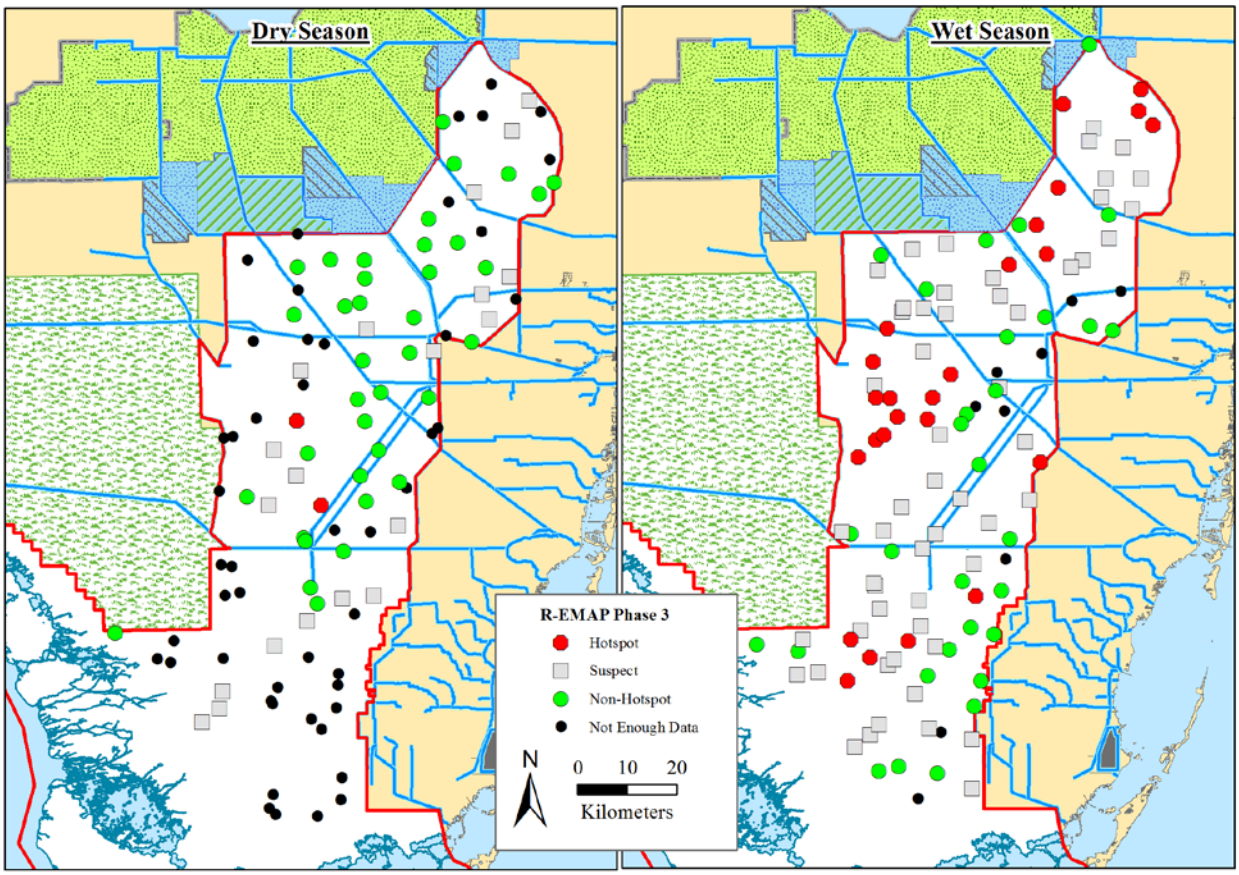

Figure 3B-24. Hg biological hotspot, non-hotspot, and suspect areas identified using Phase 3 (2005) USEPA REMAP data. Hotspot locations were identified by exceeding both the sediment Hg SQG TEL and mosquitofish wildlife protection criteria.

Soil nutrient stoichiometric ratios significantly differed between categories of stations similarly to Julian et al. (submitted). Soil C:N significantly differed between categories ($\chi^2 = 6.9$ df = 2, $\rho < 0.05$) with hotspot being significantly lower than suspect and non-hotspot stations (**Figure 3B-25**). This relationship of soil C:N and hotspot category suggests that litterfall and decomposition plays a role in Hg dynamics. In forested systems, flux of Hg from litter fall to soil is a significant pathway (Demers et al. 2007). The role of litter fall and decomposition has a duel role by altering soil organic matter and contributing Hg to the soil with initial decomposition of litter causing an increase in both C and N. As litter progresses through the decay continuum, soil N concentrations increases, soil C:N decreases, and THg accumulates in the soil (Åkerblom et al. 2007, Melillo et al. 1989, Obrist et al. 2009). Therefore, soil C:N could also be used to further delineate Hg biological hotspots, although more research is needed to identify the C:N breakpoint.

Soil C:P ratios did not significantly differ between categories ($\chi^2 = 6.9$ df = 2, $\rho < 0.05$). Generally, soil C:P ratios were more variable in non-hotspot and suspect stations relative to hotspots (**Figure 3B-25**). Alternatively soil N:P significantly differed between categories ($\chi^2 = 6.2$ df = 2, $\rho < 0.05$) with hotspot being significantly greater than suspect and non-hotspot stations (**Figure 3B-25**). Generally, hotspots were observed in oligotrophic portions of the marsh with few located close to water management features meanwhile non-hotspots were located in both remote (oligotrophic) location and near water management features. Based on nutrient soil ratios it is possible that vegetation, litter fall and decomposition can also play an important role in informing Hg biological hotspot locations. Furthermore, based on the variability of soil nutrient ratios, hydrology may also play a factor.

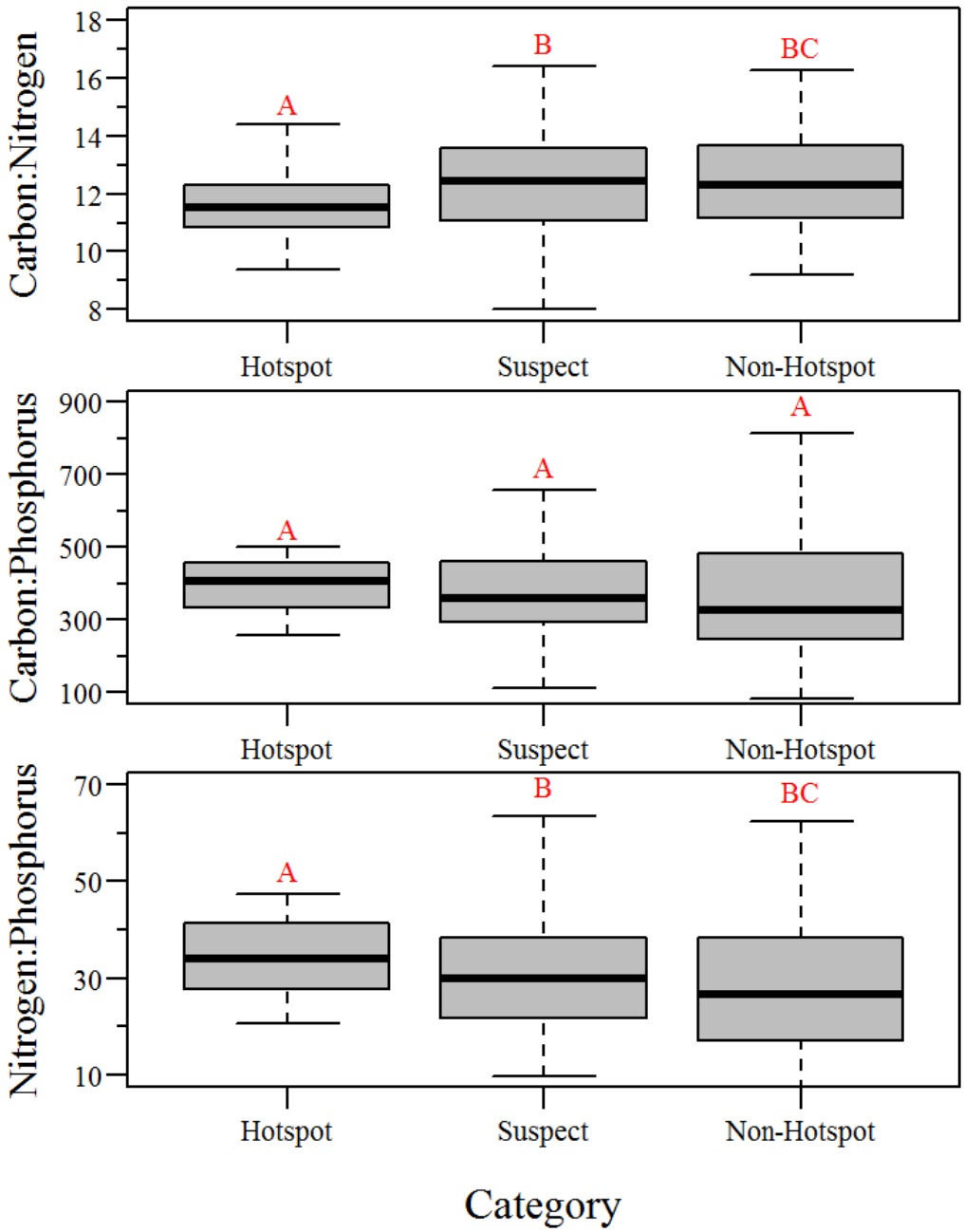

Figure 3B-25: Soil molar nutrient ratios between hotspot, suspect and non-hotspot stations within the EPA during the 2005 Phase 3 USEPA REMAP sampling effort. Different letters indicate statistically significant differences based on results from the Dunn's Multiple Comparison test.

MERCURY SOURCES TO THE EVERGLADES

ATMOSPHERIC MERCURY DEPOSITION TRENDS

Paul Julian II[1]

The source of inorganic mercury (Hg^{2+}) to the Everglades, as in most aquatic/semi-aquatic ecosystems, is through atmospheric deposition (Atkeson and Axelrad 2004, Hammerschmidt and Fitzgerald 2005, Liu et al. 2008). Due to amendments to the Clean Air Act (Public Law 1990), near-field (i.e., local) sources of Hg have been significantly reduced in the United States (FDEP 2013). However, far-field (i.e., global) sources are still relatively unregulated and are the primary sources of Hg to the Everglades (Driscoll et al. 2013). Hg^{2+} from these distant sources are transported via the atmosphere and delivered largely from wet deposition as the result of scavenging of aerosol and reactive gaseous forms of Hg (Guentzel et al. 2001). This section reports on the estimated Hg wet deposition flux and associated atmospheric load to the EPA using monitoring locations within the Mercury Deposition Network.

Methods

Hg wet deposition data was retrieved from the National Atmospheric Deposition Program (NADP) Mercury Deposition Network for the monitoring locations within the EPA (**Figure 3B-26**). The NADP is a cooperative effort among various groups—including federal, state, tribal, and local governmental agencies; educational institutions; private companies; and non-governmental agencies—to provide high quality atmospheric deposition data on various parameters. Similar to monitoring conducted for the NADP National Trend Network (discussed elsewhere in this chapter), rainfall is sampled in a specialized automated collector. Both laboratory and field data are rigorously checked according to quality assurance and quality control protocols approved by USEPA.

There are only three active Mercury Deposition Network stations located within the EPA. Monitoring was discontinued at site FL04 during October 2006, at which time site FL97 began operation. Furthermore monitoring at site FL34 was discontinued at the end of WY2015, at which time FL95 began operations. To provide a regional assessment of Hg wet deposition, monitoring locations were assigned to different regions of the EPA. Monitoring station FL34 and FL95 were used to assess Hg deposition for the northern EPA (WCA-1 and WCA-2), FL97 and FL04 were used to assess Hg deposition within the central EPA (WCA-3), and FL11 was used to assess Hg deposition within the southern EPA (ENP). It is acknowledged that data from three sites at any given time are difficult to justify as representative of the EPA, but what is lacking in spatial coverage is somewhat countered by long-term records and consistent quality assurance.

Monthly Hg atmospheric deposition data were summarized by water year for the POR (WY1996–WY2014). For purposes of this data analysis and summary statistics, data reported as less than the MDL were assigned a value of one-half the MDL, unless otherwise noted. Annual precipitation-weighted mean (PWM) Hg concentrations were also calculated. Atmospheric loads were estimated by multiplying annual Hg atmospheric deposition values by the area of each region within the EPA. Regions were delineated geographically with the northern region accounting for WCA-1, central region for WCA-2 and WCA-3 combined, and southern region for ENP. Water years with less than 12 months of data were not included in any statistical analysis. Trend analysis was performed on Hg deposition and PWM concentrations using the Kendall's τ correlation analysis. All statistical operations were performed with R© (Version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria) and the critical level of significance was set at $\alpha = 0.05$.

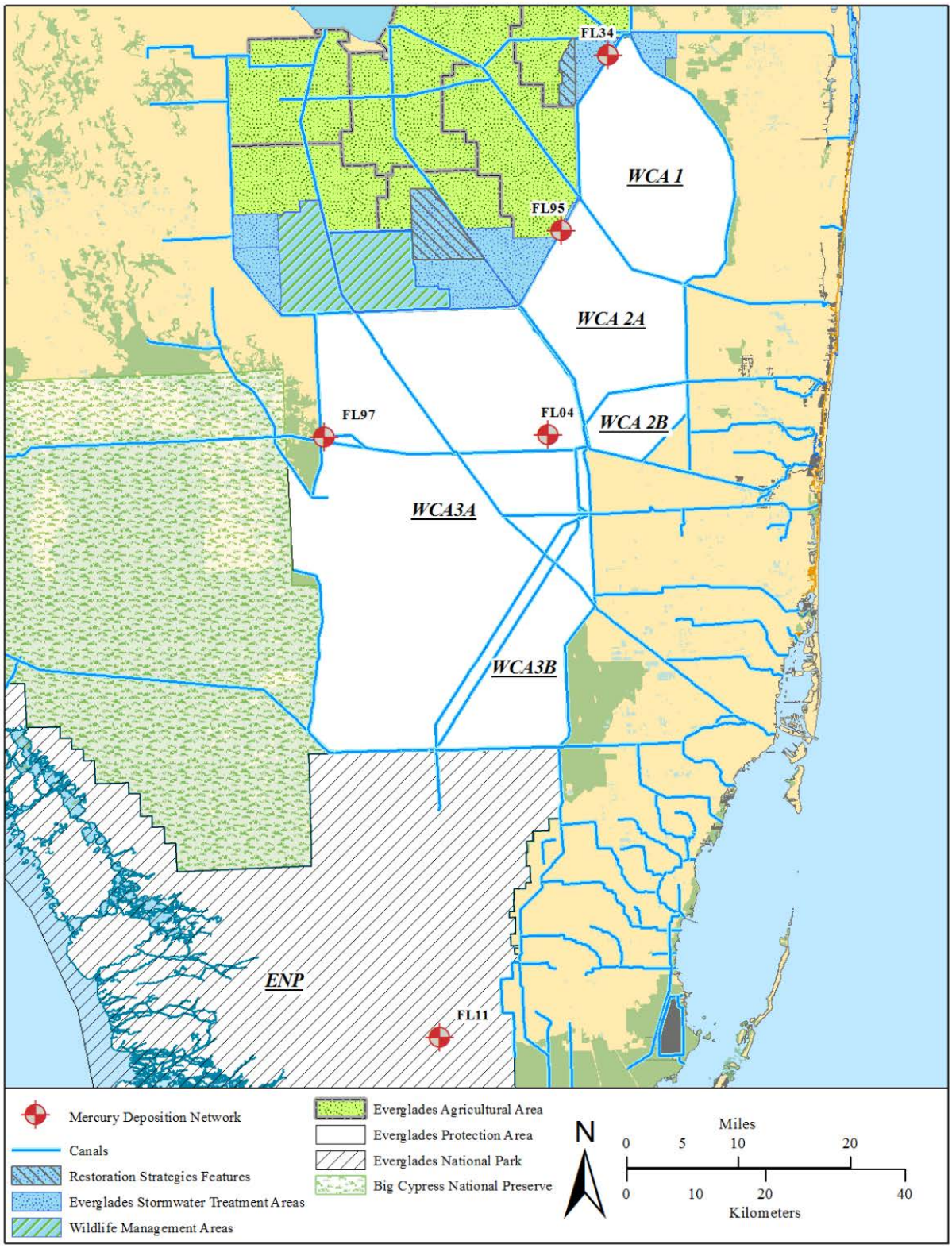

Figure 3B-26. Locations of Mercury Deposition Network atmospheric monitoring locations within the EPA (WCA-1, WCA-2, WCA-3, and ENP).

Results and Discussion

Annual Hg wet deposition has remained relatively constant (for water years with an entire data set) throughout the current POR for the northern, central, and southern EPA, as indicated by no apparent significant trends for the different regions of the EPA (**Table 3B-4**). During WY2015, wet deposition values ranging from 16.4 to 20.1 micrograms per square meter (µg/m^2) for all regions (**Figure 3B-27**) with an overall mean value of 18.1 ± 1.1 µg/m^2. Wet deposition appears to follow a slight south-to-north gradient, with the southern region exhibiting the highest POR mean (WY1996–WY2014) Hg wet deposition (17.4 ± 0.4 µg/m^2), followed by the central (17.4 ± 0.7 µg/m^2), and northern (14.9 ± 0.5 µg/m^2) regions. As the primary source of atmospheric Hg is international, the deposition patterns could potentially be driven by local weather patterns.

Table 3B-4. Kendall's τ trend analysis results for both Hg wet deposition and PWM concentrations for each region of the EPA for the entire POR (WY1996–WY2014).

	Wet Deposition			PWM Concentration		
Region	**Statistic**	**Kendall's τ**	**ρ-value**	**Statistic**	**Kendall's τ**	**ρ-value**
Northern	105	0.23	0.17	0.98	0.16	0.16
Central	95	0.11	0.53	1.30	0.22	0.22
Southern	92	-0.12	0.46	0.82	0.41	0.13

Similar to the wet deposition trend, Hg PWM concentrations remained relatively constant throughout the POR for the southern and central regions of the EPA, with the northern region experiencing slight fluctuations among years (**Table 3B-4**). During WY2015, annual PWM concentrations ranging from 16.2 to 17.5 nanograms per liter (ng/L) with an overall mean concentration of 16.9 ± 0.4 ng/L.

During WY2015, atmospheric loading in the EPA was highest within ENP followed by WCA-3, WCA-1, and WCA-2 (**Table 3B-5** and **Figure 3B-27**). Furthermore, data presented within **Table 3B-5** supports the claim that the primary source of Hg to the Everglades is through atmospheric deposition with percent atmospheric contribution range from 95 to 98 percent throughout the EPA. Hammerschmidt and Fitzgerald (2006) suggested that accumulation of Hg in fish population is linked to atmospheric Hg loading. When taking these deposition values in context to fish tissue Hg concentrations (other sections of this report), there is a qualitatively weak relationship between fish tissue Hg and deposition. While more quantitative analysis is needed, limited data and small regional differences limit investment in more detailed data collection and analysis.

Table 3B-5. Atmospheric load of THg to each region of the EPA during WY2015 and mean (± standard error) surface water load estimated during WY2001–WY2008.

Region	Atmospheric Load (kilograms)	WY2001–WY2008 Surface Water Inflow Load[a] (kilograms)	Percent Atmospheric Contribution
WCA-1	11.4	0.27 ± 0.03	98
WCA-2	10.8	0.51 ± 0.06	95
WCA-3	42.7	1.89 ± 0.18	96
ENP	65.0	1.12 ± 0.21	98

a. Load calculated using annual mean THg concentrations and flow data reported in Chapter 3A of this volume.

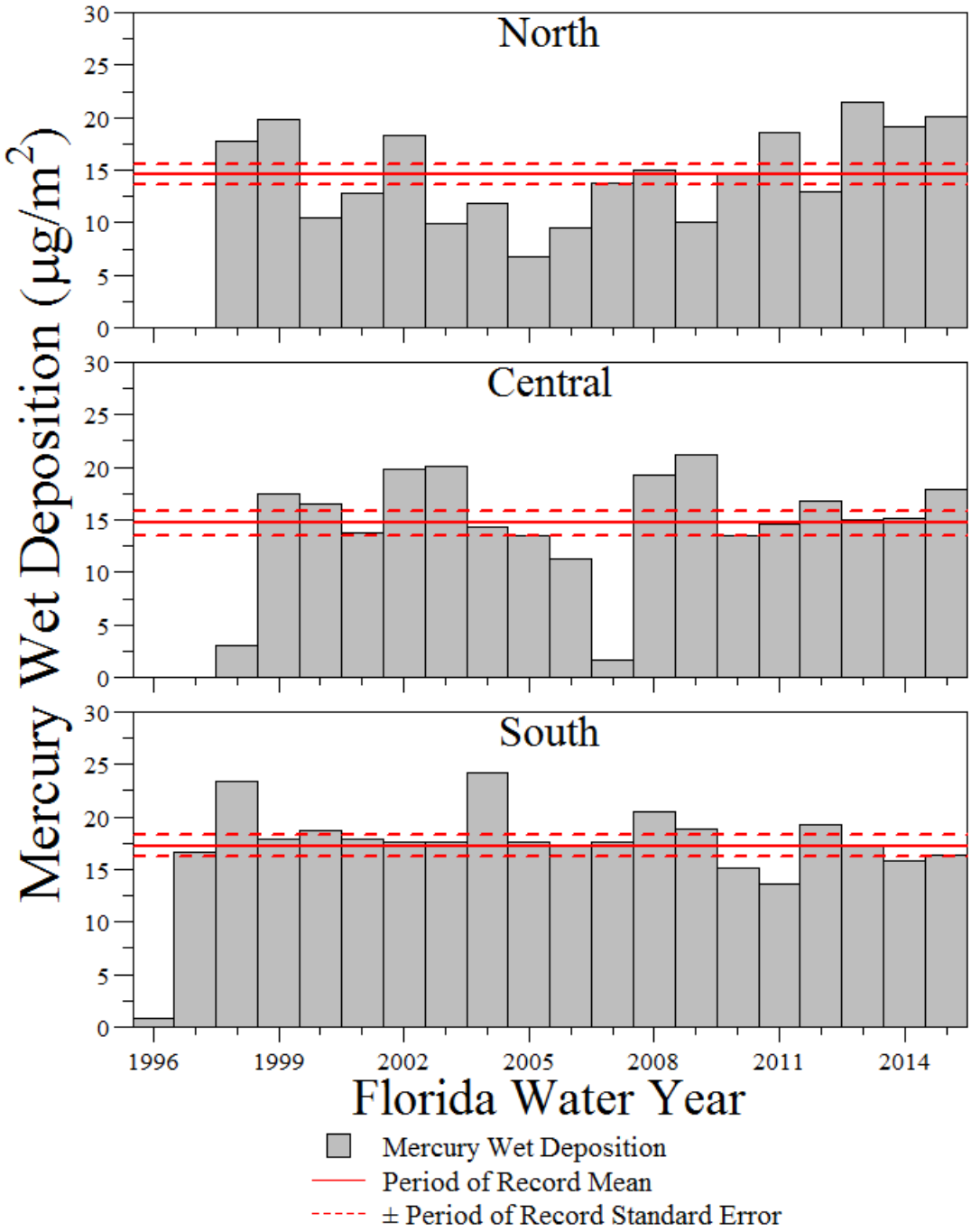

Figure 3B-27. Annual Hg deposition relative to the POR mean (± standard error) for the (A) northern (Stations FL34 and FL95), (B) central (Stations FL04 and FL97), and (C) southern (Station FL11) regions of the EPA during WY1996–WY2015.

Annually wet deposition rates and concentrations were relatively consistent, with the southern and central regions experiencing very little change from year to year. While the northern region has experienced a slight increase in recent years after WY2006, qualitatively, it seems that there is a latitudinal gradient, with the southern and central regions receiving higher atmospheric deposition fluxes relative to the northern region. It should be noted that these values are derived from one or two stations within each region; therefore, these values should be used with caution. Additional analyses should be considered including error analysis associated with the use of these stations to estimate wet Hg deposition as well as exploratory Hg dry deposition analyses to quantify the dry Hg deposition quantities to the Everglades in an effort to refine atmospheric Hg deposition estimates.

Unfortunately, due to the data release schedules, this assessment only spans WY1996–WY2015. Therefore, values presented for WY2015 should be used with caution and are expected to be updated in the following SFER.

SULFUR SOURCES TO THE EVERGLADES

SULFATE WITHIN THE EVERGLADES PROTECTION AREA

Paul Julian II[1]

The primary source of Hg to the Everglades is through global transport and atmospheric deposition, as previously noted. Once deposited, Hg can be converted to MeHg, primarily by reducing bacteria, particularly SRB which utilize SO_4^{2-} to metabolize organic matter under anaerobic conditions. During this process, some SRB have been observed to methylate THg (Gilmour et al. 2013, 1998). The exact quantitative role that SO_4^{2-} plays in the S/Hg biogeochemical cycle in Everglades marshes is still not clear; biogeochemical cycling of Hg 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 SO_4^{2-} can potentially influence the Hg/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 SO_4^{2-} 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 SO_4^{2-} within the EPA, although its role in the Hg problem is not certain at this time.

Methods

A regional synoptic approach similar to that used for water quality evaluations in previous SFERs was applied to SO_4^{2-} data for WY2015 to provide an overview of SO_4^{2-} 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 SO_4^{2-} concentrations within the EPA during WY2015 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–WY2014), and (4) the current water year (WY2015).

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 implementation of the EAA BMP Program was increasing, 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, WY2016) 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., H, J, K, N, O, V, Q, Y, or Z) 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 analytical analysis was flawed (qualifier 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 SO_4^{2-} 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 SO_4^{2-} 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 3.1.2, R Foundation for Statistical Computing, Vienna, Austria) and the critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Sulfate Concentrations

S is an essential plant macro-nutrient (Bellinger and Van Mooy 2012) and enters the Everglades ecosystem primarily as SO_4^{2-} (Orem et al. 2011), but the role of organic S in the total mass of S entering the region remains undetermined. As stated above, SO_4^{2-} is of concern due to its ability under some circumstances to influence biogeochemical processes that lead to Hg methylation and support the production of reduced S compounds under anaerobic conditions. SO_4^{2-} monitoring results are presented in this section to provide an overview of current concentrations and evaluate temporal and spatial patterns. SO_4^{2-} summary statistics relative to the Baseline, Phase I, Phase II, and current year (WY2016) are shown in **Table 3B-6**.

Similar to other water quality parameters within the EPA, SO_4^{2-} follows a general north-to-south concentration gradient (**Figure 3B-28**). This gradient is apparent for inflow regions within the EPA for WY2016, with the highest mean SO_4^{2-} concentrations observed in LNWR (43.8 mg/L), followed by WCA-2 (38.8 mg/L), WCA-3 (11.6 mg/L), and ENP (8.8 mg/L) inflows. Inflows into WCA-2, WCA-3, and ENP experienced a slight decrease in annual mean (arithmetic and geometric) and median SO_4^{2-} concentrations relative to the Phase II period. Meanwhile, annual mean (arithmetic and geometric) and median inflow SO_4^{2-} concentrations into LNWR were elevated relative to the Phase II period.

Table 3B-6. Summary statistics of SO_4^{2-} concentrations in mg/L for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), Phase II (WY2005–WY2015), and WY2016 periods.

Region	Class	Water Year 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	6.2	82.3	28.8	455.8
		1994-2004	309	55.6	34.7	48.9	5.6	50.7	6.7	460.7
		2005-2015	449	51.8	21.0	47.1	5.4	50.5	10.0	115.0
		2016	63	43.8	18.4	40.7	5.2	40.1	18.4	103.0
	Interior	1979-1993	340	16.6	21.5	10.3	4.9	10.0	2.5	220.2
		1994-2004	1205	5.0	11.5	1.0	5.8	1.0	<0.05	110.0
		2005-2015	2474	4.9	11.5	0.6	8.4	0.5	<0.05	95.1
		2016	282	7.5	12.2	0.9	11.3	0.8	<0.05	54.8
	Outflow	1979-1993	61	45.1	36.6	35.8	5.5	34.4	7.3	257.2
		1994-2004	70	50.5	50.8	38.8	5.8	40.6	4.2	418.9
		2005-2015	142	30.5	19.6	22.9	5.5	26.9	1.4	85.4
		2016	22	27.1	17.7	21.1	5.3	22.5	2.0	63.3
	Rim	1979-1993	66	42.2	37.1	25.2	6.5	34.4	2.5	139.8
		1994-2004	345	57.2	26.9	51.0	5.6	49.6	1.6	210.0
		2005-2015	237	50.8	23.6	44.8	5.5	46.3	3.5	185.0
		2016	48	41.8	15.0	39.5	5.1	38.4	22.5	83.4
WCA-2	Inflow	1979-1993	73	75.8	114.9	53.6	6.1	53.8	7.3	945.3
		1994-2004	127	55.3	38.9	48.2	5.6	52.3	7.8	418.9
		2005-2015	467	49.0	17.4	44.2	5.6	48.4	0.1	106.0
		2016	59	38.8	16.0	34.1	5.4	39.6	2.0	67.9
	Interior	1979-1993	742	42.2	35.9	32.7	5.6	36.9	2.5	344.3
		1994-2004	1354	43.1	23.5	35.1	5.7	40.9	<0.05	180.0
		2005-2015	1595	42.3	18.9	36.6	5.5	44.0	1.8	128.0
		2016	186	40.1	16.3	36.1	5.3	38.6	3.9	73.6
	Outflow	1979-1993	103	41.2	21.0	36.4	5.3	38.7	7.6	131.7
		1994-2004	95	28.6	10.9	26.2	4.8	27.9	5.8	54.3
		2005-2015	350	28.7	15.9	23.7	5.1	27.2	3.9	74.7
		2016	47	26.2	12.1	23.6	4.8	23.4	7.8	60.4
WCA-3	Inflow	1979-1993	268	36.7	35.2	24.2	5.9	29.8	1.0	286.0
		1994-2004	182	20.6	16.6	13.3	5.5	16.3	0.5	62.9
		2005-2015	570	20.7	19.8	8.1	8.4	11.7	<0.05	74.7
		2016	167	11.6	13.4	6.5	4.7	4.7	1.1	60.4
	Interior	1979-1993	450	14.9	17.3	10.5	4.6	10.7	2.0	261.5
		1994-2004	1620	10.8	34.8	3.8	6.6	4.5	<0.05	1300.0
		2005-2015	1208	13.2	15.9	3.2	10.3	4.9	<0.05	126.0
		2016	74	11.8	12.3	4.5	7.6	4.2	<0.05	52.7
	Outflow	1979-1993	137	15.9	16.7	10.3	5.0	12.4	1.0	107.6
		1994-2004	134	6.9	7.7	2.5	7.1	4.5	<0.05	36.5
		2005-2015	103	8.9	10.4	1.6	12.3	3.9	<0.05	39.3
		2016	30	9.6	5.3	6.4	5.3	11.6	0.3	17.5
ENP	Inflow	1979-1993	142	15.4	16.3	10.1	4.9	11.5	1.0	107.6
		1994-2004	134	7.4	7.2	3.7	5.9	6.0	<0.05	36.5
		2005-2015	128	7.5	8.1	2.8	7.3	4.6	<0.05	35.8
		2016	38	8.8	5.1	6.4	4.5	9.3	0.7	17.5
	Interior	1979-1993	572	9.0	19.5	4.3	4.4	4.3	0.8	205.5
		1994-2004	864	5.5	17.7	2.1	4.9	2.6	<0.05	403.0
		2005-2015	740	5.6	21.4	1.0	6.6	1.3	<0.05	242.0
		2016	56	1.6	1.7	0.6	4.5	0.8	<0.05	6.8

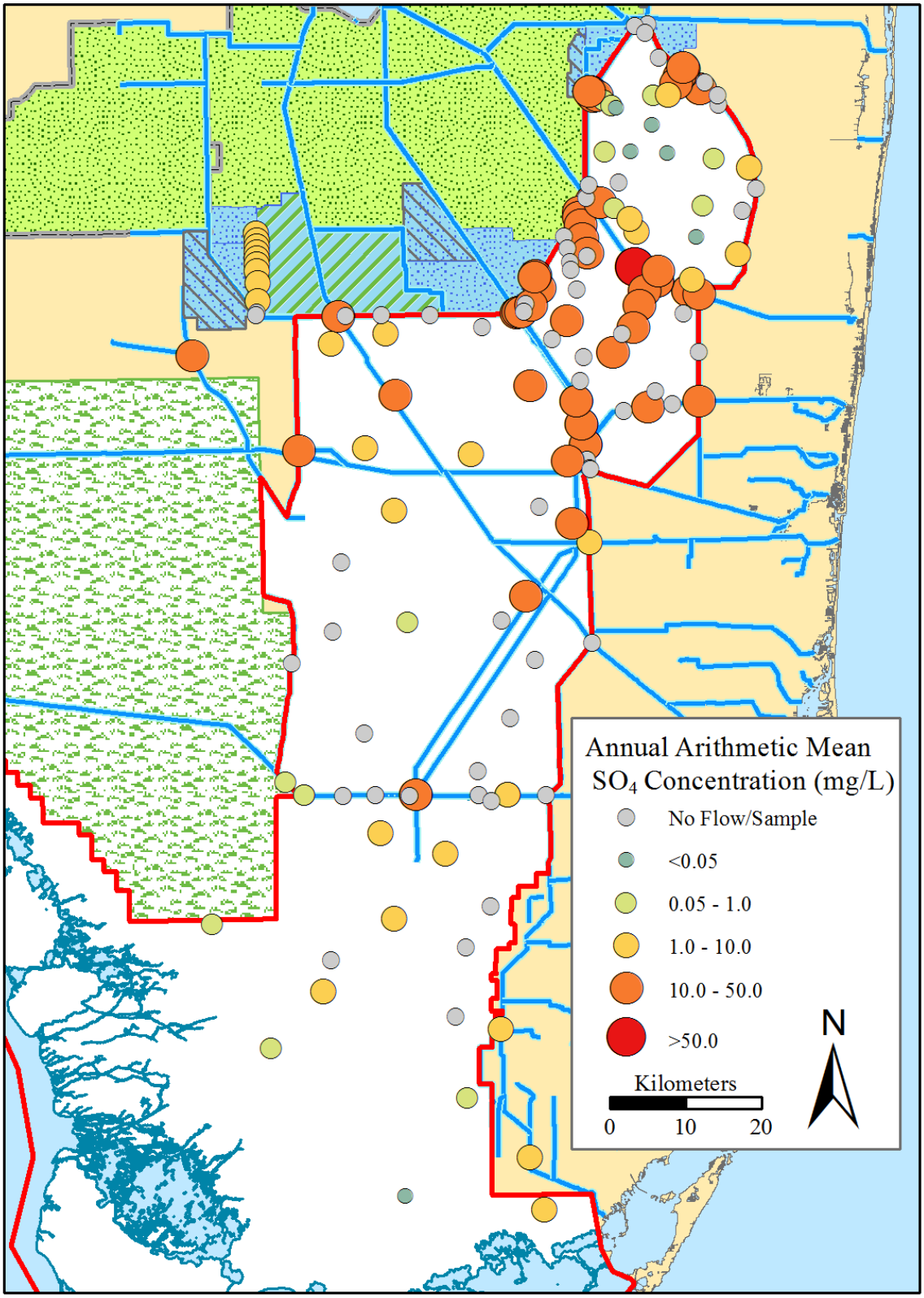

Figure 3B-28. Annual arithmetic mean SO_4^{2-} concentrations for all classifications at stations across the EPA in WY2015.

Although LNWR experienced a slight increase of SO_4^{2-} concentrations in recent years, the entire POR (WY1979–WY2016) reflects a statistically significant decrease in mean concentrations were observed for LNWR inflow and interior regions. WCA-3 and ENP inflow regions also experienced a significant decreasing trend in mean SO_4^{2-} concentrations. No significant trend in annual mean concentration was observed for both WCA-2 inflow and WCA-2 and WCA-3 interior regions (**Table 3B-7**). Due to the abundance of relative low concentration stations, data distribution, and higher values being spread out over a wide range, most valid data within the POR is extremely right-skewed with concentrations ranging from at or below the MDL (0.05 or 0.10 mg/L, depending on the time period) to 103.0 mg/L for the current water year.

Table 3B-7. Kendall's τ annual arithmetic mean SO_4^{2-} concentration trend analysis results for each region's inflow and interior classification within the EPA for the entire POR (WY1979–WY2016). Statistically significant ρ-values are italicized.

		POR (WY1979–WY2016)		
Area	**Class**	**Kendall's τ**	**ρ-value**	**Sen's Slope Estimate[a]**
LNWR	Inflow	-0.35	*<0.01*	-0.92
	Interior	-0.41	*<0.01*	-0.19
WCA-2	Inflow	-0.16	0.18	-0.28
	Interior	-0.14	0.25	0.12
WCA-3	Inflow	-0.42	*<0.01*	-0.49
	Interior	-0.09	0.46	-0.20
ENP	Inflow	-0.34	*<0.01*	-0.06
	Interior	-0.27	*<0.05*	-0.13

a. Expressed as mg/L per water year.

Some annual trends are more pronounced than others, as shown in **Figure 3B-29.** The trend in annual mean SO_4^{2-} concentrations entering seem 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 SO_4^{2-}, 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 SO_4^{2-} 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.

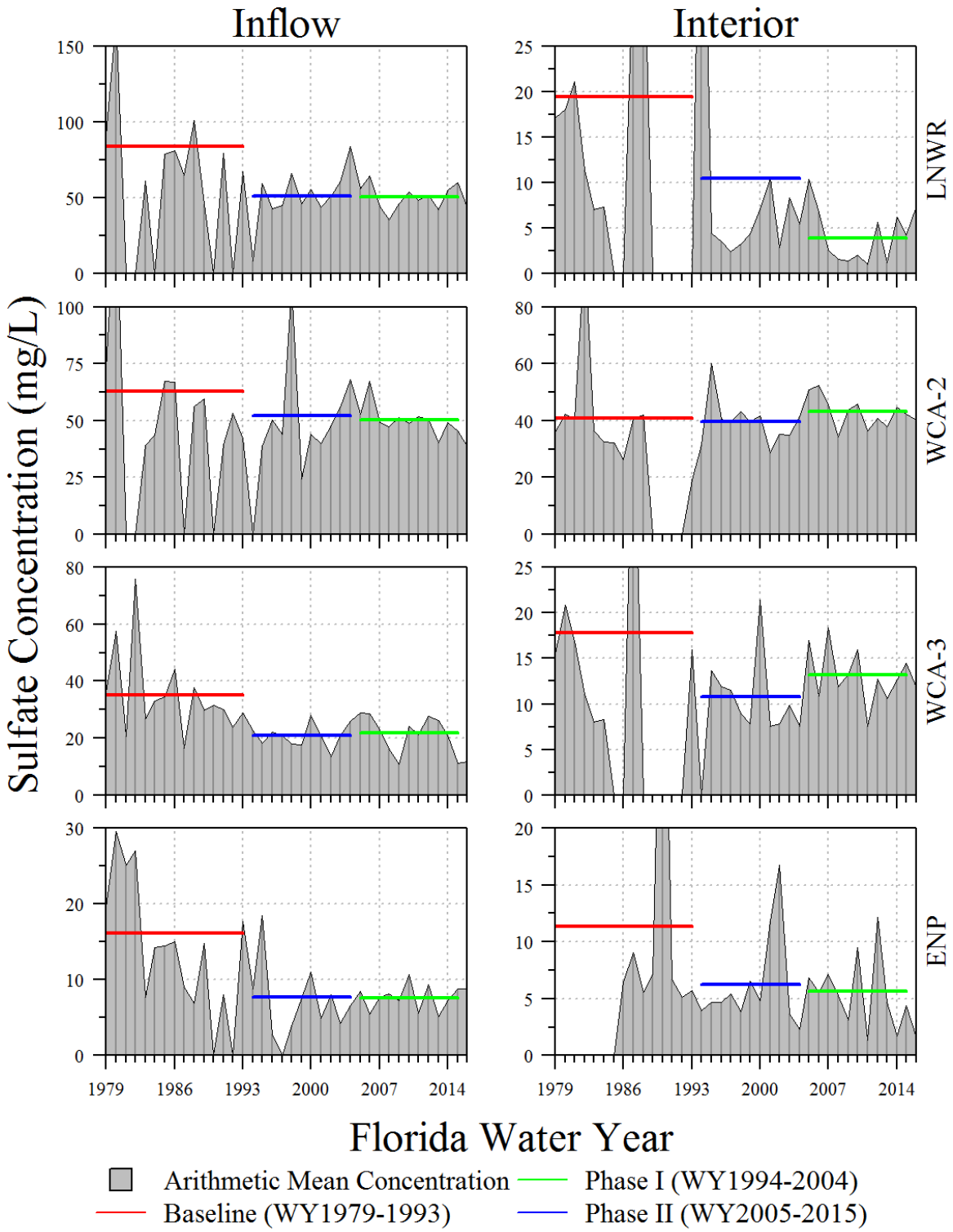

Figure 3B-29. Annual arithmetic mean SO_4^{2-} concentrations for inflow (left panel) and interior (right panel) areas of the LNWR, WCA-2, WCA-3, and ENP during WY1978–WY2016. Bars indicate arithmetic mean when flowing for inflow locations. The horizontal lines indicate the mean annual geometric mean SO_4^{2-} concentrations for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), and Phase II (WY2005–WY2015) 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 somewhat muted at interior monitoring stations of the EPA. During WY2016, WCA-2 had the highest annual mean SO_4^{2-} concentration of 40.1 mg/L, followed by WCA-3 (11.8 mg/L), LNWR (7.5 mg/L) and ENP (1.6 mg/L). Furthermore, LNWR and ENP have experienced statistically significant decreases in annual arithmetic mean SO_4^{2-} concentrations, all other areas experiencing non-significant trends (**Table 3B-7**). SO_4^{2-} concentrations within LNWR significantly decreased during the Phase II period (**Figure 3B-29**), 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 SO_4^{2-} concentrations and loads by a small portion, approximately 10 percent of the SO_4^{2-} from the water column (SFWMD unpublished data). Other factors influencing this trend of decreasing SO_4^{2-} concentrations with time could be changes in water management, establishing and managing BMPs within the EAA, and potential decreasing application of elemental S as a soil amendment to agricultural fields. It should be noted that the BMPs implemented were not used to directly mitigate SO_4^{2-}; however, these BMPs could have provided ancillary benefits. It is also important to note that SO_4^{2-} concentrations within LNWR spiked after extended periods of dry conditions as observed between WY1985 and WY1994. This was not just isolated to water column SO_4^{2-}, but phosphorus and nitrogen also spiked during these periods (Chapter 3A of this volume). The very low SO_4^{2-} concentrations observed for the interior portion of LNWR indicate that either assimilation of $SO_4^{2-}$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 SO_4^{2-} 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 SO_4^{2-} dynamics, sulfur speciation and a more in depth analysis of iron biogeochemistry is needed (Julian et al. in press).

Annual mean SO_4^{2-} concentrations within WCA-2 are approximately twice that of other regions with the EPA. This is due to historical EAA runoff containing both local and regional inputs of SO_4^{2-}. The hydrology of WCA-2A spreads the canal inflow broadly, and WCA-2 soils have relatively high concentrations of S. In fact, samples collected between 2003 and 2004 at limited locations within each region indicate that WCA-2A soils had the greatest concentration of S (14,025 ± 1,173 mg/kg; mean ± standard error), followed by WCA-3A (9,100 ± 576 mg/kg) and LNWR (8825 ± 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. 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 S in WCA-2 and around the periphery of WCA-1. This high concentration of S within the soils could result in excessive internal loading, which explains why interior mean concentrations are higher than inflow mean concentrations. Due to this relatively high marsh concentration within eutrophic/impacted portions of the WCA-2, it is reasonable to suggest that growth of macrophytic biota within this region of the EPA is not S or SO_4^{2-} limited, corresponding to 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 SO_4^{2-} in the EPA. As explained above, the S-Hg biogeochemical cycle has proven to be altered by many environmental factors in the EPA. As a result, empirical evaluation of Hg and SO_4^{2-} data provides little predictive power to link water column concentrations or loads to environmental Hg 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 S inputs can reduce Hg methylation at all or even shift methylation hot spots on the landscape or regional scale. Appendix 3B-1 of this volume summarizes recent data from a District-FDEP hotspot investigation.

In an effort to provide more information on the role of SO_4^{2-} in Hg cycling, FDEP is funding research to investigate Hg methylation potential at low SO_4^{2-} concentrations in surface water. The results will provide evidence on the importance of background SO_4^{2-} levels on methylation and will also help to assess if reduction of S or SO_4^{2-} will cause a positive or negative ecological response. So far this study has yielded interesting results that shows that relatively low SO_4^{2-} additions (i.e., 0.5 to 1.0 mg/L) significantly increases water column MeHg concentrations indicating that non-abatable sources of SO_4^{2-} could support meaningful MeHg production in the presence of bioavailable inorganic Hg (Dierberg et al. 2014, Jerauld et al. 2015).

The commonly referenced 1 mg/L SO_4^{2-} CERP performance measure for the Greater Everglades was developed to indicate background marsh concentrations that would be consistent with S limitation of Hg bioaccumulation. However, this performance measure was proposed without detailed technical support. While concerns have been raised that concentrations above this level could stimulate significant Hg methylation, the 1 mg/L SO_4^{2-} goal is not consistently associated with any particular level of Hg in the Everglades (Julian et al. 2014, 2015a, 2015b). 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 SO_4^{2-} 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.

TRENDS IN ATMOSPHERIC DEPOSITION OF SULFATE

Paul Julian II[1]

Acid precipitation and the associated deposition of acid anions have been widely recognized as an environmental issue in the United States and abroad since the mid-1970s (Dochinger and Seliga 1976). Since that time, several atmospheric deposition monitoring networks have been established to provide data to guide lawmakers in developing environmental policy and law to reduce main anthropogenic causes of acid precipitation. Assessment of the data generated from the atmospheric deposition monitoring network allowed the United States Congress to amend the Clean Air Act in 1990 (Public Law 1990). This amendment was designed to reduce the adverse effects of acid precipitation in the United States through phased reductions in emissions (Lynch et al. 2000).

Elevated atmospheric SO_4^{2-} deposition has resulted in its accumulation in soils and increased loading to surface waters (Alewell et al. 2000). This accumulation within ecosystems such as the Everglades is significant especially as SO_4^{2-} has the potential to interact with several biogeochemical cycles including the phosphorus, iron, and Hg cycles (Gilmour et al. 1992, Reddy and DeLaune 2008). To date, very few studies have focused on the quantity and timing of atmospheric SO_4^{2-} deposition. Studies that have investigated wet SO_4^{2-} deposition have reported rainwater SO_4^{2-} concentrations ranging from 0.5 to 2.5 mg/L (Orem et al. 2011). Orem et al. (2011) concludes that rain water may be an important source of SO_4^{2-} to unenriched areas, but cannot account for its high loads and concentrations in canals or SO_4^{2-} enriched marshes.

This section reports on the estimated SO_4^{2-} deposition flux and associated atmospheric load to the EPA by using data collected from the several atmospheric deposition monitoring locations shown in **Figure 3B-30**.

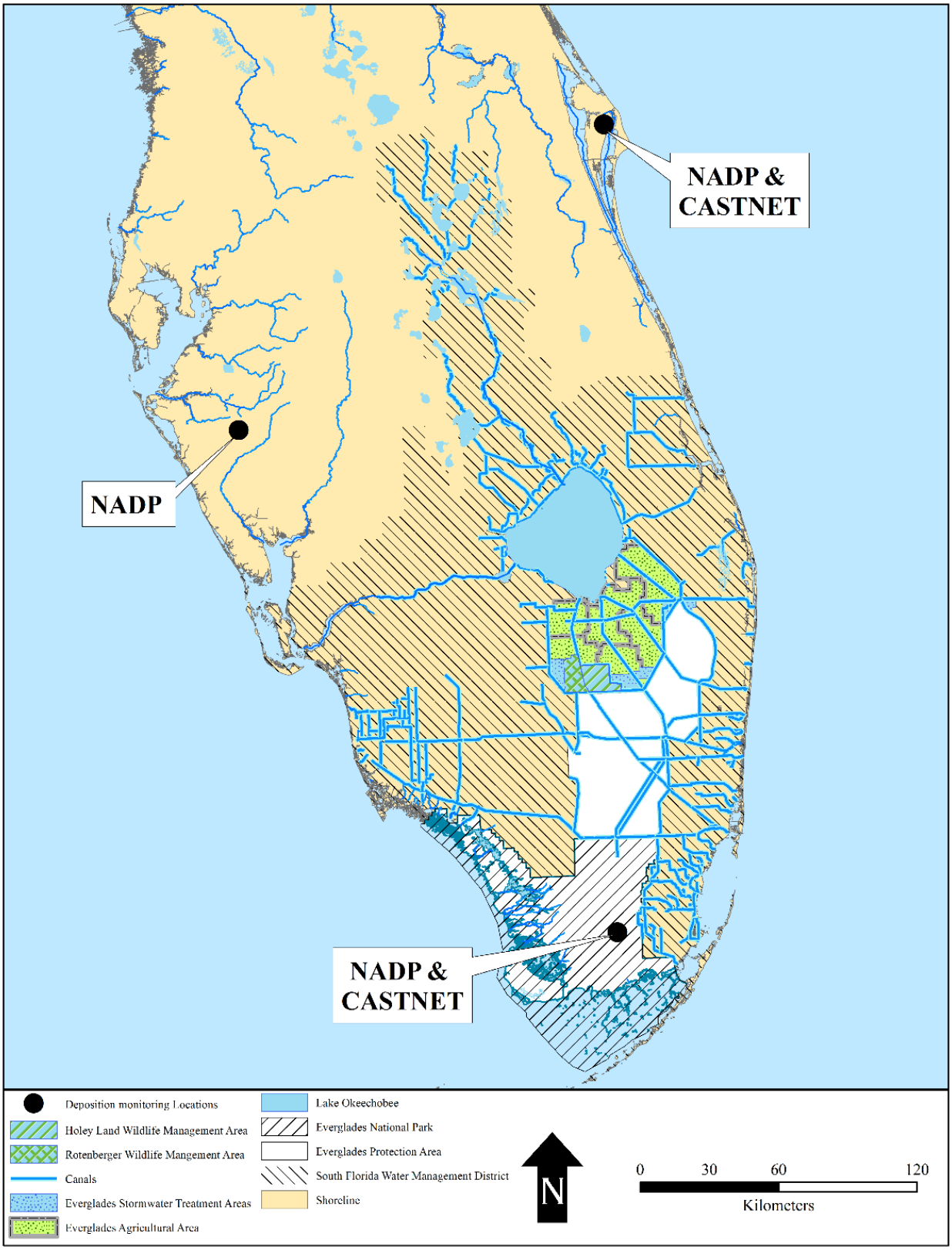

Figure 3B-30. NADP and Clean Air Status and Trend Network stations used in this analysis.

Methods

Atmospheric deposition data was retrieved from the NADP National Trend Network and USEPA Clean Air Status and Trends Network for several monitoring locations identified in **Figure 3B-30**. Due to the limited number of stations located within the Everglades region, two other stations outside of the region were used in conjunction with the Everglades station. As previously noted, the NADP is a cooperative effort between many different groups and stakeholders to provide high quality atmospheric deposition data on various parameters. The NADP and its associated laboratory as part of the National Trend Network measures free acidity (H^+ as pH), conductance, calcium, magnesium, sodium, potassium, SO_4^{2-}, nitrate, chloride, and ammonium. Both laboratory and field data are rigorously checked according to quality assurance and quality control protocols approved by USEPA. Similar to the NADP National Trend Network, the Clean Air Status and Trend Network is a national air quality monitoring network designed to provide dry deposition data to assess trends in air quality, atmospheric deposition, and ecological effects due to changes in air pollutant emissions.

Annual mean SO_4^{2-} fluxes were calculated using data collected from the deposition monitoring locations identified in **Figure 3B-30**. These three sites were identified due to their distribution across the region and study location. As data are being used from two different atmospheric deposition networks, the PORs for each fraction and monitoring location vary slightly (i.e., wet deposition data from WY1995 to present; dry deposition data from WY1999 to present). In an effort to conduct trend analysis, the POR (WY1999–WY2014) mean dry deposition was calculated to fill in data gaps in the earlier portion of the entire data set; only water years with a complete year of data were used to calculate this average dry SO_4^{2-} flux of 85.0 ± 4.7 mg/m^2. Water years with less than 12 months of data were not included in the trend analysis. Trend analysis was performed on wet, dry, and total (wet + dry) SO_4^{2-} deposition and SO_4^{2-} PWM concentrations using the Kendall correlation analysis (Base R package). All statistical operations were performed with R© (Version 3.1.2, R Foundation for Statistical Computing, Vienna, Austria), and the critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Annual SO_4^{2-} wet deposition has significantly declined throughout the POR ($\tau = -0.71$, $\rho < 0.01$) at a rate of 37.0 milligrams per square milligrams per year (mg/mg^2/yr). Water years with complete data range from 653.1 to 1,657.1 mg/m^2/yr (**Figure 3B-31**). A flux rate of 653.1 mg/m^2/yr, the lowest annual deposition rate observed with a complete water year's worth of data. Concurrent with the decreases in annual SO_4^{2-}, rainwater pH continues to significantly increase ($\tau = 0.74$, $\rho < 0.001$) at a rate of 0.01 pH units per year with annual values ranging from 4.7 to 5.2 (**Figure 3B-32**). Annual SO_4^{2-} dry deposition has significantly declined throughout the POR ($\tau = -0.67$, $\rho < 0.05$) at a rate of 3.2 mg/m^2/yr. As a result of the observed declines in wet and dry SO_4^{2-} deposition, total deposition significantly declined throughout the entire POR ($\tau = -0.70$, $\rho < 0.01$) at a rate of 47.2 mg/m^2/yr. Since wet deposition is the majority of the SO_4^{2-} deposited (**Table 3B-8**), the overall total deposition trend and associated magnitude of change is dominated by the trend of wet deposition. However, it is important to note that the magnitude of change between dry and wet SO_4^{2-} deposition rate trends is of an order of magnitude different and that any reductions in SO_4^{2-} deposition rates is beneficial. A decline in total SO_4^{2-} deposition is recent years can likely be attributed to tighter emission controls after a lag period of approximately 10 years.

Similar to the wet deposition trend, PWM SO_4^{2-} concentrations significantly declined throughout the POR ($\tau = -0.59$, $\rho = <0.01$) at a rate of 0.02 mg/L per year, with concentrations ranging between 0.61 and 1.07 mg/L. The range of annual PWM SO_4^{2-} concentrations in this assessment is consistent with values summarized by Orem et al. (2011). Unfortunately, due to the data release schedules, the current assessment only spans WY1995–WY2014. The dry deposition data summarized for the Everglades region corresponds to data from other areas of North America, indicating that dry deposition is unlikely to be a major contributor to the total SO_4^{2-} flux (Mitchell et al. 2013, 2011).

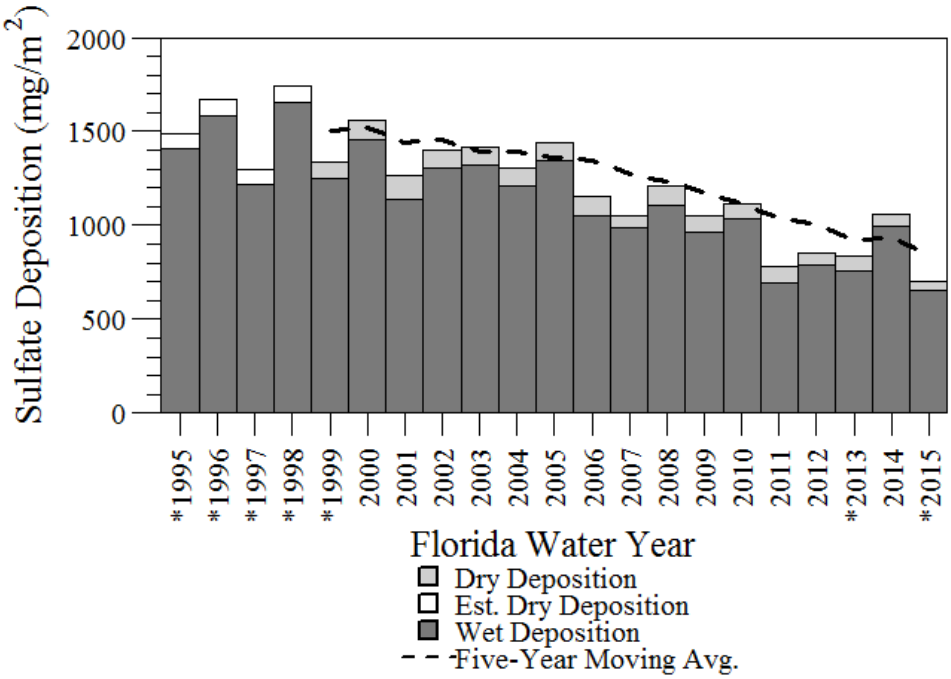

Figure 3B-31. Annual SO_4^{2-} deposition in milligrams per square meter (mg/m^2) and five-year moving average between WY1995 and WY2015. Stations with less than 12 months of data are identified with an asterisk (*).

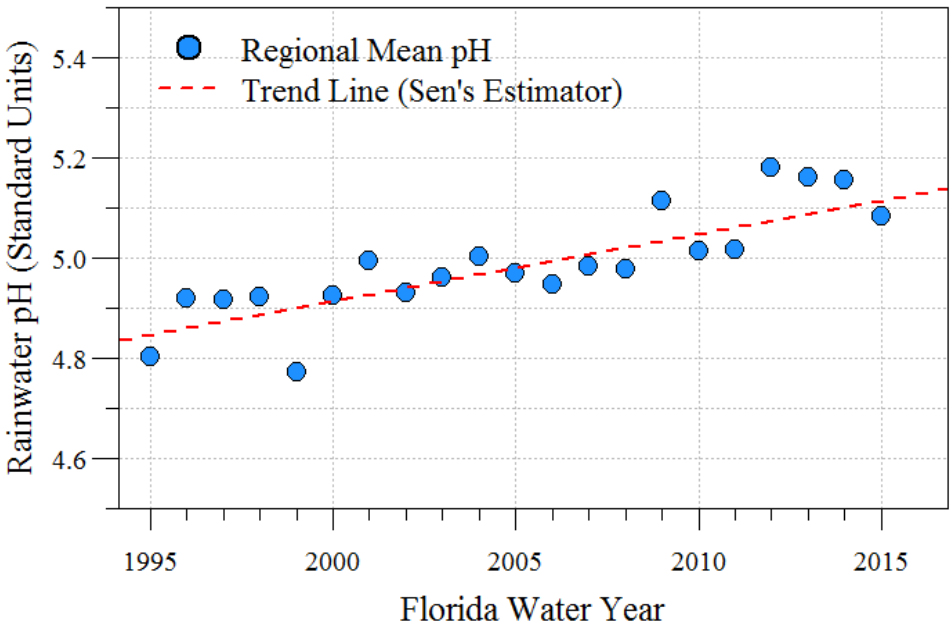

Figure 3B-32. Annual mean rainwater pH between WY1995 and WY2015.

Table 3B-8. Atmospheric SO_4^{2-} load from wet and dry deposition to each region of the EPA for WY2015.

Area	Atmospheric Deposition Load		
	Dry (metric tons)	Wet (metric tons)	Total (metric tons)
WCA-1	26.6	369.4	396.0
WCA-2	25.2	350.0	375.3
WCA-3	112.6	1,562.0	1,674.5
ENP	186.1	2,582.6	2,768.8

During WY2015, atmospheric loading to the EPA was dominated by wet deposition of SO_4^{2-} ranging from 350.0 to 2582.6 t of SO_4^{2-}. Furthermore SO_4^{2-} dry deposition amounts during WY2015 ranged from 25.2 to 186.1 t (**Table 3B-8**). Although an overall decreasing trend in SO_4^{2-}deposition has occurred through the POR (**Figure 3B-31)**, between WY2014 (7,911.1 t) and WY2015 (5214.6 t) a 41 percent decrease in total deposition load occurred.

SULFUR-MERCURY BIOGEOCHEMISTRY

EMPIRICAL EVALUATION OF THE SULFUR-MERCURY RELATIONSHIP

Paul Julian II[1], Steven Hill and Binhe Gu

The Goldilocks zone, window, or theory refers to a posited unimodal relationship between S and MeHg production that strikes a balance between SO_4^{2-} limiting and sulfide inhibition of Hg methylation (**Figure 3B-33**) (Frederick et al. 2005). This hypothesized relationship partially explained the elevated concentrations of Hg in fish from acid-impacted lacustrine lakes. Gilmour and Henry (1991) suggested that freshwater sediments impacted by SO_4^{2-} deposition may be prime locations for microbial methylation of Hg. Later studies hypothesized that this conceptual model could be applied in the Everglades ecosystem to explain elevated THg concentrations in biota and sediment (Gilmour et al. 1998, Benoit et al. 1999a, Krabbenhoft et al. 2000, Orem et al. 2011). These studies conclude that S induces Hg methylation to some degree, a point most could agree to, however, some of these studies go as far as indicating that SO_4^{2-} and Hg exhibit a unimodal relationship within the Everglades ecosystem with little to no statistically evidence to support a unimodal relationship. As a result, this hypothetical S-Hg relationship within the Everglades ecosystems has only been explained qualitatively. The objective of this study is to statistically evaluate the hypothetical S-MeHg relationship by Gilmour and Henry (1991) within the Everglades ecosystem.

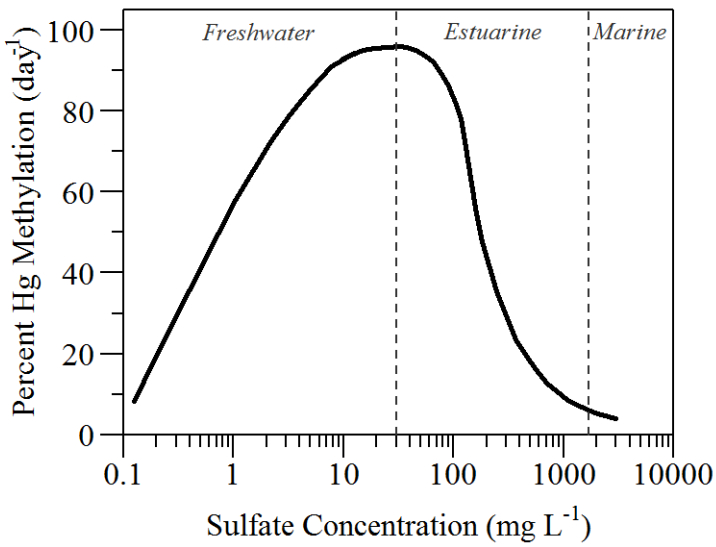

Figure 3B-33: Hypothetical sulfur-mercury unimodal relationship proposed by Gilmour and Henry (1991).

Methods

Data Sources

This study utilized two separate data sets, including a simulated data set consistent with the hypothetical unimodal relationship Gilmour and Henry (1991) referred to as the goldilocks relationship in common press and technical reports (Axelrad et al. 2013, Gilmour 2011, Orem et al. 2011) and data collected as part of the USEPA REMAP sampling effort between 1995 and 2005.

Simulated Goldilocks Data. Using the hypothetical relationship presented by Gilmour and Henry (1991) (**Figure 3B-33**), data was simulated along this hypothetical line in an effort to evaluate statistical tests to determine unimodality. SO_4^{2-} concentrations were simulated from 0.1 to 3000 mg/L which spans the freshwater, brackish, and marine continuum presented in Gilmour and Henry (1991). Hg methylation rate, expressed as a percentage per day was simulated along the hypothetical line across the SO_4^{2-} continuum using a beta distribution. Beta distribution was selected since this distribution is intended to represent proportions and is defined on the continuum between 0 and 1. This data will be used to evaluate statistical tests with data consistent with the hypothetical S-MeHg relationship.

Everglades REMAP Data. In an effort to characterize the Everglades ecosystem with respect to nutrients, Hg, and other parameters of concern, a series of large-scale probabilistic sampling design biogeochemical surveys were completed by USEPA during 1995, 1996, 1999, and 2005. All available REMAP data was retrieved from USEPA (https://www.epa.gov/everglades/environmental-monitoring-everglades) within the freshwater portion of the Everglades ecosystem. For purposes of data analysis and summary statistics, values reported less than the MDL were assigned values equal to the MDL, unless otherwise noted.

Statistical Analyses

There are very few straightforward ways to test whether any given relationship between two variables is unimodal. Therefore, this study utilizes several statistical tests including linear modeling and the Hartigan's Dip test.

Dip Test. (Hartigan and Hartigan 1985) developed a statistical method that measures the departure of the sample from unimodality. This test was coined the "dip test", which produces a statistic that is the maximum difference between the empirical distribution function, and the unimodal distribution function that minimized the maximum difference. Each parameter was evaluated separately using the Hartigan's Dip test (dip() and dip.test() functions in the "diptest" R-library; Maechler 2015).

Regression Modeling. The MeHg- SO_4^{2-} relationship was modeled using a quadratic model. Quadratic (second-order) regression models produce a parabolic geometric shape with the sign of b_2 indicating the direction of the parabola. If b_2 is negative, the parabola will be concave, meanwhile if b_2 is positive, the parabola will be convex (Zar 2010). Therefore, if the quadratic regression model indicates a statistically significant model with a negative b_2 value it can be concluded that the relationship is unimodal. Linear model assumption were tested for quadratic regression models using the Global Validation of Linear Model Assumptions package in R (gvlma library) (Peña and Slate 2006). Local linear non-parametric regression analysis with cross-validated bandwidth selection (Hurvich et al. 1998, Li and Racine 2004) was also applied to the data in an effort to identify a significant S-Hg relationship. All models were tested for significance using methods developed by Racine (1997).

All statistical operation were performed with the base R statistical package. All statistical operation were performed with R© (Version 3.1.2, R Foundation for Statistical Computing, Vienna Austria), the critical level of significance was set at $\alpha = 0.05$, unless otherwise noted.

Results and Discussion

The simulated S-MeHg data based on the hypothetical relationship proposed in the distinguished paper by Gilmour and Henry (1991)(**Figure 3B-33**) was evaluated as a proof of concept before fitting real world data to the statistical tests. The simulated S-MeHg data spanned SO_4^{2-} concentrations from fresh water (i.e., < 1.0 mg/L SO_4^{2-}) to marine (i.e., > 3,000 mg/L SO_4^{2-}) with Hg methylation rates ranging from < 1.0 percent MeHg per day to 100 percent MeHg per day. According to the dip test, the Hg methylation rate was significantly unimodal (D = 0.06, N = 600, ρ-value e< 0.001) with a significant increase in Hg methylation rate between 2.1 and 9.4 percent MeHg per day. Meanwhile, log- transformed SO_4^{2-} was also unimodally distributed (D = 0.07, N = 600, ρ-value<0.01) with a significant change in SO_4^{2-} concentrations between 148 and 2,981 mg/L.

The quadratic model resulted in a significant trend ($R^2 = 0.80$, $F(2,597) = 1,214$, ρ-value < 0.001; **Figure 3B-34**). However, the data and resulting model does not conform to the assumption of linear models (H_0: Data fit assumption of linear models; Global Statistic: 282.18, $\rho < 0.001$). Furthermore, inspection of regression diagnostics confirmed the global validation statistic with the fitted versus residual plot indicated that the model was biased homoscedastic due to its general shape and scatter of the data (**Figure 3B-35**). Additionally, the Q-Q plot indicated that the residuals were not normally distributed due to the deviation from the line at the upper and lower theoretical quantiles (**Figure 3B-35**). Non-parameteric regression of the simulated Hg methylation rate and SO_4^{2-} concentration resulted in a significant relationship with a high goodness-of-fit ($R^2 = 0.92$, ρ-value < 0.001; **Figure 3B-34**). Based on the non-parametric regression, the Hg methylation rate maxima occurs at a SO_4^{2-} concentration of 24.8 mg/L. Furthermore, upon inspection of the regression line (**Figure 3B-34**), one can clearly see a unimodal relationship. While non-parametric regression is not dependent upon a large host of statistical assumption similar to linear models, to determine unimodality is purely qualitative.

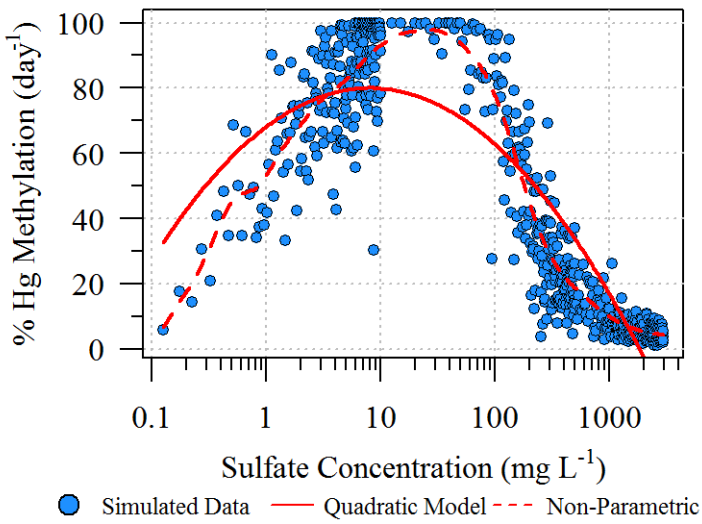

Figure 3B-34. Simulated sediment Hg methylation rate and surface water SO_4^{2-} concentration consistent with the hypothetical S-Hg relationship proposed by Gilmour and Henry (1991) with quadratic and non-parametric regression models.

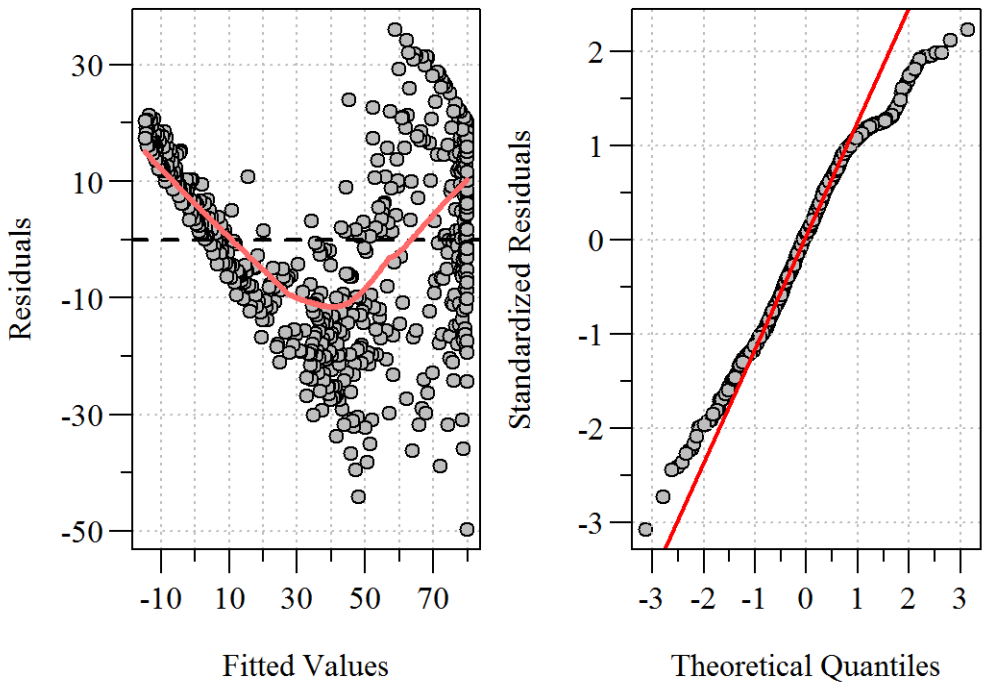

Figure 3B-35. Simulated data curvilinear model diagnostic plots: (left) fitted values versus model residual plot and (right) Q-Q plot of the hypothetical S-MeHg data quadratic model.

Several studies (Benoit et al. 2003, Gilmour et al. 2012, Axelrad et al. 2013, Julian et al. 2014) have substituted Hg methylation rate (i.e., kmeth) with percent sediment MeHg in an effort illustrate the S-Hg unimodal relationship within the Everglades ecosystem. Percent sediment MeHg ranged from < 0.1 to 19.01 percent across all phases of the REMAP monitoring effort. Percent sediment MeHg did not exhibit a unimodal relationship (D = 0.008, n = 808, ρ-value = 0.98). Furthermore, SO_4^{2-} concentrations range from 0.01 to 140 mg/L which corresponds to the "freshwater" band of the hypothesized S-Hg relationship (**Figure 3B-33**) with a significant unimodal distribution (D = 0.02, n = 741, ρ-value < 0.05) with a significant increase occurring at 2.0 mg/L. One drawback to the Dip test is that only considers one variable at a time. However, this analysis aggregates all three phases of the REMAP data set, which spans four years (i.e., 1995, 1996, 1999, and 2005) and two seasons (wet and dry). While aggregating the data sets covers various ecological conditions, breaking the data sets down into each year and season could yield a different result and, if done systematically, could be a valid approach. Therefore, additional analysis is planned to incorporate variability between years and seasons

The results of the quadratic regression applied to percent sediment MeHg and surface water SO_4^{2-} concentration from the REMAP data set resulted in a non-significant relationship with a high degree of variability around the regression line (R2 = 0.002, F(2,688)= 0.7, ρ-value = 0.49; **Figure 3B-36**). Much like the simulated data set, the data and resulting model does not conform to the assumption of linear models as indicated by the results of the global model validation analysis (Global Statistic: 9580.07, ρ < 0.001). Furthermore, the regression diagnostics plots indicate the fitted versus residual plot was biased and not homoscedastic and the Q-Q plots demonstrated the residuals were not normally distributed (**Figure 3B-37**). Additionally, non-parametric regression of the REMAP data resulted in non-significant relationship with a similar coefficient of determination relative to the quadratic model presented above (R2=0.002, ρ-value = 0.22; **Figure 3B-36**). The non-parametric model and the quadratic regression model overlap to the point of almost being indistinguishable with no perceived increase along the entire REMAP SO_4^{2-} continuum. The scatter of the data and the resulting models suggests a highly variable (high unpredictable) relationship between S and MeHg.

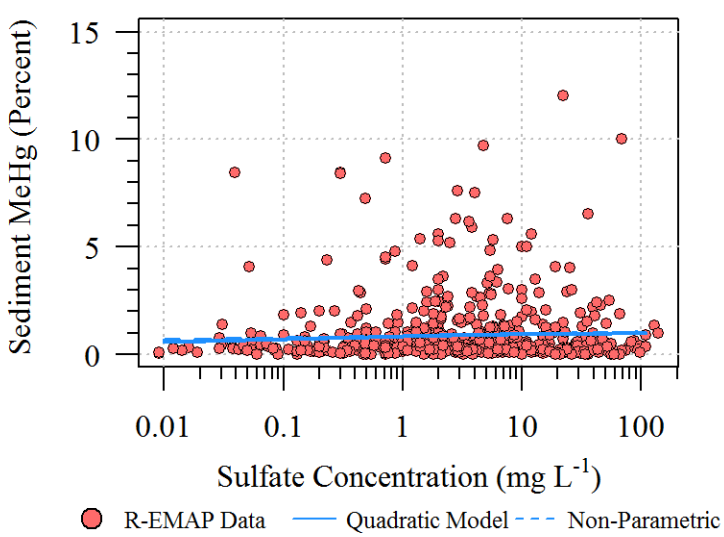

Figure 3B-36. USEPA R-EMAP percent sediment MeHg and surface water SO_4^{2-} concentration with quadratic linear model and non-parametric regression models.

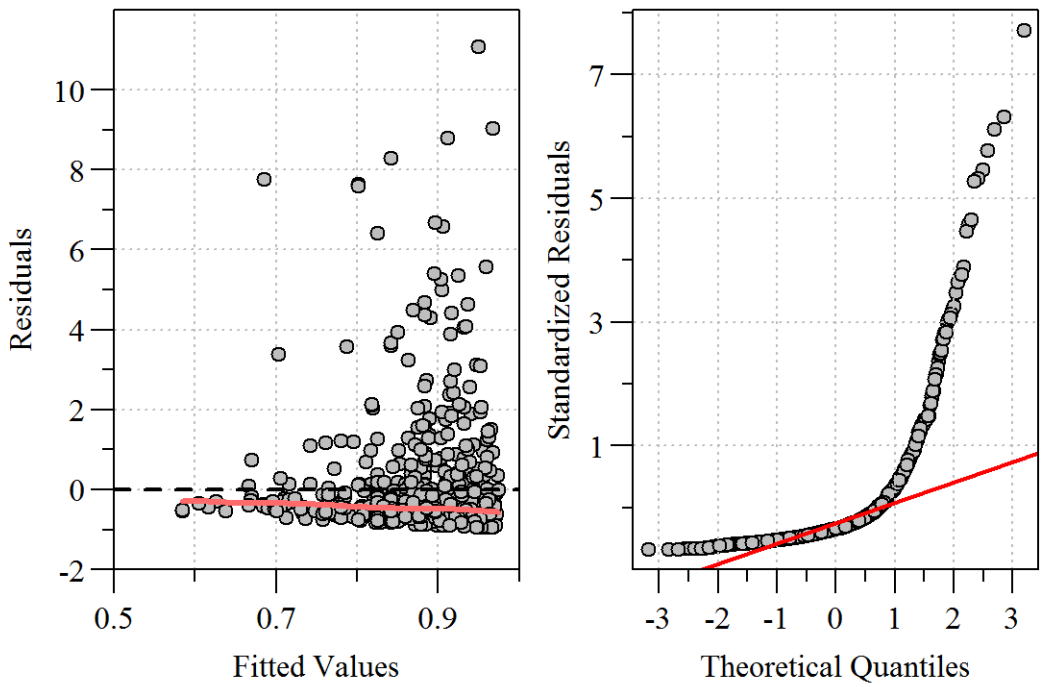

Figure 3B-37. REMAP data curvilinear model diagnostic plots: (left) fitted values versus model residual plot and right*:* Q-Q plot of the hypothetical S-MeHg data quadratic model.

In the original paper that hypothesized the S-Hg relationship, Gilmour and Henry (1991) acknowledged that other water quality conditions such as pH, DOC, oxygen (proxy for redox condition), and SO_4^{2-} all influence net Hg methylation in freshwater ecosystems. Studies elsewhere have demonstrated that other parameters such as porewater iron, soil iron, soil reactive S species, surface water chloride, porewater alkalinity, soil texture, and structure all indirectly influence the Hg methylation by regulating the availability of Hg for methylation or methylation rates directly (Ullrich et al. 2001, Mitchell et al. 2008, Marvin-DiPasquale et al. 2014, Zheng et al. 2013). Furthermore, there are a host of factors that also influence demethylation dynamics (Oremland et al. 1991, Marvin-DiPasquale and Oremland 1998, Liu et al. 2012). Therefore, inclusion of these parameters in a multi-parameter model (i.e., multiple regression) could increase the predictability of Hg methylation rates or percent sediment MeHg in natural systems with models that adhere to the assumptions of linear models thereby reducing Type I error. However, more study is needed to determine which parameters should be considered that provides the highest explanatory power within the Everglades ecosystem. Preliminary analyses has indicated that surface water SO_4^{2-} is important but not the most influence parameter driving Hg methylation in the Everglades ecosystem (Dierberg et al. 2014, Julian and Gu 2014, Jerauld et al. 2015).

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