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

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SUMMARY

This chapter provides an assessment of sulfur and mercury (Hg) status within the Everglades Protection Area (EPA), Holey Land and Rotenberger Wildlife Management Areas (WMAs) during the Water Year 2014 (WY2014) (May 1, 2013–April 30, 2014) reporting period. The report fulfills the requirements of the Everglades Forever Act (EFA), §373.4592(13) Florida Statues. The information provided in this chapter is an update to Chapter 3B of the *2014 South Florida Environmental Report* (SFER) – *Volume I*.

The analysis and summaries provide a synoptic view of Hg and sulfur (S) in the EPA and surrounding areas on a regional scale and include the Arthur R. Marshall Loxahatchee National Wildlife Refuge [Refuge or LNWR; or Water Conservation Area 1 (WCA-1)], Water Conservation Areas 2 and 3 (WCA-2 and WCA-3, respectively), and Everglades National Park (ENP or Park). 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 is reported for the following timeframes: WY2014 for wildlife (mercury in fish tissue for largemouth bass, sunfish, and mosquitofish) and for surface water sulfate; WY2013 for atmospheric deposition of sulfate (wet + dry) and mercury (wet); and calendar year 2013 for mercury in wading bird feathers (great egrets and wood storks).

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

- During WY2014, total Hg (THg) concentrations in largemouth bass (bass; *Micropterus salmoides* Lacepéde) were collected from 15 locations within the EPA and 5 locations outside of the EPA. THg concentration from EPA sites range from 0.14 to 2.71 milligrams per kilogram (mg/kg), with a median of 0.56 mg/kg. THg concentrations for non-EPA sites range from 0.01 to 1.00 mg/kg with a median of 0.41 mg/kg.
- WY2014 THg concentrations in mosquitofish (*Gambusia* spp.) from 12 monitoring sites ranged from 0.01 mg/kg at site WCA2F1 to 0.13 mg/kg at site ROTENC, with a median concentration of 0.03 mg/kg, which is 36 percent lower than the median value of WY2013.
- In WY2014, THg concentrations in trophic level 3 (TL3) sunfish (*Lepomis* spp.) from 12 monitoring sites ranged from 0.13 mg/kg at WCA-2 F2 to 0.36 mg/kg at CA35ALT and L67F1, above the federal methylmercury (MeHg) criterion (0.077 mg/kg; TL3 fish) for protection of wildlife. THg concentrations in these species from all sites followed the period of record trend—highest in spotted sunfish (*Lepomis punctatus*; 0.26 mg/kg), intermediate in bluegill (*L. macrochirus*; 0.19 mg/kg), and lowest in redear sunfish (*L. microlophus*; 0.12 mg/kg).
- In 2013, the mean feather THg concentrations in great egrets (*Ardea alba* L.) from three colonies sampled in WCA-3 ranged from 3.8 mg/kg to 11.1 mg/kg, with an overall mean of 7.4 mg/kg.
- Additional sampling of wood stork (*Mycteria Americana* L.*)* chick feathers was conducted within ENP from three coastal colonies and one inland colony in 2013. Coastal colonies continue to experience elevated THg concentrations relative to inland colonies.
- Wet deposition is the dominate Hg source to the Everglades. During WY2013, wet deposition of THg accounted for 127.8 kilograms of Hg to the EPA[9]. No statistically significant trend was apparent in annual Hg wet deposition.
- A recent long-term mesocosm study demonstrated that long-term increases in surface water SO_4^{2-} concentrations did not result in measureable phosphorus flux from the soil to the overlying water column. This study also observed increased sulfate reduction rates with a minimal MeHg production response.
- A recent microbiological study using molecular techniques explored the distribution of mercury methylating genes in WCA-2A and WCA-3A. Study results showed that sulfate-reducing bacteria potentially capable of syntrophic metabolism are likely to be the dominant mercury methylators in high and low sulfate environments, although the rates of methylation will likely be lower during syntrophic metabolism.
- During WY2014, annual mean inflow SO_4^{2-} concentrations ranged from 55.1 milligrams per liter (mg/L) for the Refuge to 7.2 mg/L for ENP. The annual mean SO_4^{2-} concentrations at interior marsh regions ranged from 44.5 mg/L for WCA-2 to 1.8 mg/L for ENP.

[9] The complete dataset for WY2014 is under quality assurance review and is expected to be reported in the 2016 SFER.

- Sulfate loads from surface sources, including internal transfers to the EPA, totaled approximately 141,289 metric tons (mt), with a flow-weighted mean concentration of 40.1 mg/L. Another 9,231 mt of SO_4^{2-} are estimated to have entered the EPA through atmospheric deposition (wet + dry deposition). The 141,289 mt SO_4^{2-} load in surface inflows to the EPA represents an increase of roughly 38 percent compared to the previous year (102,337 mt in WY2013).
- Total SO_4^{2-} deposition (wet + dry) to the Everglades ecosystem (i.e., EPA) is a small contribution of SO_4^{2-} to the overall system relative to surface water inflows. Contributions for WY2013 range from 0.5 to 13.5 percent of the total SO_4^{2-} load to the EPA. During WY2013, wet deposition of SO_4^{2-} accounted for 6,525 mt to the EPA.[9]
- A multiyear field study was conducted to determine sugarcane yield response to elemental sulfur on organic soils in the Everglades Agricultural Area (EAA) in an effort to update the proposed application of elemental S to improve its management in the EAA. Notably, study results showed that S application did not significantly increase S content of pre-harvest sugarcane samples.
- Under a cooperative agreement between the South Florida Water Management District and Florida Department of Environmental Protection, ongoing studies are being conducted to evaluate factors influencing mercury methylation in South Florida marshes. Based on a long-term mesocosm study of S and Hg marsh biogeochemistry, changes in surface water and porewater chemistry caused by regular SO_4^{2-} dosing of in situ mesocosms were noticeable (see Appendix 3B-1). However, a well-defined MeHg response was not observed and the role of SO_4^{2-} in promoting MeHg production is less clear than once believed. Effective control of MeHg production and accumulation will require a greater understanding of the roles of SO_4^{2-}, Hg bioavailability, dissolved organic matter, seasonal climatic processes, and microbial consortia in this complex ecosystem.
- An EAA regional sulfur mass balance technical webinar was held on November 20, 2013, with presentations from the District, University of Florida Institute of Food and Agricultural Sciences, U.S Geological Survey, and Aqua Lux Lucis, Inc. A peer-reviewed report of this webinar was prepared, containing technical information and recommendations on the most effective ways to improve our understanding of S fate and transport relative to the EAA (see Appendix 3B-2).

AREA OF INTEREST

The Greater Everglades is a vast mixed wetland ecosystem that stretches from Lake Okeechobee to Florida Bay and the Gulf of Mexico (DeAngelis et al., 1998). The EPA, Holey Land and Rotenberger WMAs, and ENP 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 = 0.405 hectare) of former Everglades marsh and currently is divided into separate distinct shallow impoundments, or Water Conservation Areas (WCAs) (Bancroft et al., 1992). In addition to rainfall inputs, surface water inflows regulated by water control structures from agricultural tributaries, such as the 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. Holey Land and Rotenberger WMAs are located just north of the EPA, and together span 64,000 acres and consist of remnant Everglades' marsh with scattered small tree islands (Newman et al., 1998). The major features of the EPA and surrounding area are illustrated in Figure 1-1 of this volume.

MERCURY IN EVERGLADES FISH AND WILDLIFE

Elevated mercury (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* Daudin), crayfish (*Procambarus alleni* Faxon), Florida softshell turtles (*Apalone ferox* Schneider), pig frogs (*Rana grylio* Stejneger), mottled ducks (*Anas fulvigula* Ridgway), white-tailed deer (*Odocoileus virginianus* Zimmermann), and the endangered Florida panther (*Puma concolor coryi* Bangs) (Ware et al., 1991). More detailed synoptic monitoring programs identified elevated but highly variable Hg concentrations in piscivorous wildlife within the EPA including raccoons (*Procyon lotor* L.), alligators, wading birds, and Florida panthers (Porcella et al., 2004; Roelke et al., 1991; Rumbold et al., 2002; Spalding et al., 2000). Because of its large size, extensive wetlands, and relatively high rates of Hg deposition, the Everglades are considered sensitive to mercury methylation and subsequent bioaccumulation into piscivorous wildlife (Wiener et al., 2003). Methylation of inorganic Hg in Everglades wetlands leads to the formation of methylmercury (MeHg), a potent neurotoxin. MeHg accumulation in aquatic biota is of concern for both human health and wildlife who consume fish (Federick et al., 1997; Rumbold et al., 2008) 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 (Hammerschmidt and Fitzgerald, 2006). Because fish are the main MeHg exposure pathway to both human and wildlife consumers (Sunderland, 2007), monitoring is necessary to understand the ecological significance of the spatial and temporal patterns in THg bioaccumulation in the Everglades. This section summarizes the research on the status and trends of mercury in native fish and wading birds from the Everglades region. The status of Hg bioaccumulation in other wildlife species including the American alligator, Florida panther, and Burmese python (*Python molurus bivittatus* Kuhl) is available in the 2011 South Florida Environmental Report (SFER) – Volume I, Chapter 3B (Axelrad et al., 2011).

SPATIAL AND TEMPORAL TRENDS IN MERCURY LEVELS IN EVERGLADES FISH

Binhe Gu and Ted Lange[3]

Introduction

As discussed above, the Everglades ecosystem has and 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 are 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 provides an update to Hg fish tissue concentrations in native Everglades fish along a trophic gradient of species, providing the ability to evaluate spatial and temporal trends in

MeHg exposure scenarios for both wildlife and humans. Fish representing three distinct trophic levels, with varying life histories, allow for assessment of various bioaccumulation and health assessment endpoints. Mosquitofish (*Gambusia* spp.) represent short-term changes in bioaccumulation due to their relatively short life span and limited 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, *Lepomis macrochirus* Rafinesque; redear sunfish, *L. microlophus* Günther; and spotted sunfish, *L. punctatus* Valenciennes) are also common in the canal/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. Both mosquitofish and sunfish 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 largemouth bass (*Micropterus salmoides* Lacepéde) provide a spatially integrated measure of exposure to a long-lived top predator and, as such, are primarily utilized to assess human exposure to MeHg. Mercury concentrations in axial muscle tissue (fillets) from individual bass are used to assess human health risks associated with Hg exposure. Hg effects on Everglades fish health from actual environmental exposure have been documented (Scheuhammer et al., 2007; Wiener et al., 2003); however, little is known about the toxicological significance of MeHg to the native fish within the EPA.

Methods

Fish Sampling within the Greater Everglades Ecosystem

To assess THg concentrations and trends in fish, 12 monitoring stations were established within the EPA and Holey Land and Rotenberger Wildlife Management Areas (WMAs). In addition to these sites, bass were collected at seven additional locations within the EPA, Stormwater Treatment Area 1W (STA-1W), and the Kissimmee Chain of Lakes (KCOL) (**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). Bass collected from other marsh and canal sites within the EPA between WY1989 and WY2014 (May 1, 1988–April 30, 2014) were included in this assessment.

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 sub-samples 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 mm (i.e., 4 to 7 inches) 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 total length (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°C in plastic bags.

Homogenized samples of mosquitofish, sunfish, and bass axial muscle tissue collected from the EPA were analyzed by the District using United States Environmental Protection Agency (USEPA) Method 7473 (Mercury in Solids and Solutions by Thermal Decomposition,

Amalgamation, and Atomic Absorption Spectrophotometry), with a method detection limit (MDL) of 0.005 milligrams per kilogram (mg/kg). Some bass samples collected from the EPA and other areas of the Greater Everglades were analyzed by the Florida Department of Environmental Protection (FDEP or Department) Laboratory, where THg determinations were made using USEPA Method 245.6 (Mercury in Tissues by Cold Vapor AAS), with an MDL of 0.02 mg/kg. A portion of the samples were analyzed by both methods and evaluated to determine comparability. 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 and evaluated against the USEPA trophic level 3 (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.

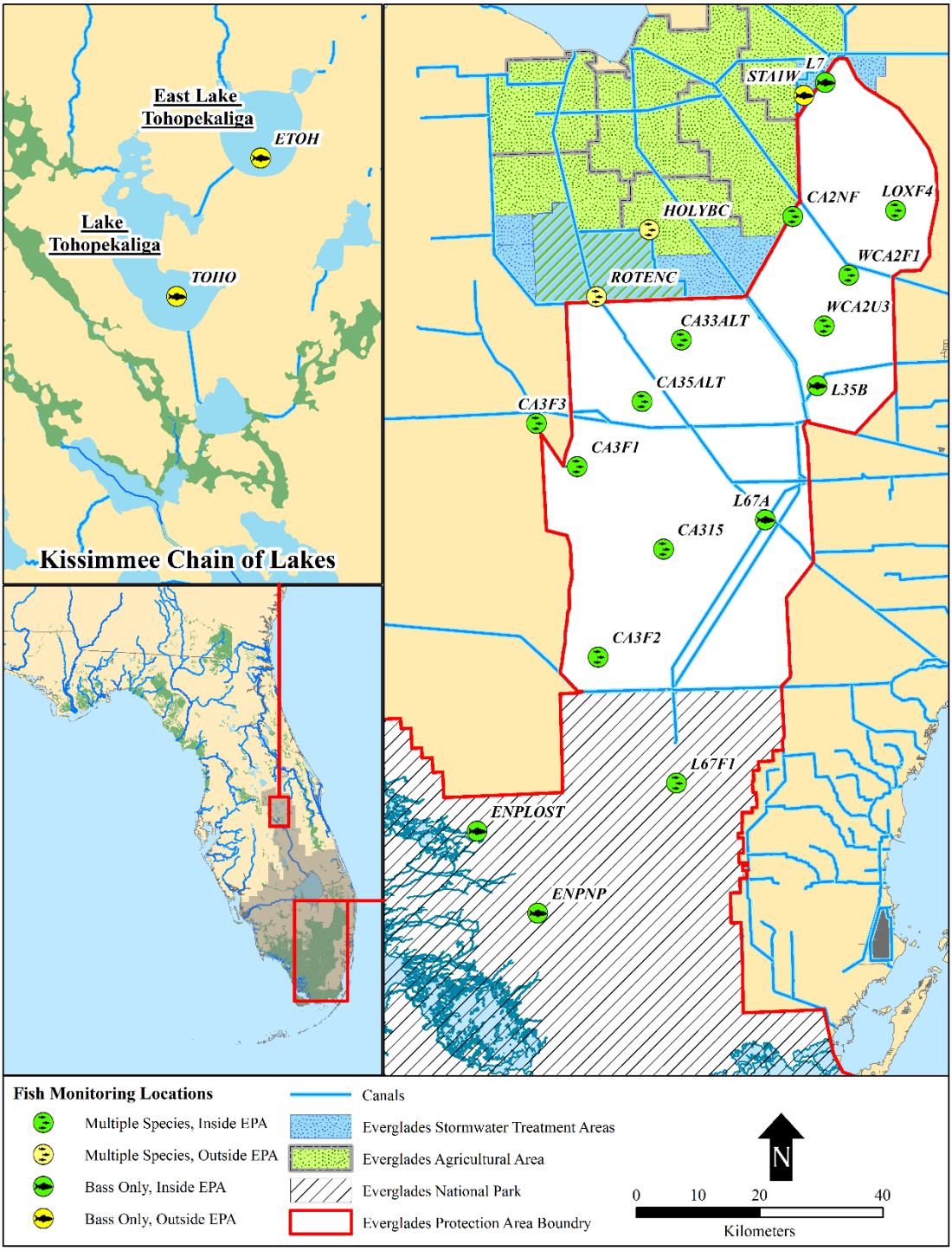

Figure 3B-1. Everglades fish long-term mercury (Hg) monitoring located within the Everglades Protection Area (EPA), Holey Land and Rotenberger Wildlife Management Areas (WMAs) Stormwater Treatment Area 1W (STA-1W) and the Kissimmee Chain of Lakes (KCOL). Fish collected at multiple species monitoring locations include mosquitofish (*Gambusia* spp.), sunfish (*Lepomis* spp.), and largemouth bass (*Micropterus salmoides* Lacepéde). Station CA3F1 is an inactive station with fish sampling activity suspended since October 2009.

Data Screening and Handling

The Hg data evaluated in this section of the chapter were retrieved from the District's DBHYDRO database. Similar to water quality analysis within this chapter and Chapter 3A of this volume, fish tissue data were screened based on laboratory qualifier codes. These qualifiers are consistent with the 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 (SFWMD, 2008). Fatal qualifiers are standard data qualifiers used by both laboratories for sample analysis and data users to indicate that the quality or accuracy of the data may not be suitable for statistical analysis. Additional data for bass were retrieved from the FWC Fisheries Research Lab (Eustis, FL) fish Hg database.

Analysis

Fish tissue THg concentrations were summarized by station, region, and species using basic descriptive statistics for the current WY (WY2014; May 1, 2013 – April 30, 2014) and the entire period of record (WY1999 – WY2014; May 1, 1998 – April 30, 2014). For mosquitofish and sunfish, spearman rank correlation analysis was applied to annual regional and station-specific fish tissue THg concentrations while Kruskal-Wallis analysis was used to test significant difference among three or more data sets. Bass THg concentrations were summarized by station, region, and water year using basic descriptive statistics. Analysis of variance (ANOVA) was used to assess longitudinal trends in THg concentrations among regions while regression analyses were utilized assess temporal trends in THg within the water conservation areas of the EPA. THg concentration values were transformed using the natural logarithm to fit the assumptions of the statistical tests.

Results and Discussion

Mosquitofish

Mosquitofish composite samples from across the EPA have been collected for THg analysis since WY1999 (**Figure 3B-2**). During WY2014, THg concentrations in mosquitofish from 12 monitoring sites ranged from 0.01 mg/kg at site WCA2F1 to 0.13 mg/kg at site ROTENC, with a median value of 0.03 mg/kg, which is 36 percent lower than the median value in WY2013. While the lowest value was also at the same site as WY2013, the highest value was at the canal of Rotenberger WMA. Similar to WY2013, the majority of the sites showed lower THg concentrations in WY2014 than the previous water year.

For the period of record, the median value of THg in mosquitofish is 0.06 mg/kg (n=685). In WY2014, THg concentrations in mosquitofish exceeded the federal criterion of 0.077 mg/kg MeHg for TL3 fish for protection of wildlife (USEPA, 1997) at 1 of the 12 monitoring sites. This is compared to three sites with THg in mosquitofish exceeding the federal criterion in WY2013. The highest median value of mosquitofish THg was observed at both WCA2AU3 and CA35ALT (0.10 mg/kg). For the period of record (POR), 36 percent of the data exceeded the federal criterion. During WY2014, Site CA2NF near the L-6 Canal and site WCA2F1 near the Hillsborough Canal had no exceedances for the monitoring period and displayed the lowest mosquitofish median THg values of 0.02 and 0.01 mg/kg, respectively. It should be noted that CA2NF and WCA2F1 are located in the northern portion of WCA-2A near the Stormwater Treatment Area 2 (STA-2) outflow and within the heavily nutrient enriched areas of the marsh. On the other hand, WCA2U3 is located in the nutrient-poor area on the mid-southern end of the

marsh. This trend supports the theory that fish tissue Hg concentration would be lower in eutrophic/nutrient enriched areas due to the relatively elevated productivity and dilution of available Hg (Lange et al., 1993).

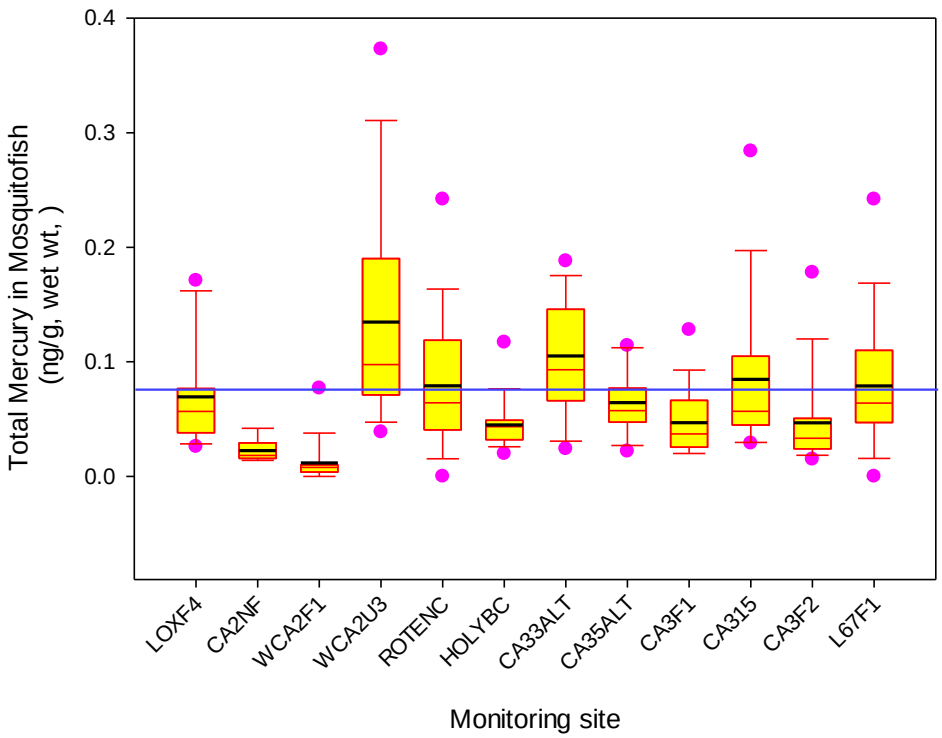

Figure 3B-2. Box plots of total mercury (THg) concentrations in mosquitofish at each monitoring site from Water Years 1999–2014 (WY1999–WY2014; May 1, 1998–April 30, 2014)). Black and red lines within each box are annual average and median value, respectively. Blue line denotes the 0.077 milligrams per kilogram (mg/kg) U.S. Environmental Protection Agency (USEPA) methylmercury (MeHg) criterion for trophic level 3 (TL3) fish for protection of wildlife.

Since WY2006, when samples were collected from all 12 sites, the change in mosquitofish THg concentrations within sites between two consecutive years was relatively high. For example, THg concentrations in the interior site (WCA2U3) of WCA-2 in WY2012 were highest (0.37 mg/kg) for the POR but decreased to 0.19 mg/kg in WY2013 and further decreased to 0.07 mg/kg in WY2014. By contrast, THg concentrations in mosquitofish collected from ROTENC were 0.02 mg/kg in WY2013 but increased to 0.13 mg/kg in WY2014, which represents an increase of more than an order of magnitude. It is not clear what factors control the dramatic temporal variations in mosquitofish THg concentration within sites. Changes in prey availability, biochemical interactions, or a combination of these factors could potentially influence an important role on controlling THg concentration in mosquitofish. Moreover, mosquitofish can vary in trophic level depending on age, habitat, and diet (Williams and Trexler, 2006), which can profoundly impact variability across the landscape.

Low THg concentrations (i.e., less than 0.077 mg/kg) in mosquitofish were found in wet years (WY2000, WY2002–WY2007, WY2011, and WY2014), and THg concentrations greater than 0.077 mg/kg were typically associated with dry years. Dry conditions and dry out have been found to promote sulfate production and Hg release from sediments and, consequently, high rates of Hg methylation in the Everglades (Gilmour et al., 2004b; Rumbold and Fink, 2006).

The annual mosquitofish median THg concentrations were 0.05 mg/kg in WCA-1, 0.03 mg/kg in WCA-2, 0.05 mg/kg WCA-3, and 0.07 mg/kg in ENP (L67F1). These differences in median THg concentrations among WCAs and Park for the POR are statistically significant (Kruskal-Wallis analysis, H=8.83, p=0.03). There was no significant temporal trend of THg in mosquitofish over the POR for any site (Spearman rank order correlation, all p>0.05). This is partly due to considerable interannual variations in THg concentrations within sites (**Figure 3B-3**).

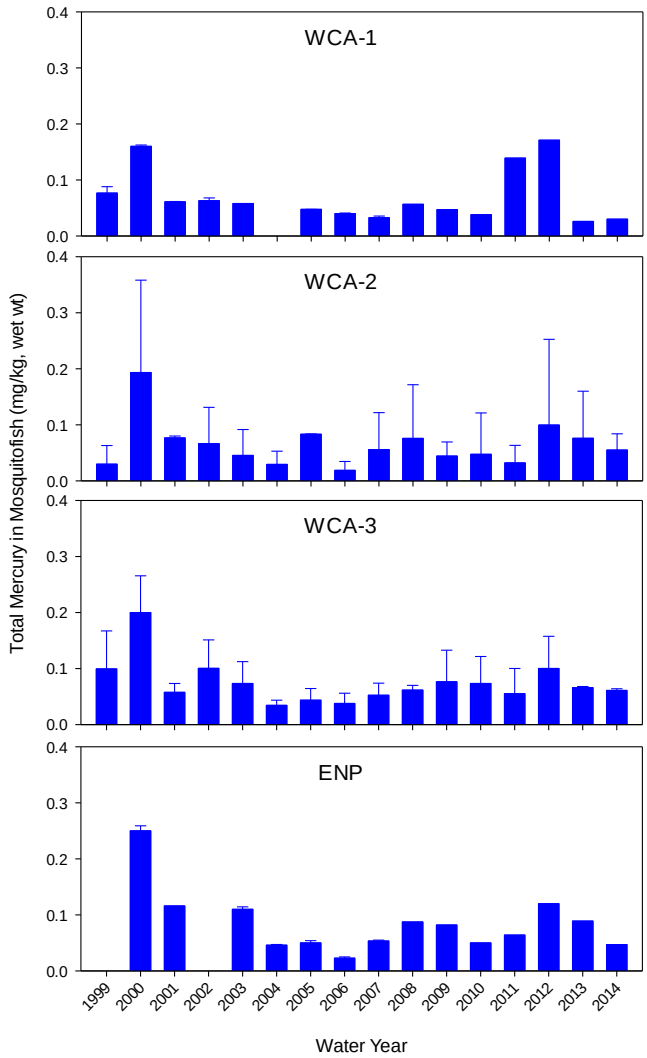

Figure 3B-3. Annual THg concentrations in mosquitofish composite samples at Water Conservation Areas 1, 2 and 3 (WCA-1, WCA-2, and WCA-3, respectively), and Everglades National Park (ENP or Park) from WY1999–WY2014. Error bars represent one standard deviation.

Sunfish

Three TL3 sunfish species—bluegill, redear sunfish, and spotted sunfish—have been sampled for THg analysis in the EPA since WY1999 (**Figure 3B-4**). The overall average sunfish whole body concentration of THg for data pooled from all sites and years is 0.18 ± 0.16 mg/kg (n=3440). These values exceeded the USEPA MeHg criterion of 0.077 mg/kg for TL3 fish for protection of wildlife. Except WCAF1, all other sites displayed an average THg concentration greater than the USEPA criterion.

Average THg concentrations for the POR were highest at the ENP site L67F1 (0.39 mg/kg) and lowest (0.04 mg/kg) at a WCA-2A site (WCA2F1) near the Hillsborough Canal (**Figure 3B-4**). This distribution pattern of THg concentrations in sunfish is consistent with that in mosquitofish. All interior sites in WCA-2 and WCA-3 displayed high long-term average THg concentrations (~0.20 mg/kg). THg concentrations in sunfish tended to increase from north to south (**Figure 3B-4** and **Figure 3B-5**). There was no significant temporal trend of THg concentrations in all three sunfish species within each site (Spearman rank order correlation, all $p>0.05$). However, when only THg data from the target range (102–178 mm) of the dominant sunfish (bluegill) are used, two sites displayed significant trends over time, with increases in THg concentrations in the interior sites of WCA-2 (U3) and WCA-3 (CA33ALT) (**Figure 3B-6**).

During WY2014, the average THg concentrations in the three TL3 sunfish species from 12 monitoring sites was 0.20 mg/kg, with a range from 0.13 mg/kg at WCA2F2 to 0.36 mg/kg at CA35ALT and L67F1. Compared to WY2013, THg concentrations in sunfish on average increased by 0.03 mg/kg. THg concentration in these species from all sites for the POR was highest in spotted sunfish (0.26 mg/kg), followed by bluegill sunfish (0.19 mg/kg), and redear sunfish (0.12 mg/kg), possibly due to feeding preferences among the three species (**Figure 3B-7**).

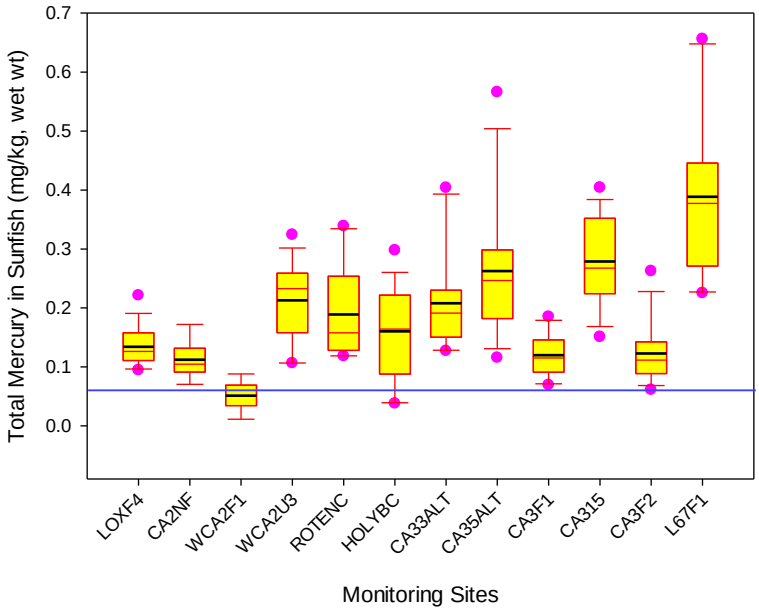

Figure 3B-4. Box plots of annual THg concentrations sunfish at each EPA monitoring site from WY1999–WY2013. Black and red lines within each box are annual average and median value, respectively. Blue line denotes the 0.077 mg/kg USEPA MeHg criterion for TL3 fish for protection of wildlife.

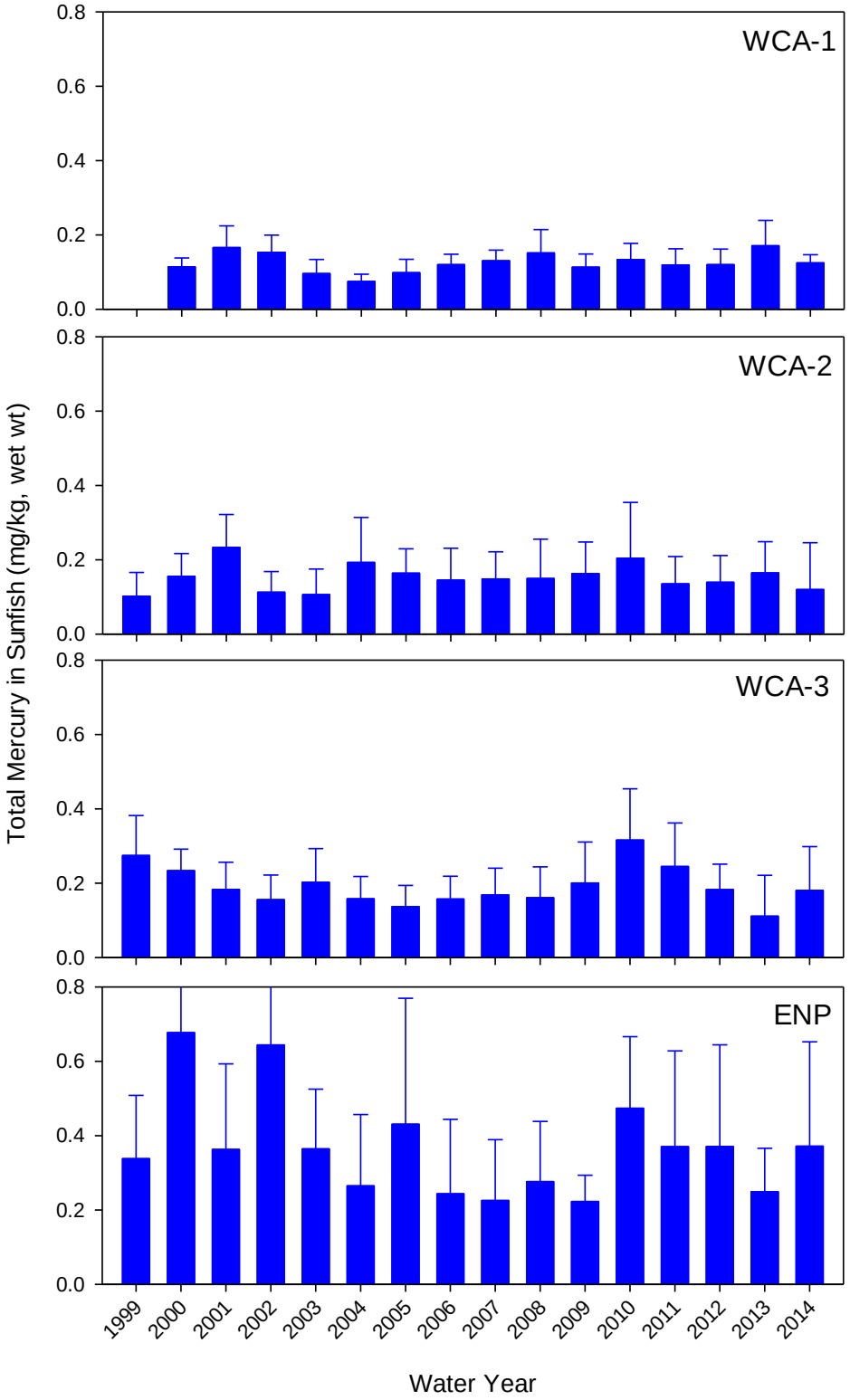

Figure 3B-5. THg concentrations TL3 sunfish at WCA-1, WCA-2, WCA-3, and ENP from WY1999–WY2013. Error bars represent one standard deviation.

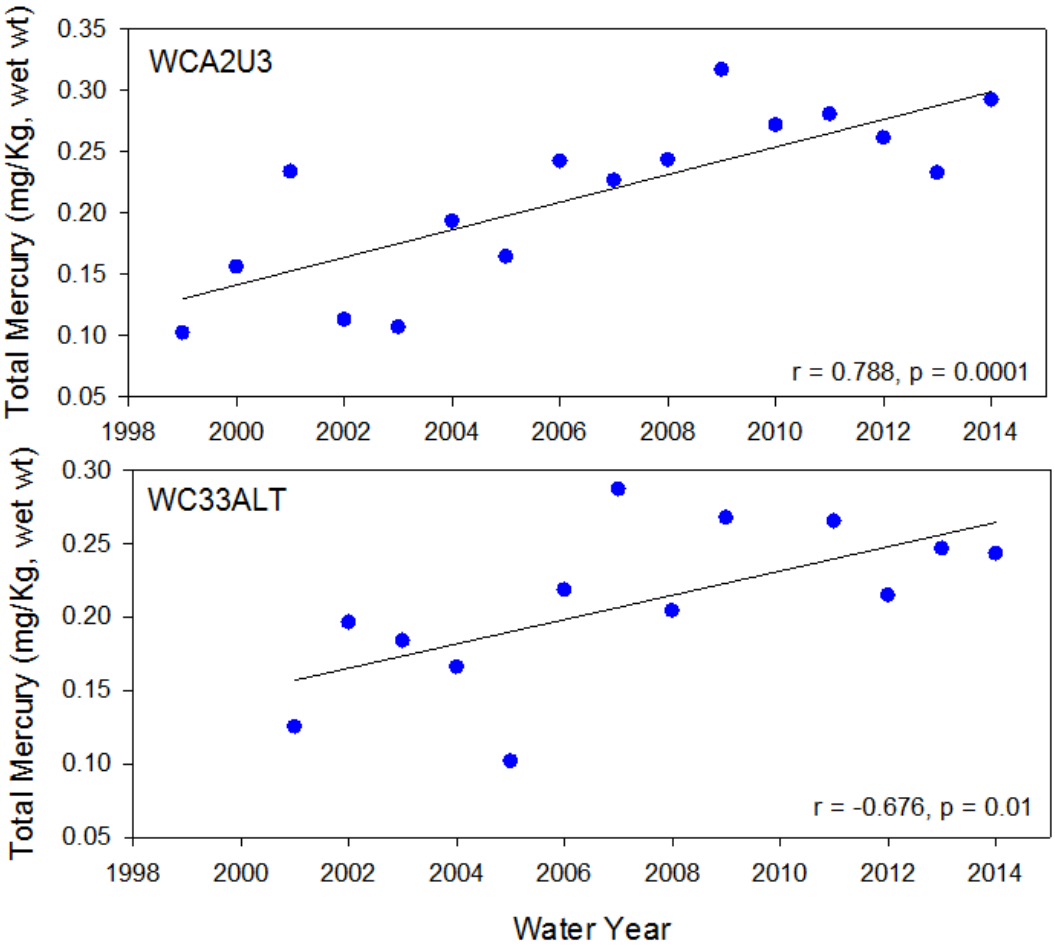

Figure 3B-6. Changes in THg concentrations of bluegill sunfish of the target range [102–178 millimeters (mm)] from WY1999–WY2013 at two EPA monitoring sites. There was a significant increase or decrease of THg concentration over time at these sites. Sites with no significant correlation are not shown.

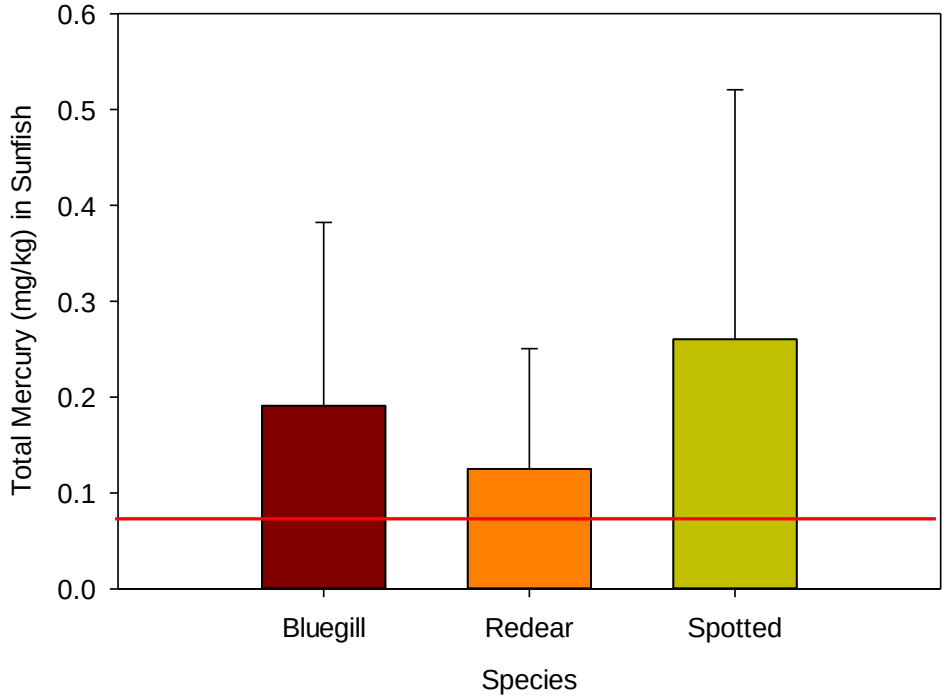

Figure 3B-7. Mean (± standard error) THg concentration for each sunfish species from WY1999–WY2013. Red line indicates the USEPA TL3 fish MeHg.

In summary, the overall median value of THg in mosquitofish across the EPA is below the federal criterion for wildlife protection while the overall median value of THg in sunfish exceeded the criterion. For WY2014, mosquitofish THg concentrations exceeded the USEPA criterion in 1 of the 12 monitoring sites. By contrast, sunfish THg concentrations exceeded the federal criterion in 11 of the 12 monitoring sites. Most sites did not exhibit an increasing trend in THg concentrations over the past 15 years. Mosquitofish THg concentrations in the southern portion of the EPA (WCA-3 and ENP) were generally higher than those in the central and northern WCAs. Similarly, there is a clear north-to-south trend of increasing THg concentrations in sunfish in the EPA, consistent with the distribution pattern of Hg levels in bass (Julian et al., 2014a).

Largemouth Bass

The POR for largemouth bass collections within the EPA extends back to WY1989 when assessments determining the range and extent of Hg contamination in fish and wildlife were conducted by cooperating state agencies (Ware et al., 1991). During this period, 43 sites were sampled including the long-term monitoring sites identified in **Figure 3B-1**, where most bass samples were collected. Between WY1989 and WY2014, THg concentrations in individual bass ranged from 0.01 to 4.80 mg/kg, with a median of 0.54 mg/kg (n=4991). Similarly, at five locations outside the EPA sampled between WY1990 and WY2014 (ETOH, TOHO, STA1W,

HOLYBC, and ROTENC), THg concentrations were slightly lower, with individual bass ranging from 0.001 to 2.31 mg/kg and a median of 0.41 mg/kg (n=1970).

During WY2014, bass were collected from 15 sites within the EPA and an additional five from other waters of the Everglades (**Table 3B-1**). THg concentrations in individual bass at EPA sites ranged from 0.14 to 2.71 mg/kg with a median of 0.56 mg/kg (n=257) while at non-EPA sites concentrations in individual bass were lower, ranging from 0.01 to 1.00 mg/kg with a median of 0.41 mg/kg (n=100). Both medians exceeded the USEPA-recommended MeHg criterion for the protection of human health of 0.35 mg MeHg/kg in fish tissue (USEPA, 2001) as well as 82 and 63 percent of all bass from the EPA and non-EPA sites, respectively. Median THg concentrations did not exceed the human health criterion at only two locations during WY2014 including the artificial wetland within STA-1W and marsh site LOXF4 in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge or LNWR) **(Table 3B-1**). It should be noted that recreational fishing is not allowed in either area.

It is evident that a strong north-to-south gradient in Hg bioaccumulation exists across the Greater Everglades (**Figure 3B-2** and **Figure 3B-8** top panel). Within the EPA, median THg concentrations ranged from a low of 0.24 mg/kg in the LNWR marsh at site LOXF4 to 1.25 mg/kg in the estuarine portion of ENPSRS at site ENPNP (**Figure 3B-8**; bottom panel). Despite variations among sites within regions (i.e., individual WCAs), significant differences ($F_{1,348}$=73.38.62, $P<0.001$) were observed in mean THg among regions within the EPA with LNWR<WCA2=WCA3<ENPSRS (**Figure 3B-9**). Across the Greater Everglades region, there was no difference between regions KISS and LNWR, suggesting that high rates of MeHg bioaccumulation extend to the extreme northern Everglades drainage. Similarly, to the south, there were no significant differences observed among WCA2, WCA3, and ENPLOST, which appear to represent MeHg bioaccumulation in areas of ENP outside of the Shark River Slough (ENPSRS). There was a significant increase among sites from north to south (**Figure 3B-9**), while notably there were high rates of MeHg bioaccumulation consistently observed in the Shark River Slough (ENPSRS) where sunfish and bass THg concentrations exceeded all other areas of the EPA and the Greater Everglades. Also, there have been low THg concentrations in bass from STA-1W consistently. The WY2014 median THg in STA-1W bass was 0.04 mg/kg, which was significantly lower ($F_{1,348} = 73.38$, $P<0.001$) than all other regions. Since monitoring started in WY1995, STA-1W bass have been lower in THg than all EPA regions, and median THg concentrations have remained well below the USEPA human health criterion. Early concerns that the Everglades Nutrient Removal (ENR) Project—the precursor to STA-1W—would promote high rates of MeHg bioaccumulation due to inundation of organic-rich soils proved unfounded. Ultimately, the man-made marsh has functioned as a THg and MeHg sink, removing about 70 percent of the inflow mass (Miles and Fink, 1998).

Table 3B-1. Summary of total mercury (THg) concentrations in largemouth bass (*Micropterus salmoides* Lacepéde) by locations sampled during Water Year 2014 (WY2014) (May 1, 2013–April 30, 2014). Grouping by region and sites located within the Everglades Protection Area (EPA) are identified. No bass were collected from site WCA2F1. Sites are arranged by latitude from north to south.

				WY2014 THg mg/kg)		
Region	**Within EPA**	**Site Name**	**Description**	**N**	**Median**	**Range**
KISS	NO	ETOH	East Lake Tohopekaliga	21	0.41	0.26 - 0.93
KISS	NO	TOHO	Lake Tohopekaliga	20	0.33	0.15 - 0.76
STA1W	NO	STA1W	Man-made marsh, Cell 3	19	0.04	0.01 - 0.18
LNWR	YES	L-7	Canal	21	0.49	0.18 - 1.40
LNWR	YES	LOXF4	Marsh	20	0.24	0.16 - 0.51
HOLYBC	NO	HOLYBC	Holeyland WMA, North Borrow Canal	20	0.51	0.17 - 1.00
ROTENC	NO	ROTENC	Rotenberger WMA, East Borrow Canal	20	0.60	0.43 - 0.88
WCA2	YES	CA2NF	Marsh	20	0.30	0.17 - 0.73
WCA2	YES	WCA2F1	Marsh	NS		
WCA2	YES	WCA2U3	Marsh	20	0.61	0.17 - 1.20
WCA2	YES	L-35B	Canal	21	0.92	0.14 - 2.00
WCA3	YES	CA33ALT	Marsh	1	0.54	
WCA3	YES	CA35ALT	Marsh	9	0.94	0.66 - 1.29
WCA3	YES	CA3F3	Marsh	20	0.35	0.25 - 1.07
WCA3	YES	L-67A	Canal	20	0.88	0.42 - 1.80
WCA3	YES	CA315	Marsh	25	0.66	0.25 - 1.50
WCA3	YES	CA3F2	Marsh	20	0.38	0.14 - 1.33
ENPSRS	YES	L67F1	Canal/Marsh	20	1.13	0.30 - 2.71
ENPSRS	YES	ENPNP	Estuarine Creek-North Prong Creek	20	1.25	0.50 - 2.10
ENPLOST	YES	ENPLOST	Estuarine Creek-Lostmans Creek	20	0.54	0.24 - 1.20

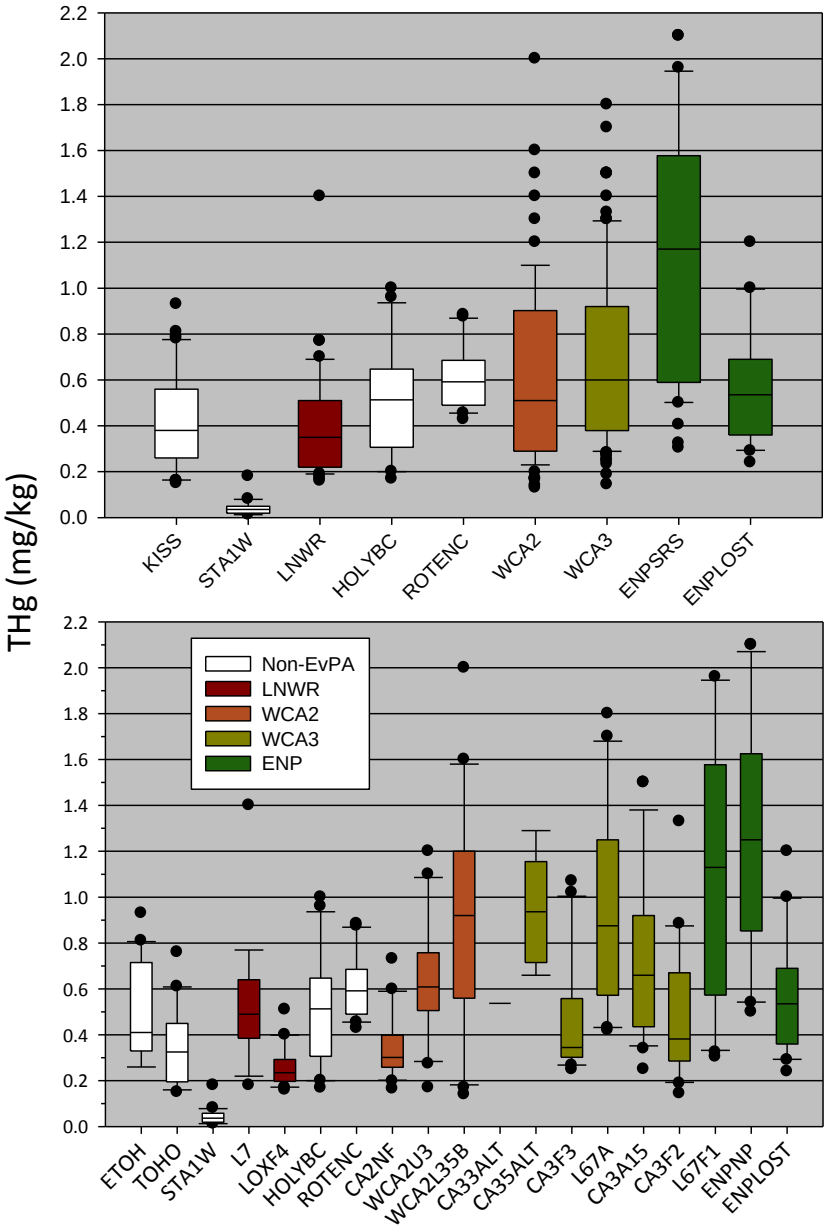

Figure 3B-8. THg concentrations in axial muscle tissue of 357 largemouth bass collected from the Everglades during WY2014, summarized by region (top panel; see **Table 3B-1**) and by individual sample sites (bottom panel). Box plots represent the median and 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and points are outliers. Sites are arranged from north to south.

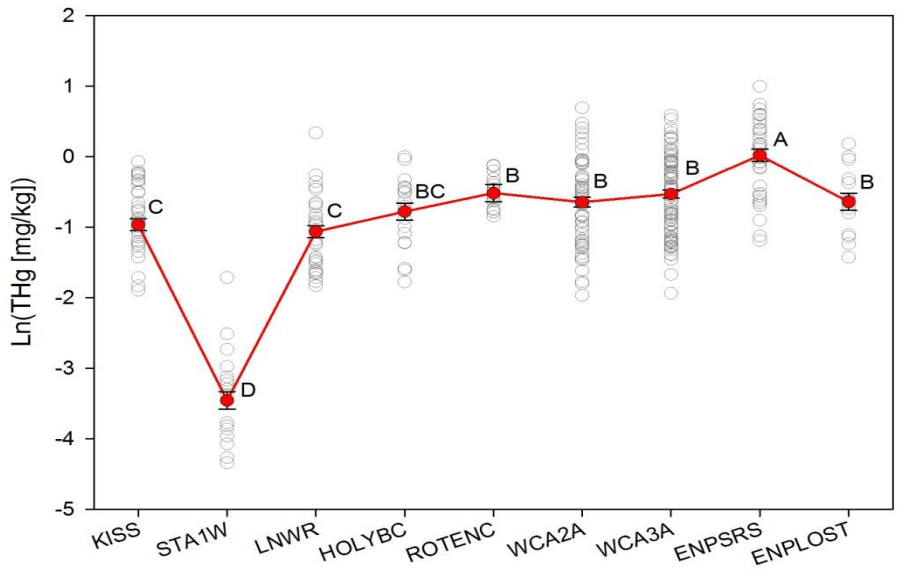

Figure 3B-9. Predicted and observed natural logarithm, or ln(THg) in 348 bass collected during WY2014. Significant differences among region means are indicated by dissimilar letters ($P < 0.05$). Error bars represent the standard error.

THg concentrations in bass have generally decreased in the greater Everglades since WY1989; however, distinct regional trends are evident. Based on differences among regions, where LNWR<WCA2=WCA3<ENPSRS, decreasing trends are evident in LNWR and WCA2-3 (**Figure 3B-10**) while no apparent trend is evident for ENPSRS. In LNWR, the median THg in bass collected prior to WY2001 was 0.50 mg/kg with a range of 0.05 to 2.14 mg/kg (n=262) while for the period since WY2001, the median has declined to 0.24 mg/kg with a range of 0.02 to 1.50 mg/kg (n=554). Overall, this represents a decrease in MeHg concentration of 52 percent between the two periods. Although individual bass continue to exceed the USEPA human health criterion of 0.35 mg/kg, over the past decade the median THg has remained below the criterion during all years except WY2014 (**Figure 3B-10**) when the median reached 0.35 mg/kg (n=41). Similarly in WCA2 and WCA3 combined, prior to WY2001, the median THg in bass was 0.80 mg/kg, with a range of 0.09 to 4.36 mg/kg (n=855). Since WY2001, the median THg in WCA2 and WCA3 decreased to 0.44 mg/kg, with a range of 0.01 to 3.20 mg/kg (n=2346). This difference among periods represents a 45 percent decline; however, the median THg since WY2001 has remained in excess of the USEPA criterion during all years and reached its maximum value of 0.68 mg/kg (n=224) during WY2011.

Considered as a whole, bass THg concentrations from LNWR, WCA2, and WCA3 declined significantly between WY1989 and WY2012 (t_{4023}= -27.86, P<0.0001), while a significant increase in THg occurred between WY2002 and WY2014 (t_{4023}=5.38, P<0.0001). These represent a 5.2 percent per year decline during the early period and a 2.2 percent per year increase during the past decade. A similar POR increase was reported for sunfish THg concentrations at sites WCA2U3 and CA33ALT (**Figure 3B-8**) and is evident in bass at select sites within the EPA. For example, after declines during the 1990s, a significant increase ($F_{1,11}$=6.11, P=0.597) in bass THg occurred between WY2002 and WY2014 in bass at site WCA2U3.

At the same time, bass THg concentrations within ENPSRS (sites L67F1 and ENPNP combined) remained relatively high with annual medians exceeding 1 mg/kg during most years (**Figure 3B-10)**. Between WY1990 and WY2002, there was no significant trend among annual means (t_{694} = -0.54, P<0.60); however, between WY2002 and WY2014 a significant decline (t_{694}= -2.19, P<0.029) in bass THg occurred, representing an annual decline of 9.1 percent. Due to much annual and interannual variability in results, the R^2 value was low (0.01). Nonetheless, MeHg bioaccumulation rates remain high within the ENPSRS with WY2014 median THg concentrations of 1.13 and 1.25 mg/kg at sites L67F1 and ENPNP, respectively (**Table 3B-1**).

Whether THg concentrations in fish are remaining constant over the past decade as reported in previous SFERs or whether recent increases in fish tissue THg concentrations are occurring, it is evident that Hg continues to be a significant water quality issue within the EPA and Greater Everglades as a whole. For the most part, THg concentrations in bass have declined from the levels observed during the late 1980s and early 1990s, are highly variable across the landscape (even within individual WCAs), and continue to exceed criteria concentrations for the protection of piscivorous wildlife and humans. Because future rates of Hg emissions and atmospheric deposition are highly uncertain (Krabbenhoft and Sunderland, 2013) and response of fisheries to Hg load reductions could take decades (Munthe et al., 2007), a better understanding of system controls on methylation and bioaccumulation on appropriate scales is needed to develop a comprehensive strategy to manage the Everglades Hg issue.

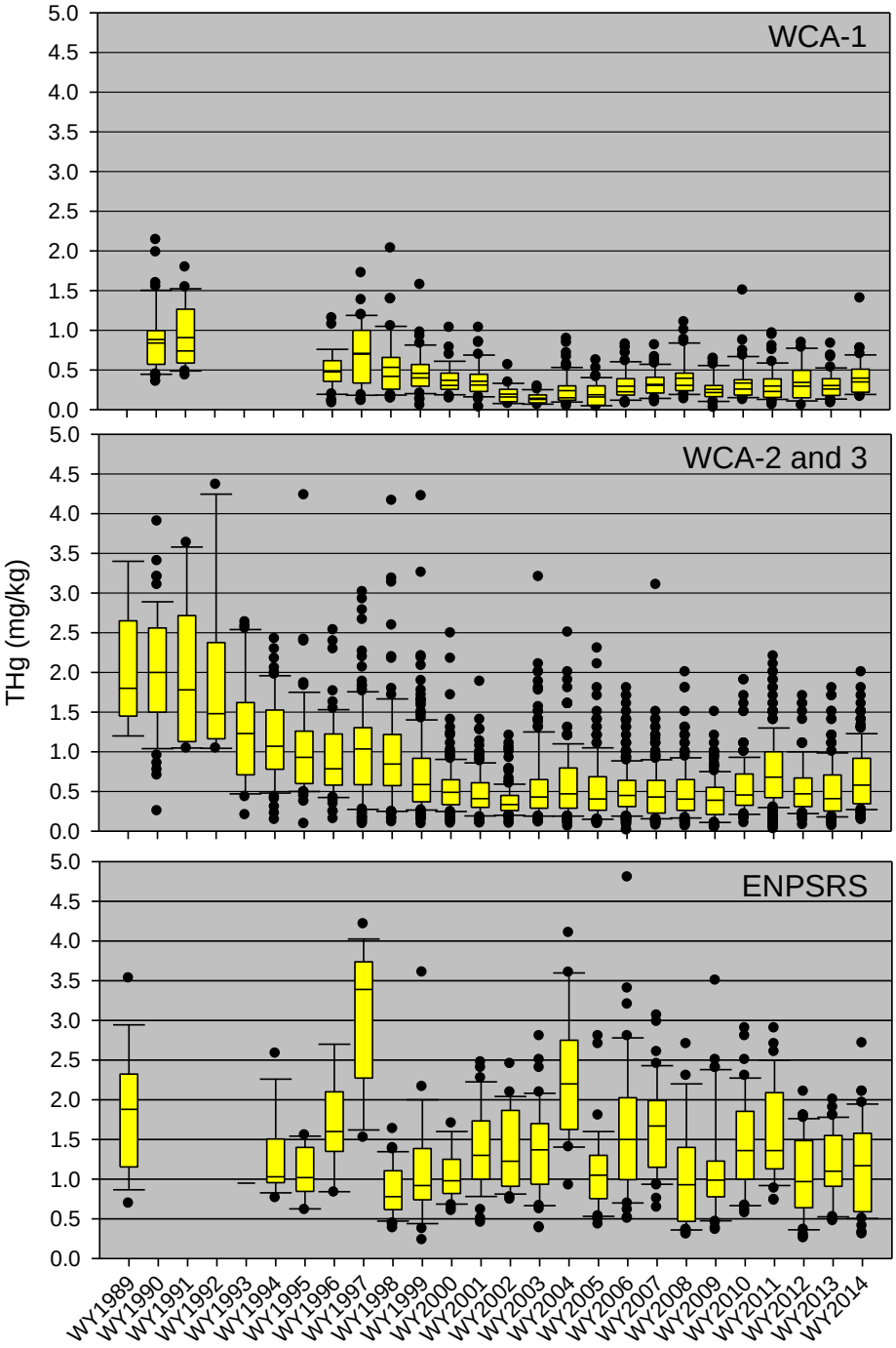

Figure 3B-10. THg concentrations in muscle tissue of largemouth bass collected from regions within the EPA between WY1989 and WY2014. Box plots represent the median and 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and points are outliers.

TEMPORAL TRENDS IN MERCURY LEVELS IN EVERGLADES WADING BIRDS

Peter Frederick[4]

Introduction

As discussed previously, accumulation of Hg in aquatic ecosystems is of concern for Everglades wildlife, especially piscivorous species positioned near the top of the aquatic food web (Rumbold et al., 2001). Several studies have documented elevated Hg concentrations in wading birds from the Everglades region (Frederick et al., 2004; Heath et al., 2005; Rumbold et al., 2001; Sundlof et al., 1994). Using a combination of museum and field specimens, Frederick et al. (2004) observed a drastic increase in feather mercury concentration in several wading bird species after the late 1970s, for great egrets (*Ardea alba* L.), where a 150 percent mean percent difference was observed in feather Hg concentrations between pre-1980s and post-1990 periods.

This section provides an update to feather Hg concentrations within great egrets and woods storks (*Mycteria americana* L.) within the Everglades region (previously reported by Axelrad et al., (2013). The use of wading birds, especially great egrets in the Everglades, as bio-monitors of environmental contamination offer several advantages including the ability to collect a large amount of data in a small area due to communal nesting behaviors, its relative trophic position, and the amount of available data on their population biology in the Everglades (Kushlan, 1993; Rumbold et al., 2001).

Methods

To assess THg concentration trends in bird feathers, 17 long term monitoring stations have been established within the central Everglades flow path (i.e., WCA-3A, WCA-3B, and ENP; **Figure 3B** - 11). Feathers are sampled from great egret nestlings (approximately 28 to 35 days old), typically between April and June, depending on nesting patterns annually. Great egret chicks were sampled due to their physiological exposure to Hg; food consumption is highest per gram of body biomass in young birds. From each colony, 8 to 15 young are sampled each year, all from different nests. Feather samples were submitted to the laboratory and analyzed for THg, similar to fish species nearly all (>95 percent) of the Hg in feather tissue is in the form of MeHg (Frederick et al., 2004).

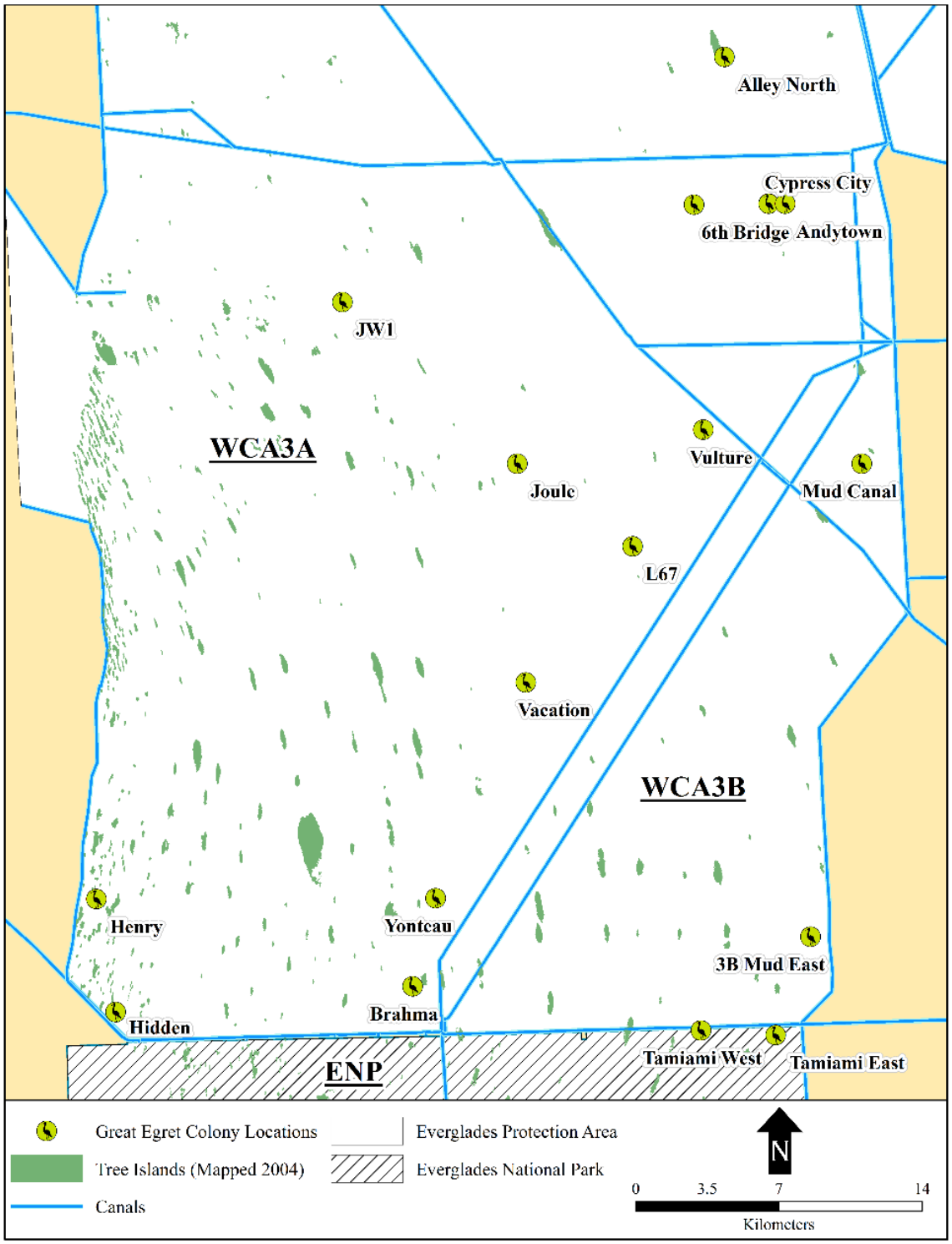

Figure 3B - 11. Everglades great egret (*Ardea alba* L.) colony monitoring locations where feathers from nestlings were sampled from 1994–2013.

Mercury Levels in Great Egrets

Mercury has been sampled in great egret nestlings continuously since 1994 over much of the central Everglades (**Figure 3B** - 11). Feathers are excellent accumulators and integrators of Hg body burden, are easily sampled, and the approach is less destructive than other tissue types (Burger, 1993; Furness et al., 1986). Furthermore great egrets have been identified to be excellent sentinel wading bird species due to their trophic position, representing a high trophic level predator, feeding almost exclusively on small to medium-sized fishes (Federick et al., 1997; Rumbold et al., 2001). The broad trend in the data is that THg concentrations fell sharply from a high in 1997 (mean across colonies = 20.7 mg/kg fresh weight, or fw) to a low in 2003 (mean=2.1 mg/kg fw; **Figure 3B-12**). Since 2003, THg concentrations have risen slightly annually to a mean of 6.8 mg/kg in 2013 (**Figure 3B-12**). It is important to note that there is relatively high variance in measurements at colonies from year to year, and the colonies sampled have also changed over time, partly due to logistics of sampling and mostly because some of the colonies are no longer active (**Figure 3B-13**). This results in uncertainty about the absolute magnitude of change over these years, as evident by highly variable annual standard deviations (**Figure 3B-12**).

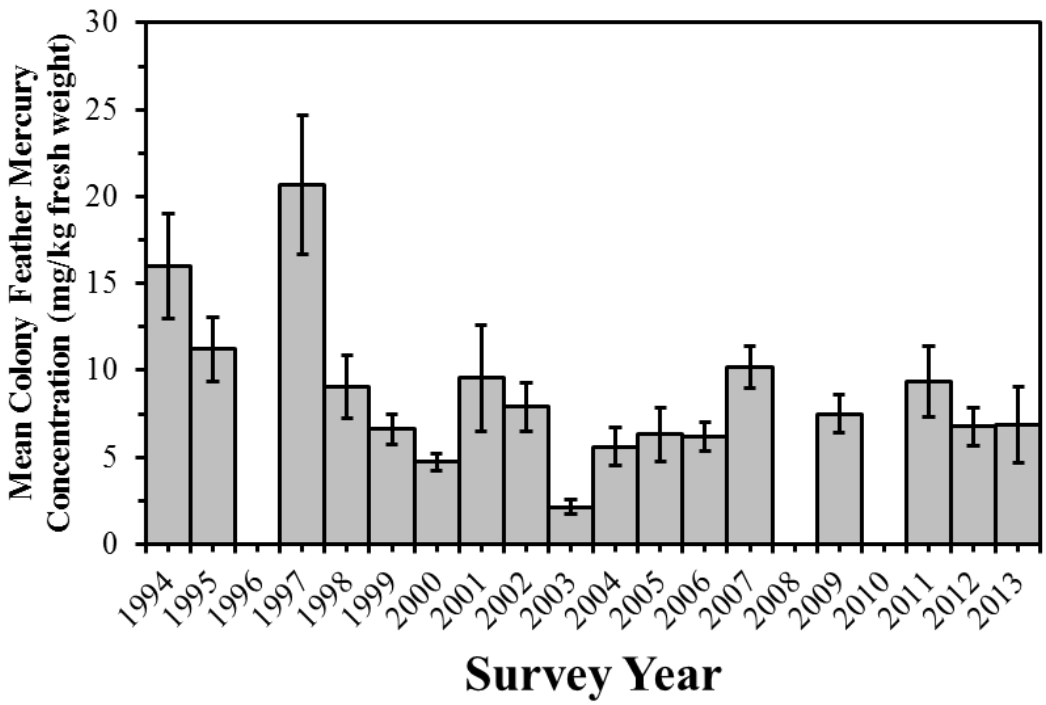

Figure 3B-12. Mean (± SE) THg concentrations in feather tissue of great egret nestlings in the central Everglades from 1994–2013. Mean values are computed from colonies spaced across the central Everglades.

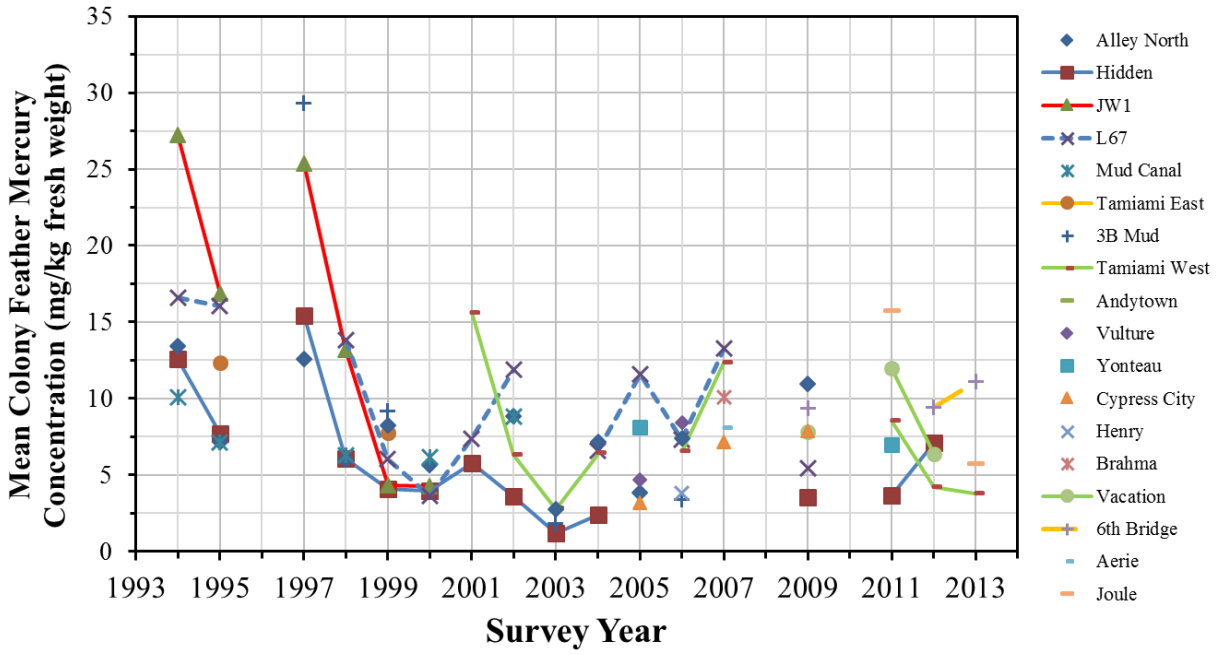

Figure 3B-13. Mean annual THg concentrations in feather tissue of great egret nestlings by colony in the central Everglades from 1994–2013. Different symbols represent different colonies; lines represent continuous data from individual colonies for two or more continuous years.

Coastal versus Inland Mercury Levels in Wood Storks

Feathers from wood stork chicks have been sampled from inland colonies from 2002–2013 using the same methods as for great egrets. However, colonies in the coastal parts of the Everglades had not been sampled, despite increasing numbers of wading birds nesting in that area. In 2011 and 2013, a cooperative project was initiated between the University of Florida and Everglades National Park. Wood stork chicks were sampled in coastal colonies (Paurotis Pond, Cuthbert Lake, and Broad River) and the inland Tamiami West colony.

These data suggest that feather Hg concentrations in the Tamiami West colony has fluctuated considerably over time, ranging from a low of 2.1 mg/kg (2003), to a high of 7.9 mg/kg (2002) (**Table 3B-2**). Coastal portions of the ENP colonies in 2011 showed particularly high concentrations of feather THg, with mean THg concentrations of 9.6 mg/kg (Cuthbert Lake), 8.4 mg/kg (Paurotis Pond), and 5.1 mg/kg (Broad River). While the mean feather THg concentrations in coastal colonies were generally lower in 2013 (7.2 mg/kg), they still remained relatively high. Additionally, the difference between coastal and inland colonies was approximately 77 percent larger in 2013 than in 2011.

These high concentrations in coastal colonies are of particular concern since the coastal zone is the area expected to have the most dramatic restoration of prey base and consequently, wading birds, in the system. In part, this is because historically this was the area that hosted the greatest nesting numbers. In 2005, the Comprehensive Everglades Restoration Plan's (CERP) Restoration

Coordination and Verification (RECOVER) team developed interim goals or targets (referred to as interim goals/interim targets) for several parameters throughout the Greater Everglades system as well as specific sub-regions (Lake Okeechobee, Northern Estuaries, Florida Bay, Biscayne Bay, etc.). RECOVER developed the system-wide wading bird nesting patterns indicator, which revolves around several variables including number of nesting birds and locations of nesting colonies. The desired restoration condition is to substantially increase nesting of wading bird species in the Southern Everglades estuaries. Therefore, the occurrence of increased levels of contamination in these areas is not a positive development for restoration. However, additional research is required further define trends and mechanisms involved with marine versus freshwater Hg dynamics in wading bird species.

Table 3B-2. Mean THg concentrations in wood stork nestling feathers (± 1 SD) at the freshwater Tamiami West colony and coastal locations. Note that coastal locations were sampled for the first time in 2011. Coastal ENP is an average of all samples collected at Paurotis Pond, Cuthbert Lake, and Broad River colonies.

Region	Survey Year	THg Feather Concentration (mg/kg fw) Mean	Standard Deviation
Freshwater ENP	2011	6.5	2.7
	2013	3.8	1.2
Coastal ENP	2011	8.0	3.0
	2013	7.2	2.4

MERCURY SOURCE TO THE EVERGLADES

ATMOSPHERIC MERCURY DEPOSITION TRENDS

Paul Julian II[1]

Introduction

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)(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 (MDN; **Figure 3B-14**).

Methods

Hg wet deposition data was retrieved from the National Atmospheric Deposition Program (NADP) MDN for the monitoring locations within the EPA (**Figure 3B-14**). 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 (NTN; 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/quality control (QA/QC) protocols approved by the USEPA.

There are three active MDN stations located within the EPA. Monitoring was discontinued at site FL04 during October 2006, at which time site FL97 began operation. To provide a regional assessment of Hg wet deposition, monitoring locations were assigned to different regions of the EPA. Monitoring station FL34 was 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 four sites 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 and includes both wet (May–October) and dry (November–April) seasons 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 analyses were conducted using JMP® (Version 10.0.0, SAS, Cary, NC, USA) and the critical level of significance was set at $\alpha = 0.05$.

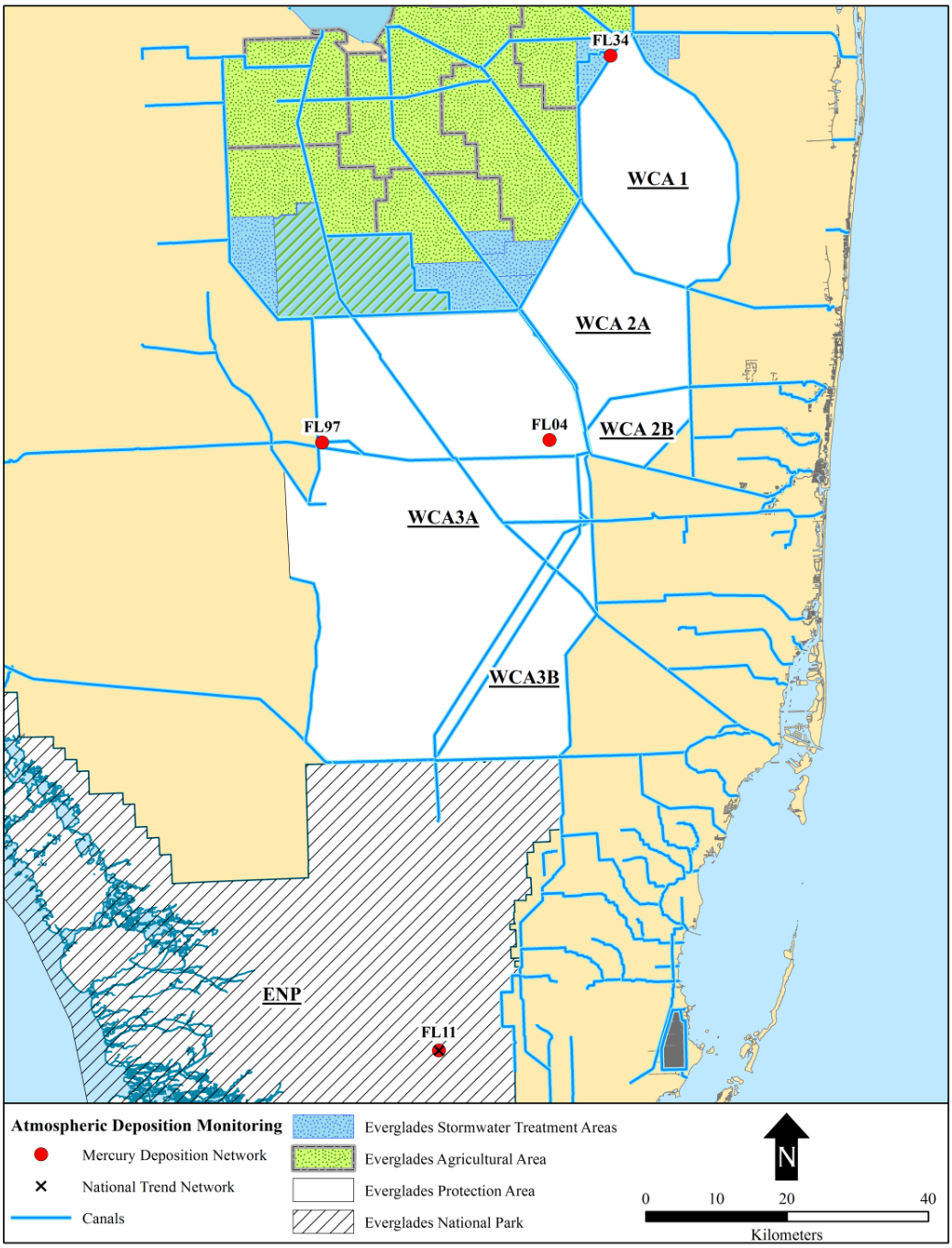

Figure 3B-14. Locations of atmospheric monitoring locations within the EPA (WCA-1, WCA-2, WCA-3, and ENP) and their associated monitoring networks.

Results and Discussion

Annual Hg wet deposition has remained relatively constant (for WYs with an entire dataset) 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-3**). Wet deposition values ranging from 9.6 to 21.5 micrograms per square meter (μg/m^2) for all regions (**Figure 3B-15**). Wet deposition was significantly different between the southern and northern regions and the northern and central regions, respectively, but not between the southern and central regions. Wet deposition appears to follow a south-to-north gradient, with the southern region exhibiting the highest POR mean (WY1996–WY2013) Hg wet deposition (17.6 ± 0.6 μg/m^2), followed by the central (17.1 ± 0.5 μg/m^2), and northern (14.5 ± 1.2 μg/m^2) regions. As the primary source of atmospheric Hg is international, the deposition patterns could potentially be driven by local weather patterns.

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-2**) and with PWM concentrations ranging from 10.3 to 17.1 nanograms per liter (ng/L).

Table 3B-3. Kendall's τ trend analysis results for both mercury (Hg) wet deposition and precipitation weight mean (PWM) concentrations for each region of the EPA.

Wet Deposition		
Region	**Kendall's τ**	**ρ value**
Northern	0.21	0.34
Central	-0.21	0.46
Southern	-0.06	0.78
Precipitation Weight Mean Concentration		
Region	**Kendall's τ**	**ρ value**
Northern	0.06	0.79
Central	0.21	0.46
Southern	-0.15	0.50

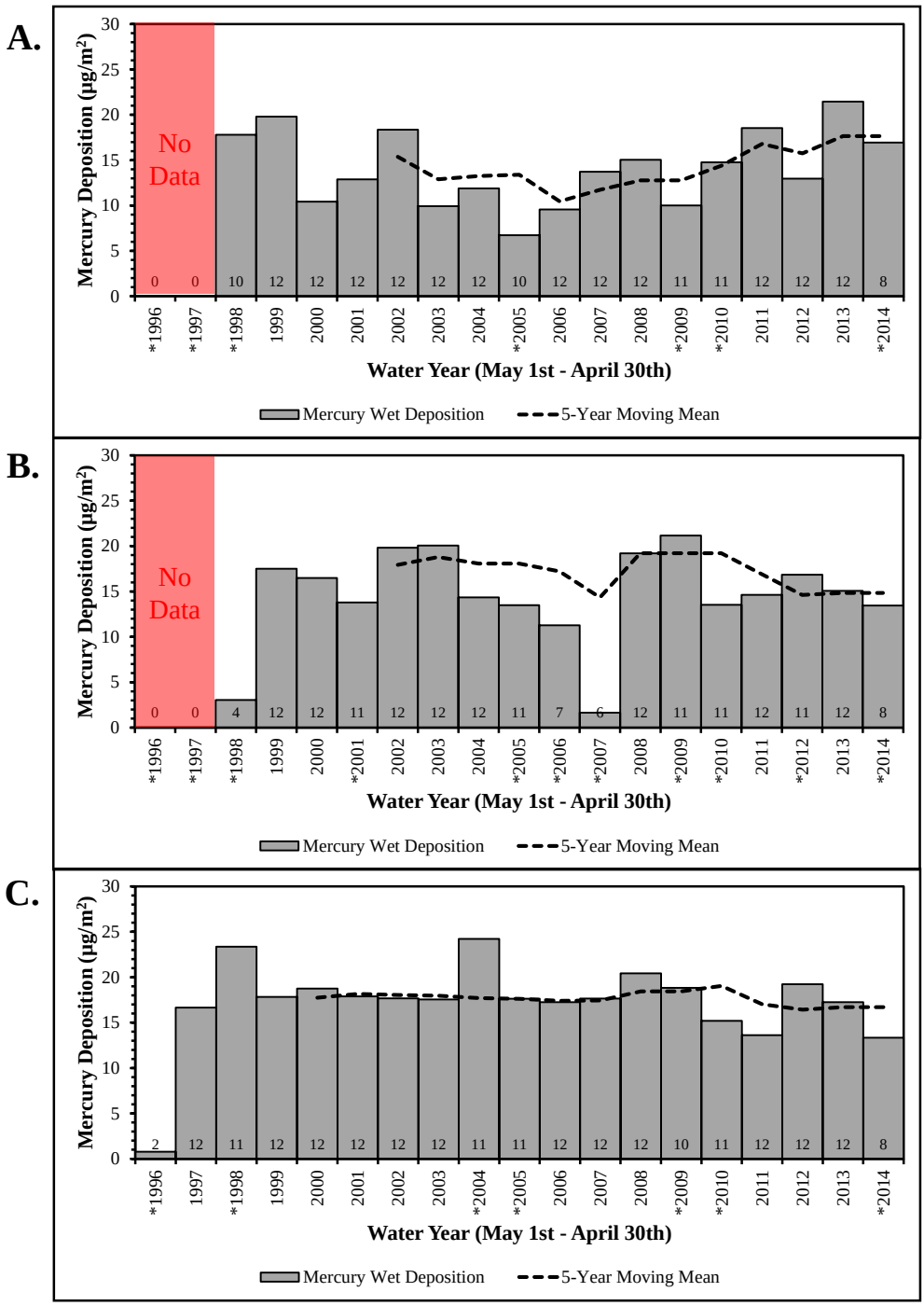

Figure 3B-15. Annual and five-year moving average Hg deposition [in micrograms per square meter (µg/m^2)] for the (A) northern, (B) central, and (C) southern regions of the EPA for WY1996–WY2014. Stations with less than 12 months of data are identified with an asterisk (*).

During WY2013, atmospheric loading was highest within ENP between all regions of the EPA, followed by WCA-3, WCA-1, and WCA-2 (**Table 3B-4**). Furthermore, data presented within **Table 3B-4** supports the claim that the primary source of Hg to the Everglades is through atmospheric deposition with percent atmospheric contribution range from 96 to 98 percent throughout the EPA. When taking these deposition values in context to fish tissue Hg concentrations (other sections of this report), it is interesting to see qualitatively that a potential correlation exists between fish tissue Hg and deposition, a more quantitative analysis is needed. This relative relationship have been observed before, (Hammerschmidt and Fitzgerald, 2006) suggested that accumulation of Hg in fish population is linked to atmospheric Hg loading.

Annually and the five-year moving average wet deposition were relatively consistent, with 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 single 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–WY2013. **Figure 3B-15** includes data for WY2014, but it should be noted that this dataset is currently incomplete. Therefore, values presented for WY2014 should be used with caution and are expected to be updated in the following SFER.

Table 3B-4. Atmospheric load of total mercury (THg) to each region of the EPA, including the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge), Water Conservation Areas 2 and 3 (WCA-2, and WCA-3), and Everglades National Park (ENP), during WY2013 and mean (± standard error) surface water load amount during WY2001–WY2008.

Region	Atmospheric Load (kilograms)	WY2001–WY2008 Surface Water Inflow Load* (kilograms)	Percent Atmospheric Contribution
Refuge	12.1	0.27 ± 0.03	98
WCA-2	11.5	0.51 ± 0.06	96
WCA-3	36.0	1.89 ± 0.18	95
ENP	68.2	1.12 ± 0.21	98

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

SULFUR AND MERCURY BIOGEOCHEMISTRY

REVIEW OF SULFATE EFFECTS ON INTERNAL PHOSPHORUS RELEASE IN EVERGLADES MARSHES

Forrest E. Dierberg[6], Thomas A. DeBusk[6] and Mike Jerauld[6]

Sulfur, in the form of sulfate, has been shown to promote the release of soil and detrital phosphorus (P) into the water column, a process commonly referred to as internal eutrophication (Lamers et al., 2002; Smolders et al., 2006). Given this and the observation that SO_4^{2-} concentrations in much of the Everglades are elevated (Stober et al., 2001) due to human activities in upstream watersheds (Bates et al., 2002), considerable concern has been raised over the potential contribution of anthropogenic SO_4^{2-} to the well-defined problem of P enrichment in the Everglades (Axelrad et al., 2008, 2007, 2006; Gu et al., 2012; Orem et al., 2011).

To date, three studies attempting to quantify sulfate-induced P release in this environment have been reported (Dierberg et al., 2012, 2011; Gilmour et al., 2007b). The study described by Gilmour et al., (2007b); data reported in Axelrad et al., (2007) suggested that P concentrations were elevated in surface waters and shallow (2-5 cm) porewaters of in situ mesocosms at the WCA-3A site WCA-3A-15 dosed with SO_4^{2-} (20–100 milligrams per liter, or mg/L). However, data from only a single sampling date were reported and those results were never presented beyond preliminary form in the 2007 SFER – Volume I, Chapter 3B, and no supporting metadata (e.g., methods) or ancillary environmental data (e.g., iron concentrations) were published. Conversely, two peer-reviewed, published studies reported no significant P release from Everglades soils in response to SO_4^{2-}. Slurries of soils spanning the Everglades sulfate gradient (STA-2 Cell 1, WCA-2A Site U3, and 9 km from WCA-3A-15 incubated in the presence of added SO_4^{2-} (32–96 mg/L) did not release additional soluble reactive P (SRP), compared to controls (Dierberg et al., 2011). Moreover, in situ porewater SRP in the sulfate- and P-enriched STA-2 Cell 1 did not co-vary with porewater sulfate or sulfide (S^{2-}) (Dierberg et al., 2012). These findings contradict results reported in other North American and European wetlands (Geurts et al., 2008; Roden and Edmonds, 1997; Smolders et al., 2006; Zak et al., 2006), but illustrate that the unique biogeochemical conditions of the Everglades reduces the vulnerability of soil P to SO_4^{2-} effects.

Smolders et al., (2006) outlined four inter-dependent mechanisms by which excess SO_4^{2-} can mobilize soil P through (1) direct competition of SO_4^{2-} with phosphate (PO_4^{3-}) for anion-sorption sites; (2) increases in pH due to alkalinity generated during the microbial reduction of SO_4^{2-} to S^2, creating conditions more favorable to organic matter decomposition (specifically, P mineralization); (3) increased soil organic matter decomposition by sulfate-reducing bacteria (SRB) stimulated by sulfate availability; and (4) redox-mediated reduction and dissolution of ferric iron minerals and the associated P. The latter mechanism is coupled with and exacerbated by the precipitation of reduced (ferrous) iron (Fe) with S^{2-} as FeS_x minerals, which have relatively low affinities for P. Recent studies have revealed how these P-release processes are neutralized in the Everglades (Dierberg et al., 2012, 2011; Gilmour et al., 2012).

First, displacement of exchangeable P by SO_4^{2-} is not thought to be prominent in most ecosystems, as the number of anion-binding sites is typically small (Lamers et al., 2002; Smolders et al., 2006). Indeed, the mean potassium chloride (KCl)-extractable SRP

concentrations in the Everglades is low, ranging from 0.3 to 1.1 micrograms per cubic centimeter, or $\mu g/cm^3$ ($\leq$ 1.5 percent of total phosphorus, or TP) in soils of the three WCAs which contain most of the remnant Everglades marsh (Reddy et al., 1998). Exchangeable P was present in similarly small pools in the WCA soils examined by Dierberg et al., (2011).

Second, alkalinity is high in most Everglades marshes, which likely reduces the relative importance of any alkalization from sulfidogenesis. Everglades waters are well-buffered with respect to alkalinity by the diel cycle of mineral $CaCO_3$ precipitation and dissolution mediated by photosynthetic algae (Scinto and Reddy, 2003), so changes in alkalinity may be difficult to induce. Although Dierberg et al., (2011) observed alkalinity increases during their closed-flask incubation, this alkalization was attributed to pH-induced dissolution of $CaCO_3$, rather than to SO_4^{2-} reduction based on concomitant increases in calcium (Ca) and decreases in pH. Therefore, it was concluded that sulfidogenic alkalization did not constitute an environmentally relevant P release mechanism in the Everglades.

Third, SO_4^{2-} appears not to increase the rate of organic matter mineralization in Everglades soils. Despite observing SO_4^{2-} reduction to S^{2-}, Dierberg et al., (2011) found no increase in carbon mineralization (measured by carbon dioxide and methane evolution) in slurries amended with SO_4^{2-} compared to unamended controls. This was attributed to metabolic limitation due to factors other than SO_4^{2-}, perhaps P or labile C. Similarly, Koch-Rose et al., (1994) as well as Fisher and Reddy, (2001) found no relationship between P release and SO_4^{2-} concentration or reduction in soils from WCA-2A.

Finally, the susceptibility of soils to release of Fe-bound P due to sulfidogenesis is determined by the size of the pool of P associated with Fe (Lamers et al., 2002; Smolders et al., 2006). Because the soils of the Everglades typically formed in place from accumulated organic matter in a watershed dominated by sandy soils of marine origin, a relatively small fraction of soil TP is associated with Fe (10–15 percent)(Reddy et al., 1998). Therefore, even when sulfidogenic effects reduce ferric iron stability, only a very small quantity of P is available for release.

Overall, the extant body of literature strongly indicates that SO_4^{2-} enrichment does not promote internal P release in Everglades marshes. Although the number of Everglades-specific studies is limited, laboratory (Dierberg et al., 2011) and field studies (Dierberg et al., 2012) have shown very close agreement, and the theoretical underpinnings of their findings are robust. At least one additional study has investigated this topic (Gilmour et al., 2007b) , but too little information has been presented to support a rigorous examination of those findings.

MESOCOSM STUDY TO EVALUATE THE EFFECTS OF SULFATE ON INTERNAL PHOSPHORUS RELEASE AND METHYLMERCURY CONCENTRATION IN CENTRAL WCA-3A

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William F. DeBusk[7] and Ben Gu

Introduction

Sulfate has been implicated in contributing to two major biogeochemical processes identified as threats to the well-being of the Everglades: P enrichment and MeHg contamination (Gilmour et al., 1998; Orem et al., 2011).

Enhanced P mobilization, referred to as internal eutrophication (Lamers et al., 1998), may occur by several mechanisms in sulfate-enriched wetlands (Smolders et al., 2006). Despite considerable concern over the import of these processes in the Everglades (Axelrad et al., 2008, 2007, 2006; Gu et al., 2012; Orem et al., 2011), to date, only three studies attempting to quantify their effects in this environment have been reported (Dierberg et al., 2012, 2011; Gilmour et al., 2007b)(see discussion in preceding section).

Studies of MeHg production in various wetland and aquatic ecosystems have demonstrated a strong relationship between SO_4^{2-} and Hg methylation by SRB (Gilmour, 2011; Orem et al., 2011). This is thought to occur as a result of increased metabolic activity of SRB in the presence of SO_4^{2-} (Gilmour, 2011). Although most regions of the Everglades exhibit excessive tissue Hg levels in fish and predator animals (Orem et al., 2011), the overall role and relative importance of SO_4^{2-} (as well as S^{2-}) in the production and bioaccumulation of MeHg in the Everglades is not well understood (Julian et al., 2014a; Liu et al., 2008). Myriad interactions among other factors such as inorganic Hg bioavailability and water/porewater constituents such as dissolved organic matter (DOM) and dissolved Fe have also been found to influence or control Hg cycling in other ecosystems (Hsu-Kim et al., 2013). Evidence for the importance of inorganic Hg loading (Pollman, 2012), DOM (Aiken et al., 2011; Liu et al., 2008), and a suite of other surface water constituents (Zheng et al., 2013) on MeHg production and accumulation in the Everglades has emerged recently. In particular, the seasonal drydown/reflood cycle may be especially important to the Everglades Hg cycle by liberating key ingredients for Hg methylation, e.g. SO_4^{2-}, inorganic Hg, DOM (Gilmour et al., 2004a; Krabbenhoft and Fink, 2001; Zheng et al., 2013).

Several studies have reported links between SO_4^{2-} concentration and Hg concentrations (surface water MeHg or mosquitofish THg) in the Everglades based on broad-scale environmental monitoring data (Gabriel et al., 2014; Orem et al., 2011). However, attempts to establish this relationship experimentally have not proved definitive due to ambiguous results (Cleckner et al., 1998; Gilmour et al., 1998) or insufficiently rigorous reporting (Gilmour et al., 2007a, 2004a).

Most current knowledge of the role of SO_4^{2-} in the Everglades is based on data from research in other ecosystems or regions, and on data collected from field monitoring of water, soils, and biota in the Everglades. As such, there is a paucity of hypothesis-driven experimental data on which to base ecosystem models and management decisions for the Everglades. To address this data gap, a multiyear mesocosm field study was conducted in a relatively unimpacted region of the Everglades using a sulfate dose-response approach to assess biogeochemical changes under sulfate-enriched conditions. A summary of research methods and findings is presented below, with further details on this study presented in Appendix 3B-1.

The primary objective of the mesocosm study was to determine changes in surface water, porewater, and soil pools of sulfur (SO_4^{2-}, S^{2-}, and total S), THg and MeHg, nutrients, and dissolved organic carbon (DOC), and concurrent changes in relevant physicochemical parameters, at varying levels of SO_4^{2-} enrichment.

Methods

In late 2009 and early 2010, in situ mesocosm enclosures were established in a ridge and slough community 9 km NNW of long-term water quality monitoring station CA315 in south-central WCA-3A, located in a relatively unimpacted (i.e., typically, annual average surface water TP concentration of ~4 µg/L and SO_4^{2-} concentration of <1 mg/L) region of the Everglades. This site was selected to utilize the long record of continuous monitoring data, and, in part, to facilitate comparison with previous investigations of sulfate effects in the Everglades, since most included

or focused on CA315 or nearby sites (Cleckner et al., 1999, 1998; Gilmour et al., 2007a, 2007b, 2004a, 2004a, 1998; Li et al., 2009).

Mesocosm enclosures were batch-fed slough water and experimental chemical amendments on a biweekly basis for a total of three wet seasons (2010–2012). The experimental design included three control mesocosms (no amendments) and three groups of three mesocosms, which each received biweekly doses of SO_4^{2-} amendments (3, 12, 24, or 48 mg/L). Approximately two weeks after amendments were added, surface waters in each enclosure (including the unamended control enclosures) and unenclosed, unamended control areas in the ridge and slough outside the enclosures, were sampled for a suite of analytes. After water samples were collected, the mesocosm water was exchanged and amendments were added to initiate the next cycle. Surface water and soil samples were analyzed for THg and MeHg on four occasions: beginning of Monitoring Season 2 (following a regional dry down period), twice at the end of Monitoring Season 2 (Feb 2012, before and after a spike of inorganic Hg) and at the end of Monitoring Season 3 (project closeout). No effect of the Hg spike was detected in MeHg concentrations, so Hg data from February 2012 were averaged within treatment groups for subsequent analyses. Further details on sampling procedures and timing of Hg sampling events are documented in the Appendix 3B-1.

Results

Sulfate additions effectively altered sulfur chemistry in the mesocosms. The range of SO_4^{2-} amendment levels created a robust and distinct gradient in the concentrations of surface water SO_4^{2-}, and porewater SO_4^{2-} and S^{2-} among the treatments (**Figure 3B-16**, panels a, c, and d). Furthermore, the loss of SO_4^{2-} from the water column, presumed to approximate the SO_4^{2-} reduction rate, was positively correlated to the SO_4^{2-} amendment rate (**Figure 3B-16b**). Finally, soil redox potentials displayed an inverse relationship with the sulfate amendment rate, and were markedly depressed (-100 to - 175 mV) in mesocosms that received SO_4^{2-} doses relative to enclosed and unenclosed controls (approximately -50 mV; not shown; see **Appendix 3B-1**).

Within the mesocosms, no response of P to SO_4^{2-} amendments was observed. Mesocosms receiving SO_4^{2-} amendments produced TP concentrations equal to or less than the TP concentration in the unamended control mesocosms (**Figure 3B-17**). The same pattern was reflected in all P species. Ammonia-N, a possible indicator of internal eutrophication (Gilmour et al., 2007a), was elevated by a factor of 2 to 3 in all surface waters of all mesocosms compared to the unenclosed controls, but showed no response to SO_4^{2-} addition (not shown).

Overall, there was no treatment effect on surface water MeHg (one-way ANOVA: $F[5,37] = 1.66$, $p = 0.17$; **Figure 3B-18**, panel a), although surface water MeHg was weakly but significantly correlated to SO_4^{2-} concentration among individual mesocosms ($r = 0.3$, $p = 0.03$; Error! Reference source not found.). The conditions during Hg sampling events were diverse, so each event was considered separately. Only in the February 2012 sampling event was a significant treatment effect detected (one-way ANOVA: $F[4,11] = 5.35$, $p =0.03$). In that event, surface water MeHg was positively related to the SO_4^{2-} dosing rate (**Figure 3B-18**, panel a). There was never a detectable treatment effect on soil MeHg (one-way ANOVA overall, and within sampling events: $p > 0.05$; **Figure 3B-18**, panel b). In both surface waters and soils, overall average MeHg was significantly ($p<0.01$) higher in August 2011 (following site drydown/reflood) than for either of the subsequent Hg sampling events (**Figure 3B-18**).

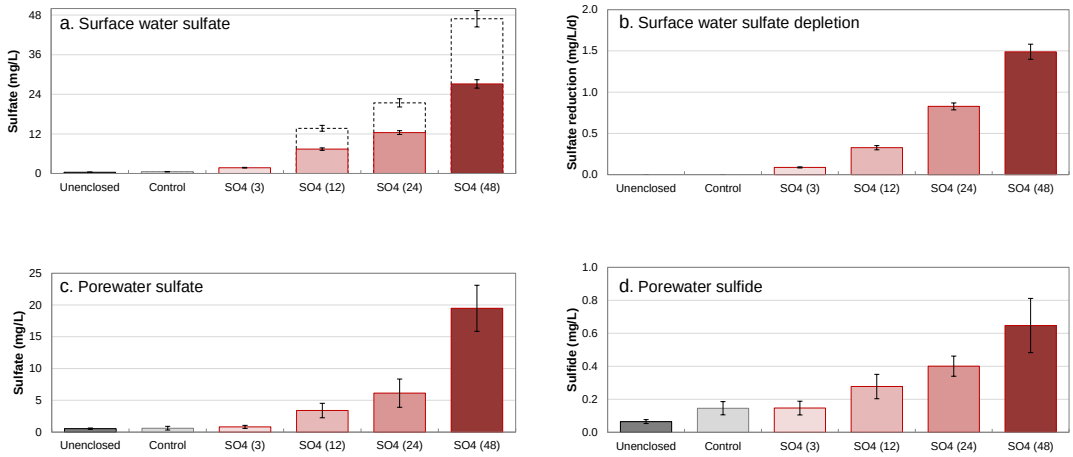

Figure 3B-16. Average surface water (a) sulfate (SO_4^{2-}) concentrations, (b) SO_4^{2-} depletion, and porewater (c) SO_4^{2-} concentrations, and (d) sulfide (S^{2-}) concentrations (mg/L) for each mesocosm treatment group and the adjacent unenclosed controls. Surface water constituents were measured two weeks after each SO_4^{2-} dose, resulting in 51 measurements of each individual mesocosm over three dosing seasons. Porewater was measured once at the end of each of three dosing seasons. Values shown here are averaged across two to six replicates within each group. Error bars give ± 1 S.E. In panel a, the dashed bars give the average surface water SO_4^{2-} concentrations measured 1 to 3 days (typically 2 days) after each SO_4^{2-} dose during Season 1.

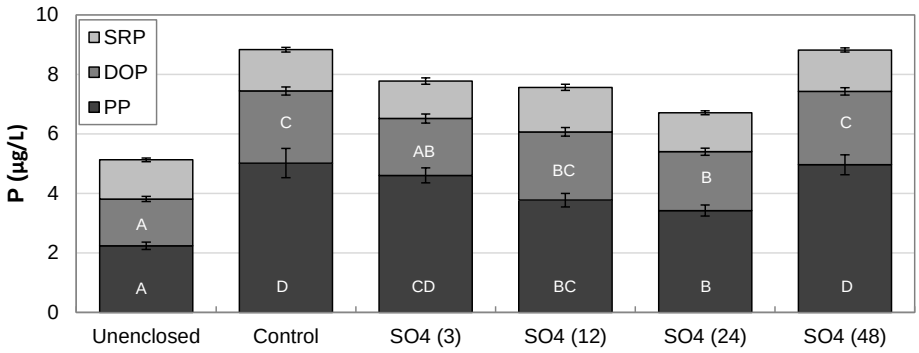

Figure 3B-17. Period of record (POR) average concentrations of P species (PP = particulate P; DOP = dissolved organic P; SRP = soluble reactive P) within each treatment group. Statistical comparison of means was conducted within each species; values sharing a common letter are not statistically different (α = 0.05). No statistical differences were detected between mean SRP concentrations. Error bars show ± 1 standard error (S.E.). Total height of each bar approximates the average TP concentration.

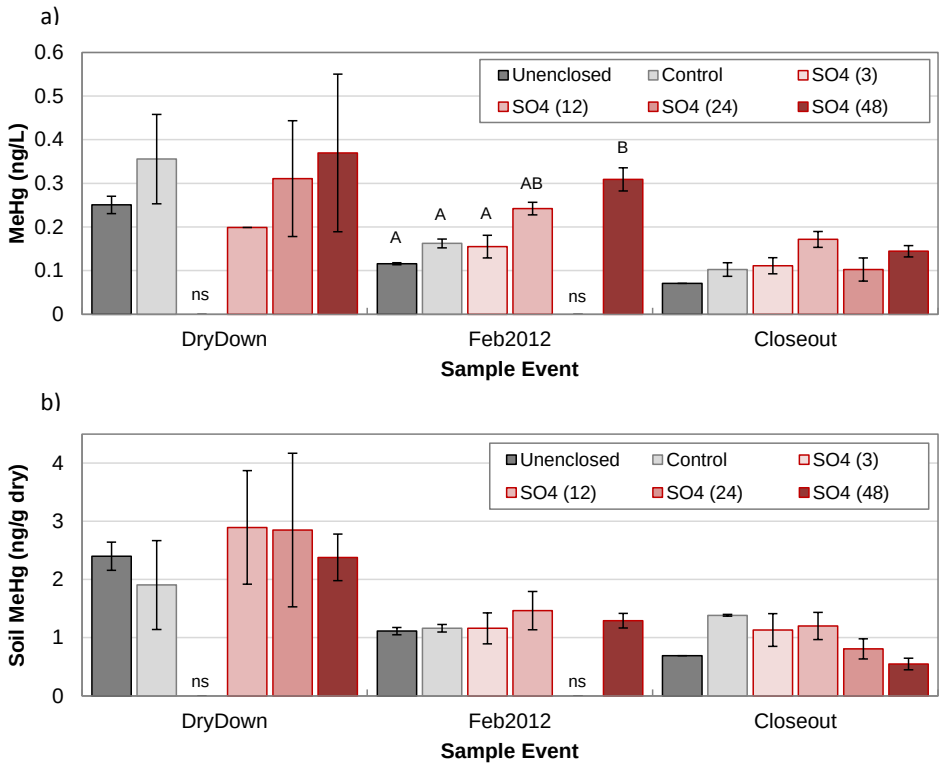

Figure 3B-18. Average (a) surface water and (b) soil (0–4 cm) methylmercury (MeHg) concentrations by experimental treatment group and each sampling event (February 2012 events combined). N = 2 for each value, except the February 2012 event, where n=4. Error bars give ± 1 S.E. Tests for treatment effect (one-way ANOVA) were conducted within each sampling event; a significant treatment effect was detected only in the surface water from February 2012. Within that event, a pairwise comparison of means (Tukey's HSD) was conducted; values sharing a common letter are not significantly different; ns= not sampled.

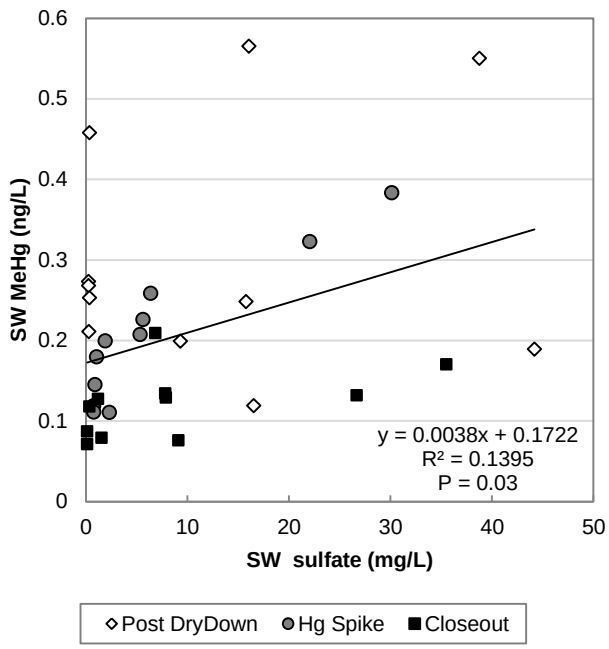

Figure 3B-19. Scatterplot of the relationship between surface water MeHg and SO_4^{2}. Each point represents the MeHg concentration in a given sampling event and the SO_4^{2-} concentration measured during the preceding monitoring season. The individual mercury sampling events are distinguished, and the data from the two events in February 2012 are averaged and presented as Hg Spike.

Discussion

Given the strong gradients in SO_4^{2-} and S^{2-} concentrations and sulfate reduction rates created by the experimental sulfate additions, this experiment was well suited to quantify the effects of exogenous sulfate on internal P release and Hg methylation at this site.

Internal Phosphorus Release

This experiment clearly demonstrated that long-term (three years) increases in surface water SO_4^{2-} concentration did not result in measureable P release from soils into the water column, contrary to the original hypothesis. Although this finding differs from those reported for other environments (i.e., that sulfate enrichment mobilizes soil P) (Geurts et al., 2008; Roden and Edmonds, 1997; Smolders et al., 2006; Zak et al., 2006), it corroborates the results of studies on the topic conducted in Everglades environments (Dierberg et al., 2012, 2011). Through these and the present investigation, it has become apparent that the unique biogeochemistry of the Everglades reduces the sensitivity of soil P to the four potential effects of SO_4^{2-} (Smolders et al., 2006) as (1) a very small pool of ion-extractable P (0.2 mg/kg; 0.05% of the soil TP) did not support the mobilization of phosphate by anion exchange with sulfate; (2) there was no detectable increase in alkalinity associated with sulfate reduction in this open system; (3) Dierberg et al., (2011) found no increase in carbon mineralization (nor associated P release) in soil slurries from

this site exposed to added SO_4^{2-}; and (4) a small pool of Fe-bound P combined with high Fe:P ratios limited the P release associated with ferric iron reduction.

Despite a superficial similarity in experimental design and infrastructure between this and the study reported by Gilmour et al., (2007a), any comparison of the results must be attempted with great caution. First, the data presented in Axelrad et al., (2007) apparently represent only a single sampling event, with no estimates of variability within treatments or over time given. Second, operational differences may have distinguished our study from that of Gilmour et al., (2007a). In particular, that study observed P release to surface waters only at a SO_4^{2-} concentration of 100 mg/L, more than double the highest concentration tested in the present experiment, and well above the highest concentrations observed in any Everglades marshes (Scheidt and Kalla, 2007). In addition, based on a limited description of methodology, it appears that their mesocosm study did not include routine surface water exchanges, which likely introduced undocumented experimental artifacts.

Mercury Methylation

Although sulfate amendments markedly increased the rate of its reduction (as evidenced by SO_4^{2-} depletion rates and porewater S^{2-} accumulation), we observed a modest, at best, MeHg response. The unimodal ("bell-shaped") MeHg response with respect to sulfate concentrations that has been predicted for ecosystems with gradients of SO_4^{2-} and S^{2-} (Gilmour and Henry, 1991; Orem et al., 2011) was not observed. Thus, net MeHg accumulation was not closely paired with sulfidogenic activity. This implies that the methylation rate was constrained only secondarily by SO_4^{2-} concentration.

Primary limitations on methylation may have included availability of labile carbon (C) or bioavailability of inorganic Hg. For example, labile C is necessary for microbial SO_4^{2-} reduction to S^{2-} (Lamers et al., 2002, 1998; Reddy and DeLaune, 2008), but low organic matter mineralization rates measured at WCA-3A (Dierberg et al., 2011) may indicate a limited supply of labile C. The bioavailability of inorganic Hg to methylating microorganisms can also be an important rate-limiting factor in the production of MeHg (Hsu-Kim et al., 2013) and is probably relevant to MeHg production in the Everglades (Aiken et al., 2011; Pollman, 2012).

Finally, the apparent stimulation of Hg methylation by drydown/reflood is consistent with earlier studies (Fink, 2003; Krabbenhoft and Fink, 2001). It is apparent that some rate-limiting constituent(s) is/are released during the drydown cycle. Focused laboratory studies are under way to resolve the mechanics of this process.

The overall response of MeHg to SO_4^{2-} additions in our WCA-3A mesocosms was not entirely consistent with previous mesocosm studies in WCA-3A conducted by the ACME research program. (Gilmour et al., 2007a, 2004a) have previously reported significant (positive) response of MeHg production to additions of SO_4^{2-} to ACME mesocosms during unpublished studies conducted at multiple Everglades field sites.

The increased response of MeHg accumulation to sulfate additions in ACME mesocosms relative to the DBE mesocosms is not readily explained by the extremely limited quantitative data made available by the ACME research group. For example, soil redox data are not currently available but may be useful for evaluating the possibility that soil redox levels in the ACME mesocosms were artificially reduced (e.g., by lack of water exchanges), thereby eliciting a greater Hg methylation response. Detailed methodological information related to the design, operation, maintenance, and sampling of the ACME mesocosms would be extremely useful for facilitating comparison of these and previous study results.

RECENT ADVANCES IN MICROBIOLOGY OF MERCURY METHYLATORS IN THE EVERGLADES

Hee-Sung Bae[8], Forrest E. Dierberg[6], and Andrew Ogram[8]

The Everglades is subject to significant amounts of atmospheric deposition of inorganic mercury (Rood et al., 1995), which may be biologically transformed to the more toxic MeHg. Bioaccumulation of MeHg in the Everglades is of great concern because of its impacts on wildlife (Cleckner et al., 1998) and potential impacts on human health (Clarkson, 1997; Mergler et al., 2007).

Although considerable research has been devoted to the identification of the complex geochemical interactions (SO_4^{2-} reduction, DOM complexation, Hg^{2+} availability) that enhance Hg methylation (Aiken et al., 2011; Gilmour, 2011; Hsu-Kim et al., 2013; Liu et al., 2009, 2008; Orem et al., 2011; Zheng et al., 2013), limited work has been conducted to identify the dominant phylogenetic groups responsible for methylation in the Everglades (Schaefer et al., 2014). The dominant methylators of Hg in the Everglades are generally considered to be SRB (Gilmour et al., 1992), although recent work indicated that diverse groups of bacteria may also contribute to Hg methylation in other anaerobic environments (Gilmour et al., 2013; Parks et al., 2013) and in low sulfate regions of the Everglades (Schaefer et al., 2014).

Recently, a study reported that the genes *hgcA* and *hgcB* are required for Hg methylation in a phylogenetically diverse group of microorganisms (Parks et al., 2013). Recent investigation has focused on the distribution of *hgcA* and *dsrB* mRNA (representing active SRB) sequences along SO_4^{2-} concentration gradients in WCAs and the relationships between *hgcA* and *dsrB* phylotypes and Hg methylation in laboratory microcosms, employing specific metabolic inhibitors that target SRB and methanogens (Bae et al., 2014). The results of this study are summarized below.

A new polymerase chain reaction primer set was developed to target *hgcA*, a gene encoding a corrinoid protein essential for Hg methylation across broad phylogenetic boundaries. This primer set was used to study the distribution of *hgcA* sequences in soils collected from three sites along a gradient in SO_4^{2-} and nutrient concentrations in the northern EPA. The sequences obtained were distributed in diverse phyla, but the *hgcA* clone libraries from all sites were dominated by sequences clustering within the order *Syntrophobacterales* of the *Deltaproteobacteria* (49 to 65 percent of total sequences). Also, *dsrB* mRNA sequences obtained from these sites were dominated by *Syntrophobacterales* (75 to 89 percent).

In a laboratory amendment study (Error! Reference source not found.), addition of SO_4^{2-} generally resulted in increased MeHg accumulation, with concomitant SO_4^{2-} reduction, suggesting that the sulfidogenic methylation was accelerated by the SO_4^{2-} addition. The addition of MoO_4^{2-} (SRB inhibitor) almost completely inhibited MeHg production and MoO_4^{2-} reduction, indicating that Hg methylation was controlled predominantly by SRB. Bromoethanesulfonate (BES), a methanogen inhibitor, did not inhibit MeHg production but rather increased its accumulation. This implies that methanogens are not the key organisms responsible for methylation in these soils. The increased concentrations of MeHg observed in the BES incubations may suggest that methanogens are responsible for Hg demethylation at this particular site (near WCA-3A-15) (Marvin-DiPasquale and Oremland, 1998). At this time, it is not clear what mechanism is responsible for the high concentrations of MeHg observed in the BES treatment. Finally, MeHg accumulated (albeit to a lesser concentration than in unamended controls) in flasks to which both MoO_4^{2-} and BES were added, indicating methylation by groups other than SRB and methanogens.

Overall, study findings reveal the potential importance of syntrophs within the SRB clades in the WCAs. Therefore, further investigation into the significance of syntrophic versus sulfidogenic metabolism in accounting for net MeHg accumulation as well as the role of non-SRB, such as iron-reducing bacteria, is warranted. Also, the degree and extent of demethylation in the Everglades have been understudied. Additional clarification of these important processes and under what environmental conditions they become significant should facilitate management actions to help minimize Hg methylation in the Everglades. For example, any strategy for managing Hg methylation in the Everglades should consider that net Hg methylation is most likely not limited to the action of SO_4^{2-} reduction.

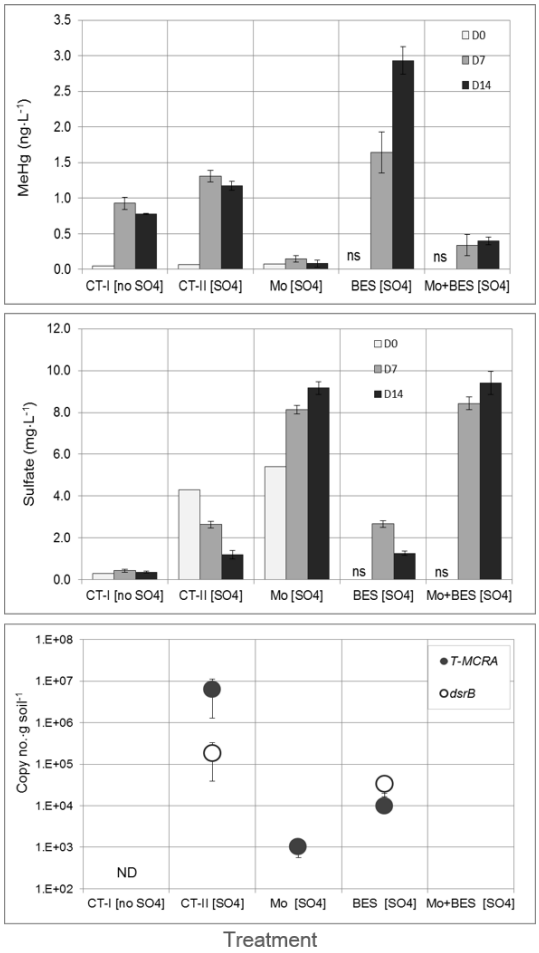

Figure 3B-20. MeHg production, SO_4^{2-} consumption, and transcript concentration of mcrA and dsrB during laboratory incubations of WCA-3A soils with and without specific inhibitors. All controls and treatments received 139 nanograms per liter (ng/L) of Hg^{2+} as $HgCl_2$ at the beginning of the incubation. D0, D7, and D14 represent the initial, day 7, and day 14 concentrations, respectively, of MeHg and SO_4^{2-} in the water column. The transcript concentrations are from the soil after 14 days of incubation. CT – Control (no inhibitors added). Mo = 20 mM molybdate (MoO_4^{2-}); BES = 50 mM bromoethanesulfonate. ND = not determined; ns = no sample. Error bars represent the standard error from triplicate incubation vessels.

SULFUR SOURCES AND EFFECTS

SULFATE WITHIN THE EVERGLADES PROTECTION AREA

Paul Julian II[1]

Introduction

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, one of which is sulfate reducing bacteria (SRB) which utilize sulfate to metabolize organic matter under anaerobic conditions. During this process some SRB have been observed to methylate THg (Gilmour et al., 2013, 1998; Gilmour and Henry, 1991). 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 complex and confounded by other variables, particularly food web dynamics, water quality and hydrological conditions (Julian, 2013; Julian and Gu, 2014). However, based on previous and ongoing research, SO_4^{2-} can potentially influence the Hg/MeHg cycle under some suite of ambient conditions. The end products of these complexities must be predictable and quantified before an effective control or management strategy can be designed and implemented. Furthermore, various SO_4^{2-} sources to the Everglades originate from both natural (oxidation of peat soil, groundwater, etc.) and anthropogenic sources (atmospheric deposition, fertilizer application, etc.). 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 sulfate data for WY2014 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, while it limits spatial analyses within each region. However, spatial analyses can be made among regions, because the majority of inflow and pollutants enter the northern third of the EPA and the net water flow is from north to south.

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** 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 (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 QA/QC 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 WY2013 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–WY2013), and (4) the current water year (WY2014).

The Baseline period corresponds to the timeframe prior to implementation of the Everglades Agricultural Area (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 Long-Term Plan for Achieving Water Quality Goals in the EPA and 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, WY2014) will be used to make comparisons with the historical periods and will be analyzed independently as the fourth period.

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.

Analysis

Unless otherwise noted, all inflow and outflow summary statistics (geometric mean, minimum, maximum, etc.) were performed using data collected on flow events only. All valid data (i.e., non-qualified data) were used to compute summary statistics for all other regions (i.e., interior and rim). Surface water sulfate concentrations were summarized for each period, region,

and classification using basic descriptive statistics including arithmetic mean, standard deviation, sample size, minimum, maximum, and median. Typically, geometric mean concentrations were employed when reporting concentrations at a given sampling location. However, due to low sample size at each station, arithmetic mean concentrations were also employed for monitoring locations. Surface water sulfate and chloride loads to each region of the EPA were calculated using the Java-based District nutrient load program. Annual flow-weighted mean (FWM) sulfate concentrations for WY2014 were also calculated using flow and load values generated from the Java-based District nutrient load program for each structure. Kendall's τ correlation statistic was applied to annual arithmetic and geometric mean sulfate concentrations of inflow and interior portions of each region of the EPA. Additionally sulfate inflow loads and FWM concentrations for each region of the EPA were assessed using the Kendall's τ correlation statistic to determine trend direction and significance. All statistical operations were performed with JMP® (Version 10.0.0, SAS, Cary, NC, USA). The critical level of significance was set at $\alpha = 0.05$.

Results and Discussion

Sulfate Concentrations

Sulfur is an essential plant macro-nutrient (Bellinger and Van Mooy, 2012) and enters the Everglades ecosystem primarily as sulfate (Orem et al., 2011), but the role of organic sulfur in the total mass of sulfur entering the region remains undetermined. As stated above, sulfate is of concern due to its ability to influence biogeochemical processes through microbial pathways that lead to Hg methylation and support the production of reduced sulfur compounds under anaerobic conditions. Sulfate monitoring results are presented in this section to provide an overview of current concentrations and evaluate temporal and spatial patterns. Sulfate summary statistics relative to the Baseline, Phase I, Phase II, and current year (WY2014) are shown in **Table 3B-5**.

Similar to other water quality parameters within the EPA, SO_4^{2-} follows a general north-to-south concentration gradient (**Figure 3B-21**). This gradient is apparent for inflow regions within the EPA for WY2014, with the highest mean SO_4^{2-} concentrations observed in the Refuge peripheral canals (55.1 mg/L), followed by WCA-2 (49.2 mg/L), WCA-3 (21.0 mg/L), and ENP (7.2 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 Refuge were elevated relative to the Phase II period. The slight increase in the annual SO_4^{2-} concentrations in the Refuge could be due to increased annual surface water flow into the Refuge (approximate 4 percent increase in volume relative to WY2013). Additionally, an increase in SO_4^{2-} concentrations entering the Refuge could be due to diversion events as a result of tropical storm activity (see Chapter 2 of this volume).

Although the Refuge experienced a slight increase of SO_4^{2-} concentrations in recent years throughout the entire POR (WY1979–WY2014), statistically significant decrease geometric mean concentrations were observed for the Refuge inflow and interior regions. WCA-3 and ENP inflow regions also experienced a significant decreasing trend in geometric mean SO_4^{2-} concentrations. No significant trend was observed for both WCA-2 interior and inflow geometric mean concentrations, inflow flow-weighted mean and loads. No significant trend was observed for ENP inflow sulfate loads (**Table 3B-6**). 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 mg/L or 0.10 mg/L, depending on time period) to 403.0 mg/L within ENP and 1,300 mg/L within WCA-3.

Some trends are more pronounced than others, as shown in **Figure 3B-22**, with the Refuge trend being less apparent than all other areas. 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 a previous SFER (Axelrad et al., 2013) connate seawater could potentially be a relatively large source of sulfate, chloride, and dissolved solids (i.e., other minerals) to the EPA, more specifically the Refuge. Another driving factor of interior trends, are the biogeochemical process 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 flux of nutrients including sulfate from the soil to the water column occurs. This dryout/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.

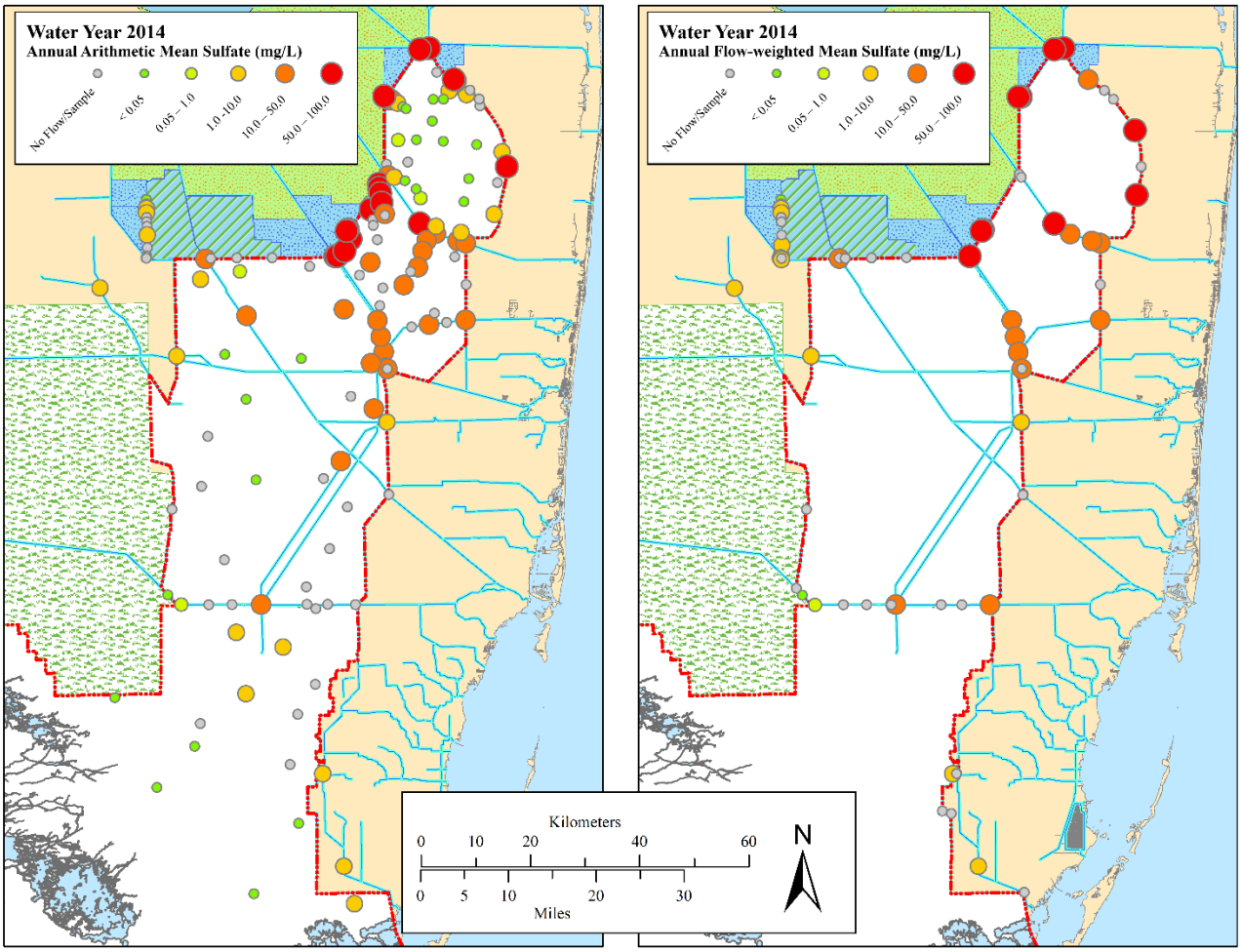

Figure 3B-21. Annual arithmetic mean sulfate concentrations (milligrams per liter, or mg/L) for all classifications (left panel) and annual flow-weighted mean sulfate concentrations at water control structures (right panel) for WY2014 at stations across the EPA.

Table 3B-5. Summary statistics of sulfate (SO_4^{2-}) concentrations in milligrams per liter (mg/L) for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), Phase II (WY2005–WY2013), and WY2014 periods.

Region	Class[1]	Period	Sample Size	Arithmetic Mean	Standard Deviation	Geometric Mean	Geometric Standard Deviation	Median	Min.	Max.
Refuge	Inflow	1979-1993	53	94.4	65.0	80.9	6.1	78.8	29.9	340.0
		1994-2004	309	55.6	34.7	48.9	5.6	50.7	6.7	460.7
		2005-2013	312	49.3	21.5	44.3	5.4	47.1	10.0	115.0
		2014	71	55.1	19.2	51.5	5.4	53.7	12.7	97.7
	Interior	1979-1993	500	16.6	21.5	10.3	4.9	10.0	2.5	220.2
		1994-2004	1219	5.5	20.9	1.0	5.9	1.0	<0.05	610.0
		2005-2013	638	2.1	5.7	0.3	5.6	0.2	<0.05	49.3
		2014	336	6.3	13.2	0.6	10.3	0.5	<0.05	72.1
	Outflow	1979-1993	65	46.3	35.9	37.1	5.6	36.0	7.3	257.2
		1994-2004	78	48.2	48.8	36.9	5.7	40.0	4.2	418.9
		2005-2013	102	26.4	19.4	18.8	5.4	22.6	1.4	85.4
		2014	31	35.9	22.8	27.6	5.6	30.1	4.0	80.0
	Rim	1979-1993	96	42.2	37.1	25.2	6.5	34.4	2.5	139.8
		1994-2004	341	56.9	26.9	50.7	5.6	49.4	1.6	210.0
		2005-2013	20	38.3	14.0	35.8	5.1	36.4	14.3	61.7
		2014	48	51.3	20.0	47.5	5.4	47.1	19.2	93.5
WCA-2	Inflow	1979-1993	71	66.8	78.0	51.7	5.9	53.8	7.3	644.5
		1994-2004	127	55.3	38.9	48.2	5.6	52.3	7.8	418.9
		2005-2013	300	49.8	18.7	44.0	5.7	49.2	<0.05	106.0
		2014	90	49.2	15.3	45.3	5.5	49.1	4.0	80.1
	Interior	1979-1993	1484	42.1	35.8	32.7	5.6	36.8	2.5	344.3
		1994-2004	1640	46.3	93.2	35.3	5.7	41.0	0.1	3230.0
		2005-2013	593	40.4	19.1	33.9	5.5	43.4	2.3	87.4
		2014	196	44.5	14.6	41.5	5.2	45.8	5.3	79.1
	Outflow	1979-1993	106	41.3	20.9	36.5	5.3	38.5	7.6	131.7
		1994-2004	95	28.6	10.9	26.3	4.8	27.9	5.8	54.3
		2005-2013	233	27.7	16.9	21.9	5.2	26.6	3.9	74.7
		2014	62	31.5	15.2	27.5	5.1	30.6	5.8	59.4
WCA-3	Inflow	1979-1993	258	35.1	33.0	23.1	5.9	29.6	1.0	286.0
		1994-2004	192	21.0	16.5	13.8	5.5	17.2	0.5	62.9
		2005-2013	380	24.0	19.2	13.3	6.6	21.4	<0.05	74.7
		2014	100	20.9	21.2	7.2	8.6	6.0	<0.05	68.6
	Interior	1979-1993	611	14.9	17.3	10.5	4.6	10.7	2.0	261.5
		1994-2004	1645	11.7	45.3	3.7	6.8	4.4	<0.05	1300.0
		2005-2013	325	11.7	15.7	2.9	9.5	3.7	<0.05	126.0
		2014	82	12.6	15.0	2.0	14.9	4.5	<0.05	52.0
	Outflow	1979-1993	146	16.4	16.6	10.7	5.0	12.9	1.0	107.6
		1994-2004	141	7.4	8.0	2.7	7.2	4.7	<0.05	36.5
		2005-2013	98	8.9	10.3	1.6	12.6	4.0	<0.05	39.3
		2014	5	8.0	10.5	2.2	8.1	0.9	0.4	22.8
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
		2005-2013	110	7.4	8.1	2.6	7.7	4.6	<0.05	35.8
		2014	9	7.2	7.5	3.9	5.1	6.5	0.4	22.8
	Interior	1979-1993	572	9.0	19.5	4.3	4.4	4.3	0.8	205.5
		1994-2004	1044	5.5	17.7	2.1	4.9	2.6	<0.05	403.0
		2005-2013	269	6.8	26.1	1.0	7.0	1.2	<0.05	239.0
		2014	77	1.8	2.4	0.6	5.2	0.7	<0.05	10.9

[1] Inflow and Outflow values only utilizes data when structures are flowing.

Table 3B-6. Kendall's τ geometric mean SO_4^{2-} concentrations, flow-weighted mean SO_4^{2-} concentrations, and SO_4^{2-} inflow load trend analysis results for each region's inflow and interior classification within the EPA from WY1979–WY2014. Statistically significant ρ-values are italicized.

		Annual Geometric Mean Concentration		Annual Flow-weighted Mean Concentration		Annual Load	
Area	**Class**	**Kendall τ**	**ρ**	**Kendall τ**	**ρ**	**Kendall τ**	**ρ**
Refuge	Inflow	*-0.38*	*<0.01*	*-0.40*	*<0.01*	*-0.44*	*<0.01*
	Interior	*-0.64*	*<0.01*	---	---	---	---
WCA-2	Inflow	-0.14	0.27	-0.14	0.21	0.10	0.42
	Interior	0.12	0.32	---	---	---	---
WCA-3	Inflow	*-0.46*	*<0.01*	*-0.55*	*<0.01*	*-0.24*	*<0.05*
	Interior	*-0.48*	*<0.01*	---	---	---	---
ENP	Inflow	*-0.51*	*<0.01*	*-0.33*	*<0.01*	-0.18	0.11
	Interior	*-0.63*	*<0.01*	---	---	---	---

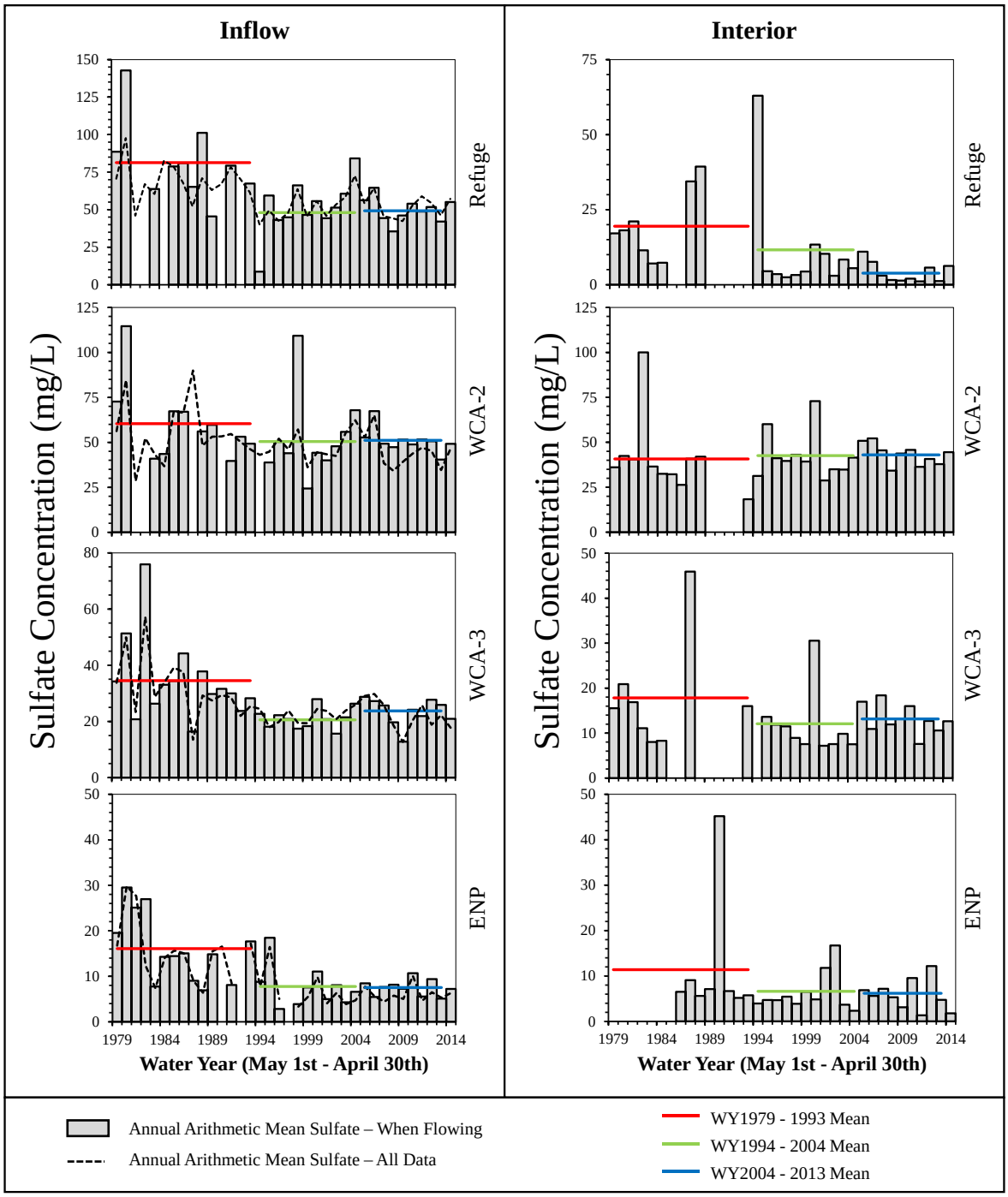

Figure 3B-22. Annual arithmetic mean SO_4^{2-} concentrations (mg/L) for inflow (left panel) and interior (right panel) areas of the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge), WCA-2, WCA-3, and ENP from WY1978–WY2014. Bars indicate arithmetic mean when flowing, dash-line indicates arithmetic mean irrespective of flow. 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–WY2013) periods. [Note: Area with no bars indicates data gaps.]

Much like other nutrients in the EPA (see Chapter 3A of this volume), the typical north-to-south gradient is somewhat disconnected but evident at interior monitoring stations of the EPA. During WY2014, WCA-2 had the highest annual mean SO_4^{2-} concentration of 44.5 mg/L, followed by WCA-3 (12.6 mg/L), ENP (1.8 mg/L), and the Refuge (6.3 mg/L). Furthermore, the only region with a significant decreasing annual arithmetic mean SO_4^{2-} concentration was the Refuge, with all other areas experiencing non-significant trends (**Table 3B-6)**. SO_4^{2-}concentrations within the Refuge significantly decreased during the Phase II period (**Figure 3B-22**), which could possibly be due to the construction and operation of STA-1W and Stormwater Treatment Area 1 East (STA-1E) in combination with the rainfall-driven hydrology. However, it has been suggested that the Everglades STAs 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 sulfur to as a soil amendment to agricultural fields. It should be noted that the BMPs implemented were not used to directly mitigate sulfate; however, these BMPs could have provided ancillary benefits. It is also important to note that SO_4^{2-} concentrations within the Refuge spiked after extended periods of dry conditions as observed between WY1985–WY1994. This was not just isolated to water column sulfate, but phosphorus and nitrogen also spiked during these periods. The very low SO_4^{2-} concentrations observed for the interior portion of the Refuge indicate that either assimilation of sulfate is occurring and potentially could be limiting due to its low availability (Bellinger and Van Mooy, 2012), or very little to no high sulfate water reached the interior portion (due to topography and hydrology) of the Refuge and these low concentrations reflect deposition-driven water column concentrations.

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 sulfate. The hydrology of WCA-2A spreads the canal inflow broadly, and WCA-2 soils have relatively high concentrations of sulfur. In fact, samples collected between 2003 and 2004 at limited locations within each region indicate that WCA-2A soils had the greatest concentration of sulfur (14,025 ± 1,173 mg/kg; mean ± standard error), followed by WCA-3A (9,100 ± 576 mg/kg) and the Refuge (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 dataset (Everglades Soil Mapping Data; Reddy et al. 2005). This larger effort showed high concentrations of soil sulfur in WCA-2 and around the periphery of WCA-1. This high concentration of sulfur 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 WCA-2, it is reasonable to suggest that growth of macrophytic biota within this region of the EPA is not sulfur/sulfate$^-$-limited, corresponding to results presented by Bellinger and Van Mooy, (2012)

Previous peer reviews of this SFER chapter (2013 and 2014 SFER Volume 1, Appendix 1-2) 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 sulfate water quality standard for the EPA. As explained above, the sulfur/Hg biogeochemical cycle has proven to be very complex and 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 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 sulfur will reduce Hg

methylation or shift methylation hot spots on the landscape or regional scale, or if it is even feasible to reduce sulfur/ sulfate entering the EPA.

In an effort to provide more information on the role of sulfate in Hg cycling, the 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 sulfur/sulfate will cause a positive or negative ecological response.

The commonly referenced 1 mg/L sulfate CERP performance measure for the Greater Everglades was developed to indicate background marsh concentrations. 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 sulfate goal is not consistently associated with any particular level of Hg in the Everglades (Julian et al., 2014a). 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/ecological integrity. Furthermore, to date, no studies have been documented to justify either a numeric sulfate criterion of 1 mg/L, or a site-specific alternative criterion that incorporates other potential factors in the methylation process, for the protection of fish and wildlife in the EPA.

Sulfate Loads

Each year, the EPA receives variable amounts of surface water inflows. These inflows contribute to loading of surface water constituents, including sulfate to the EPA system. **Table 3B-7** provides estimates of the inflow SO_4^{2-} load to each portion of the EPA for WY2014. Flows and SO_4^{2-} loads are also provided for the Baseline, Phase I and Phase II periods for comparison. Major sources of surface SO_4^{2-} loads to EPA include runoffs from EAA and Lake Okeechobee discharge (James and McCormick, 2012).

Table 3B-7. Mean flow-weighted mean (FWM) SO_4^{2-} concentrations (mg/L) and loads (metric tons, or mt) to the EPA for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), Phase II (WY2005–WY2013), and WY2014 periods.

	Area	Period				Last Five-Year Mean
		Baseline	Phase I	Phase II	Current	
		WY1979-1993	WY1994-2004	WY2005-2013	WY2014	WY2010-WY2014
Mean Annual Sulfate Load (mt)	Refuge	53,419	54,924	19,379	29,498	18,775
	WCA-2	41,773	45,319	48,029	64,131	49,106
	WCA-3	14,433	13,409	40,749	47,661	38,420
	ENP	16,035	15,979	11,723	11,057	11,136
Mean Annual Sulfate FWM (mg/L)	Refuge	86.1	69.5	54.9	62.9	56.5
	WCA-2	60.1	52.3	49.7	48.2	47.5
	WCA-3	10.1	7.3	26.0	27.7	25.7
	ENP	14.6	8.0	10.5	7.4	9.6

In addition to surface water, atmospheric deposition contributes to the SO_4^{2-} loading into the EPA (see below) and increased atmospheric deposition of sulfate has the potential to add to SO_4^{2-} loads to sediments, soils, and surface waters. Atmospheric deposition rates of SO_4^{2-}, much like other mineral and nutrient constituents, varies greatly from year to year and can be highly variable. Based on data presented within this chapter, wet deposition rates of sulfate to the EPA can range from 470.6 to 1,657.1 milligrams per square meter per year (mg m^{-2} yr^{-1}). Dry deposition rates are significantly less, ranging from 47.4 1 mg m^{-2} yr^{-1} to 127.6 1 mg m^{-2} yr^{-1}. Using these deposition rates, the EPA received approximately 9,486 ± 293 metric tons (mt; mean ± SE, WY1981–WY2013) of SO_4^{2-} through deposition.

Annual SO_4^{2-} load from surface water sources, including internal transfers to the northern portion of the EPA (Refuge, WCA-2, and WCA-3) was 141,289 mt, with a FWM SO_4^{2-} concentration of 40.1 mg/L. This represents a 38 percent increase in SO_4^{2-} load relative to the previous year (WY2013: 102,337 mt) driven by changes in hydrology (see Chapter 2 of this volume). Discharges from the EPA accounted for 64,619 mt of SO_4^{2-}, with a FWM concentration of 20.9 mg/L during WY2014. The difference between inflow and outflow loads (79,670 mt) indicates that plant and microbial uptake, burial, and gaseous sulfur emissions (i.e., hydrogen sulfide, dimethyl sulfide, etc.) after anaerobic reduction by SRB are occurring within the natural communities of the EPA. Annual SO_4^{2-} load to surface waters to ENP was 11,057 mt, with a FWM concentration of 7.4 mg/L. This corresponds to a 16 percent increase in SO_4^{2-} load from the previous year (WY2013: 13,104 mt).

Flows and loads to the EPA during Phase II and WY2014 were highly influenced by climatic extremes in the region, which experienced extended drought conditions and extended periods of heavy rainfall. The annual SO_4^{2-} load to the Refuge was 29,498 mt during WY2014, representing a 68 percent increase in load from the previous year (WY2013: 17,553 mt). The annual SO_4^{2-} load to WCA-2 also had an increase with 64,131 mt of SO_4^{2-} entering WCA-2 during WY2014, representing a 19 percent increase in load from the previous year (WY2013: 54,039 mt). WCA-3 also experienced an increase in SO_4^{2-} load with 47,661 mt of sulfate entering WCA-3, corresponding to a 55 percent increase from the prior year (WY2013: 30,745 mt).

Trends in annual inflow FWM SO_4^{2-} concentrations declined significantly throughout the period record for the Refuge, WCA-3, and ENP. However no significant trends were apparent for inflow FWM concentrations for WCA-2 (**Table 3B-6**). The majority of the trends are driven by a relatively high FWM concentration in earlier years followed by reductions in FWM concentrations with some areas experiencing larger reductions than others (**Figure 3B-23**). Similar to the FWM concentration trend analysis, inflow loads to the Refuge and WCA-3 experienced significantly declining trends throughout the POR. However, WCA-2 and ENP did not experience significant trends throughout the entire POR (**Table 3B-6**). Although SO_4^{2-} loads spiked throughout the EPA during WY1993 and WY1995 most likely due to drought and flood cycles as well as upstream management practices, the Refuge experienced significant load decreases (Julian et al., 2014a). This potentially could be due to the reduction of SO_4^{2-} loads by the STAs, even though as stated above they only remove approximately 10 percent of the sulfate entering the STAs. Other possibilities include changes in agricultural practices, water management, and improvements to BMPs within upstream basins. Furthermore, a drastic difference is apparent between Phase I and Phase II within the Refuge, while other areas have not experienced such drastic reductions in SO_4^{2-} loads (**Table 3B-7**; **Figure 3B-23**).

While the EAA and Lake Okeechobee contributes significant SO_4^{2-} loads to the EPA, they are not the sole source of surface water SO_4^{2-}. Drainage districts and other basins to the east as well as agricultural lands from the west also contribute SO_4^{2-} to the EPA. A large range of values have

been published on EAA outflow SO_4^{2-} loads (**Table 3B-8**), ranging from 31,730 mt (James and McCormick, 2012) to 348,586 mt (Corrales et al., 2011) of SO_4^{2-} discharging from the EAA. These values may represent total outflow load, which could include historical backpumping from the EAA back into Lake Okeechobee (James and McCormick, 2012). However, more recent backpumping only represents a relatively small amount of SO_4^{2-} and flow to the lake. It should be noted that values reported in (Corrales et al., 2011) are sulfur-based values; for comparison purposes, the original values were converted to SO_4^{2-} loads using a value of 3.00. This conversion factor is the ratio of the molecular weight of SO_4^{2-} (96.06 g/mol) to S (32.06 g/mol).

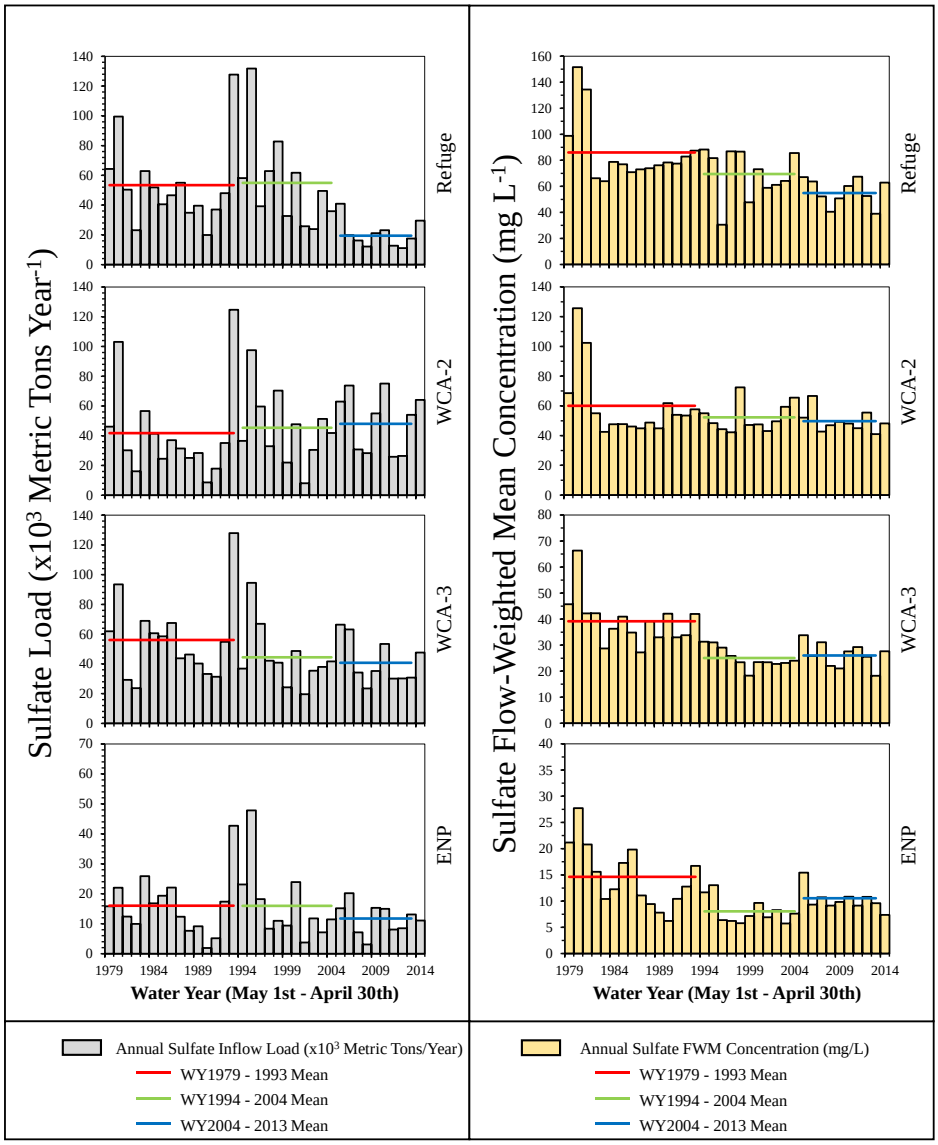

Figure 3B-23. Annual inflow SO_4^{2-} load (left panel) and FWM SO_4^{2-} concentrations (right panel) for the Refuge, WCA-2, WCA-3, and ENP from WY1979–WY2014. Horizontal lines indicate the mean annual loads and flows for the Baseline (WY1979–WY1993), Phase I (WY1994–WY2004), and Phase II (WY2005–WY2013) periods.

Table 3B-8. Estimates of annual Everglades Agricultural Area (EAA) SO_4^{2-} outflow load from peer-reviewed and technical literature in relation to EPA inflow.

		Schueneman, 2001; Wright et al., 2008	Gabriel et al., 2010[1]	Corrales et al., 2011	James and McCormick, 2012	**Reported in this Chapter[2]**
EAA Outflow	Flow Volume (1,000 ac-ft)	---	---	2,324	516	---
	Sulfate Load (1 mt/yr)	61,657	48,672 (43,729-55,406)	348,586	31,730	---
	Sulfate FWM (mg/L)	---	---	121.6	49.9	---
EPA Inflow	Flow Volume (1,000 ac-ft)	---	---	---	---	2,451 ± 174
	Sulfate Load (1 mt/yr)	---	---	---	---	113,209 ± 11,925
	Sulfate FWM (mg/L)	---	---	---	---	46.1 ± 2.3

[1] Mean (Range)
[2] Mean ± standard error of the entire period of record (WY1979–WY2014)

TRENDS IN ATMOSPHERIC DEPOSITION OF SULFATE

Paul Julian II[1]

Introduction

Acid precipitation and the associated deposition of acid anions have become 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 U.S. Congress to amend the Clean Air Act (CAA) 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 P, Fe, 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 sulfate-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 (**Figure 3B-24**).

Methods

Atmospheric deposition data was retrieved from the NADP NTN and USEPA Clean Air Status and Trends Network (CASTNET) for several monitoring locations identified in **Figure 3B-24**. 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 NTN measures free acidity (H^+ as pH), conductance, calcium, magnesium, sodium, potassium, sulfate nitrate, chloride, and ammonium. Both laboratory and field data are rigorously checked according to QA/QC protocols approved by the USEPA. Similar to the NADP NTN, CASTNET 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-24**. 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 WY1981– to present; dry deposition data from WY1999–present). In an effort to conduct trend analysis, the POR (WY1999–WY2013) mean dry deposition was calculated to fill in data gaps in the earlier portion of the entire dataset; only WYs with a complete year's worth of data were used to calculate this average dry SO_4^{2-} flux of 87.4 ± 4.5 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. All analysis was conducted using JMP® (Version 10.0.0, SAS, Cary, NC, USA) 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 (τ=-0.34, ρ= *<0.05*), WYs with complete data range from 657.1 to 1695.7 milligram per square meter (mg/m^2/year) (**Figure 3B-25**). Based on surface water inflow loads into each respective region (presented elsewhere within this chapter), atmospheric wet deposition of SO_4^{2-} is a relatively low contributor to each region of the EPA (**Table 3B-9**). No significant trend in annual SO_4^{2} dry deposition throughout the relatively short POR (WY1999–WY2013) was apparent (τ=-0.15, ρ= 0.35). However a decreasing trend in total (wet + dry) annual deposition was observed throughout the POR (τ=-0.33, ρ= *<0.05*). A decline in total SO_4^{2-} deposition is recent years can likely be attributed to tighter emission controls after a significant lag period of approximately 10 years.

Similar to the wet deposition trend, PWM sulfate concentrations declined throughout the POR (τ=--0.63, ρ=*<0.01*), with concentrations ranging between 0.57 mg/L and 1.10 mg/L. The range of annual PWM sulfate 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 WY1981–WY2013. **Figure 3B-25** includes data for WY2014 but these data are incomplete. Therefore, values presented for WY2014 should be used with caution and

are expected to be updated in the next SFER. 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).

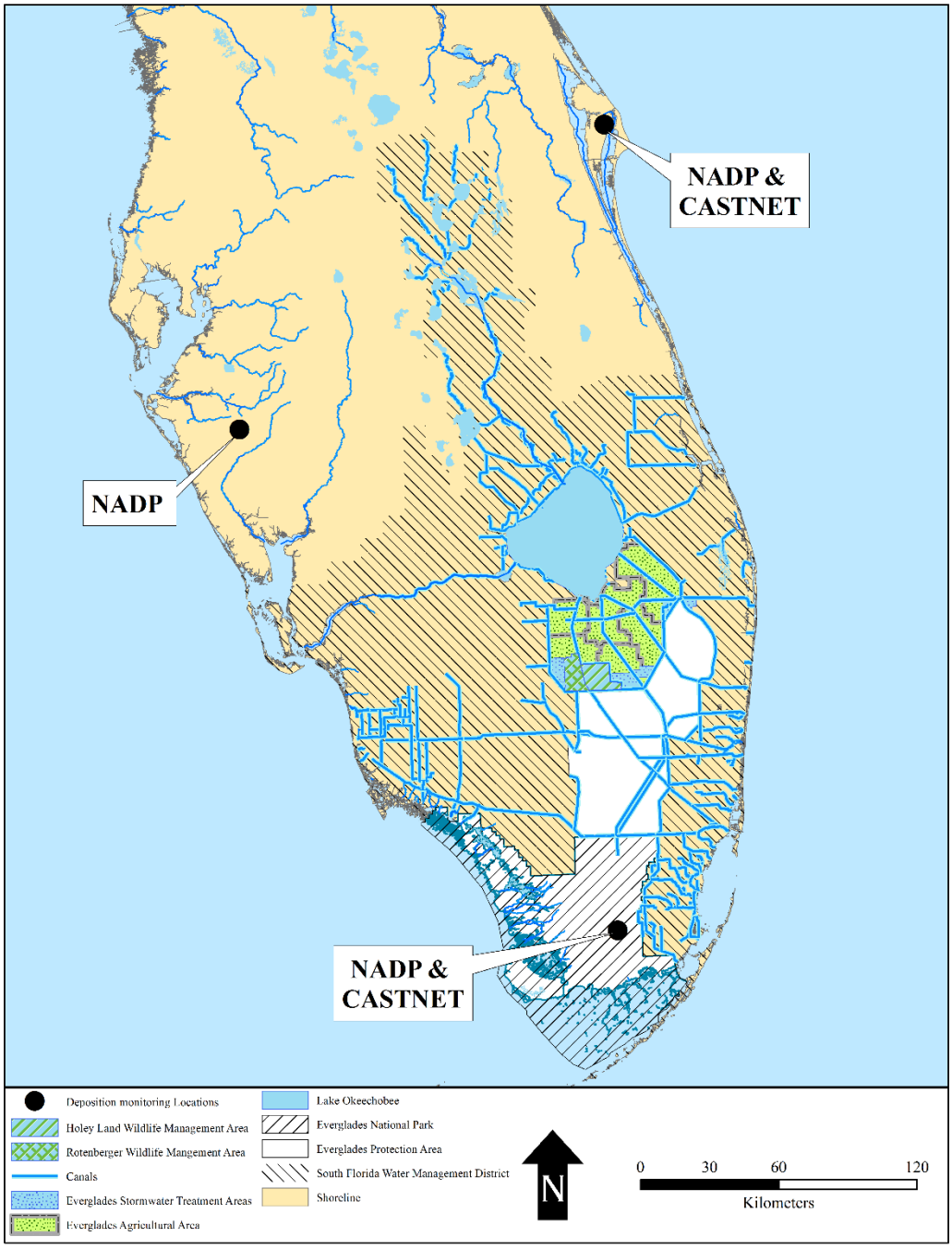

Figure 3B-24. National Atmospheric Deposition Program (NADP) and Clean Air Status and Trend Network (CASTNET) stations used in this analysis.

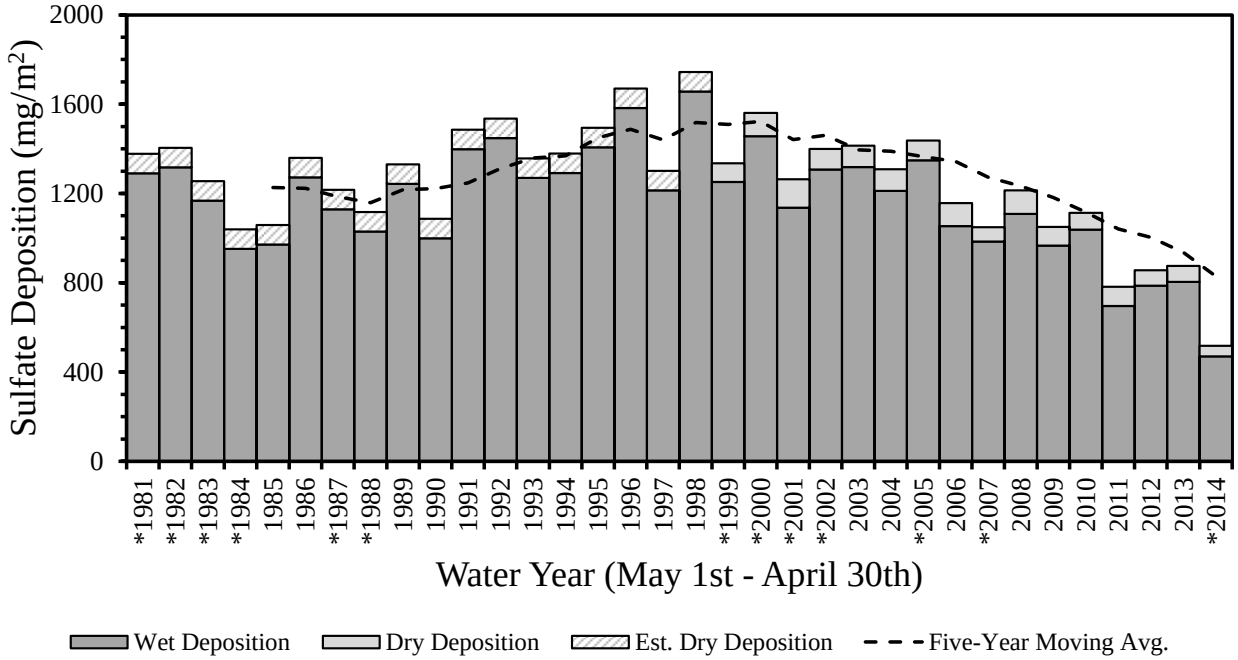

Figure 3B-25. Annual SO_4^{2-} deposition in milligrams per square meter (mg/m^2) and five-year moving average between WY1981–WY2014. Stations with less than 12 months of data are identified with an asterisk (*).

Table 3B-9. Atmospheric SO_4^{2-} load from wet deposition and surface water inflow load to each region of the EPA for WY2013.

Area	Atmospheric Load (mt)	Surface Water Inflow Load (mt)	Percent Atmospheric Contribution
WCA-1	293	17,553	1.6
WCA-2	278	54,309	0.5
WCA-3	1,239	30,745	3.9
ENP	2,048	13,104	13.5

AGRICULTURAL SULFUR USE IN THE EVERGLADES AGRICULTURAL AREA

J.M. McCray[5] and A.L. Wright[5]

Sulfur cycling in the EAA, particularly in organic soils, has received attention in several recent articles (Ye et al., 2011a, 2011b, 2010a, 2010b). Most studies focused on the effects of elemental S additions on nutrient transformations and elemental cycling, and plant growth and yield. Elemental S can be added to soils for the purpose of reducing soil pH, thereby increasing the availability of nutrients, particularly phosphorus, manganese, and other plant-essential micronutrients, to crops. It is important to understand from an agronomic perspective how much elemental S to add to soil to produce a positive response for crops and better understand the mechanisms for how the added S changes soil biogeochemical properties and processes.

A multiyear field study was conducted to determine sugarcane yield response to elemental S on organic soils in the EAA. The study consisted of six locations where rates of sulfur or various elemental S formulations were compared with no application. Soil pH related well to relative sugar yield at two locations, with an average pH>7.2 in treatments with no S application, and yield was reduced with pH>7.2, with substantial yield reductions at pH>7.5 (McCray and Rice, 2013). Proposed recommendations for banded S application in the furrow at planting suggest 280–560 kg S/ha with pH>7.5 and 112–280 kg S/ha with pH 7.2-7.4. Importantly, this research suggests that site-specific variable rate elemental S application would improve soil pH management in the Everglades.

The variable nature of pH on organic soils in the EAA could lend itself well to variable rate application of S. This result is especially important as the organic soils become shallower and in closer proximity to the bedrock limestone underlying the soils, which tend to increase the pH. Therefore, it is likely that the EAA will experience soils with higher pH in the future. Mehlich 3-extractable soil manganese (Mn) was strongly related to leaf Mn concentration and determined to be limited as pH increases, particularly at pH>7.5. Also, low extractable Mn can exist at lower pH values, suggesting that a low rate of STM5 (granular sulfur with 80 percent S and 5 percent Mn) and possibly other sources of Mn may be beneficial in those situations. Proposed recommendations suggest application of 112 kg STM5/ha with pH 6.6-7.1 and Mehlich 3-extractable Mn<5.0 g/m^3. These proposed recommendations will require approval by University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS) and represent a substantial reduction in recommended S compared to the current recommendations while targeting specific situations where sugarcane yield response can be expected. As such, estimates of elemental S application rates per unit area that have been made in the past may have overestimated the quantity of elemental S that is actually applied or needed to effect a change in soil pH and an agronomic response. These updated guidelines should improve S management in the EAA and adjust application rates to levels that have been shown to be most effective to produce a crop response while simultaneously minimizing over-application.

As part of the larger sulfur study, at one location measurements were taken of pre-harvest samples to estimate S removal by sugarcane harvest. Sulfur application did not significantly increase S content of pre-harvest samples of leaves, tops, juice, or bagasse. It was determined that sugarcane harvest removed approximately 0.26 kg S/Mg cane (kilogram of sulfur per mega-gram of cane), which would be approximately 24 kg S/ha (kilogram of sulfur per hectare) removed at harvest for a 90 Mg/ha (mega-gram per hectare) crop (40 ton cane/acre). Additionally, if 80 percent of the S in leaves and tops are lost by burning before harvest, this would remove

approximately 15 kg S/ha for a 90 Mg cane/ha crop, giving a total of 39 kg S/ha (34 lb S/acre) removed or lost from the field.

Elemental S application, along with application of iron and aluminum sulfate, has proven effective at reducing soil pH and increasing concentrations of plant-available P during the short growing seasons of leafy greens crops. These amendments were applied to soil for the purpose of reducing soil pH and increasing availability of nutrients to vegetable crops. Whereas granular forms of sulfur were applied to sugarcane, its slow reaction time in soil was insufficient to produce a positive agronomic response for short-duration vegetable crops such as lettuce. These three amendments were applied at rates up to 1,000 kg/ha to evaluate rate and source effects. Also, amendment placing was evaluated by comparing banded versus broadcast application.

There was no agronomic benefit of any amendment application beyond the 560 kg/ha rate for leafy greens. All three amendments performed similarly in terms of producing the same yield response. Banded application of the amendments produced the optimal crop response at a lower application rate due to the concentrated effects of the acidification reactions on a smaller surface area of soil. Consequently, it was quite advantageous to apply amendments only in the root zone. The powdered forms of these amendments were preferable to the granular form due to their higher surface area which enhanced reaction times in soil, resulting in a more dissolution of the amendments and more rapid decline in soil pH and increase in nutrient concentrations. This rapid reaction resulting from the use of powdered amendments produced significant yield increases.

EVERGLADES AGRICULTURAL AREA SULFUR MASS BALANCE: APPLICATION AND HARVEST ESTIMATES

Paul Julian II[1]

Elemental S application and removal (due to harvest) rates are very important parameters in the overall EAA regional sulfur mass balance model. Inflow surface water S loads account for a very small percent of the overall sulfur entering the EAA. As such outflow surface water S loads are elevated relative to inflow loads. Several processes account for the increase of S in outflow surface waters including soil oxidation, atmospheric deposition, groundwater flux, and agricultural application (see **Appendix 3B-2**). Application of elemental S is sometimes used as a soil amendment in EAA soils to reduce soil pH in an effort to increase nutrient availability to crops (Ye et al., 2010a). As such vegetation and in this case crops accumulate nutrients in there tissue. These nutrients are then directly removed from the system due to harvesting.

In the past, very little to no guidance was given to growers regarding the addition of S to soil to aid crop (i.e., sugar cane) productivity. In recent years, UF/IFAS has provided recommendations for using elemental S as a soil amendment. The current UF/IFAS soil amendment recommendation is 560 kg S/ha for soils with a pH > 6.6. However, more recent research is indicating that 280–560 kg S/ha (pre-planted in furrows) for soils with pH > 7.5, 112–280 kg S/ha for soils with pH between 7.2 and 7.4 and 112 kg S/ha using STM5 for soils with pH <7.1 every 3 to 4 years. While not an official UF/IFAS recommendation, current research indicates that less elemental S application is needed and only for specific soil pH conditions (Anderson, 1985). However, based on anecdotal evidence it seems that growers recognize that the current recommendation are not cost-effective and potentially not being utilized fully. Unfortunately, it is not known how much S is being applied across the EAA and, therefore, a reliable estimate for its application is not available at this time. Regardless, several studies have estimated agricultural S application rates, as summarized in **Table 3B-10**. The assumed application rates from various studies range from 13–41 kg/ha/yr, which range from 2.3–7.3 percent of the current UF/IFAS recommendation (**Table 3B-10**).

Conversely, S is removed from the EAA as a result of sugarcane harvest operations. Both the physical removal of the plant as well as controlled burns associated with harvest techniques remove S from the field. As discussed in the *Agricultural Sulfur Use* section above, approximate 38 percent of the S removed during harvest is removed by burning during pre-harvest operations. Various studies have attempted to quantify S removal due to sugarcane harvest ranging from 39–105 kg/ha/yr (**Table** 3B-11**)**. These estimates are ultimately dependent upon the amount of sugarcane harvested; as such, sugarcane coverage from 2000 to 2009 has fluctuated between 161,874 ha to 183,727 ha (Baucum and Rice, 2009). Additionally, the sugarcane harvest interval can range between two to four years, with a particular field producing three annual crops over this time period. Using these estimates of S removal due to harvest, sugarcane area, and harvest intervals, the amount of S being exported from the EAA could range between 1,578–9,646 mt. Interestingly, these harvest estimates range from 23 to >138 percent of the average annual S application amount (**Table 3B-10**; 6,997 mt).

Table 3B-10. Agricultural sulfur (S) application rates from various studies in the EAA. Calculated annual and three-year S application amounts are also provided.

	Schueneman (2001); Wright (2008)	Gabriel et al. (2010)	James and McCormick (2012); Landing (2014)	Corrales et al. (2011)	Current UF/IFAS Recommendation
EAA Area (ha)	283,280	280,460	230,466	290,594	230,466[1]
S Application Rate (kg/ha/yr)	13	22	27	41	560
Percent of Current Recommendation	2.3	3.9	4.8	7.3	---
Annual S Applied to EAA (kg)	3,682,640	6,170,120	6,222,582	11,914,354	129,060,960
Annual S Applied to EAA (mt)	3,683	6,170	6,223	11,914	129,061
3 Year Application (kg/ha)[1]	39	66	81	123	1680
3 Year S Applied to EAA (kg)	11,047,920	18,510,360	18,667,746	35,743,062	387,182,880
3 Year S Applied to EAA (mt)	11,048	18,510	18,668	35,743	387,183

[1] EAA area estimate from James and McCormick (2012).

Table 3B-11. S removal estimates due to harvest operations from studies conducted in the EAA, including potential minimum/maximum removal from harvest.

	EAA Sugarcane Coverage (ha)[1]	Sugarcane Harvest Interval (Years)[1]
Minimum	161,874	2
Maximum	183,727	4

Source	Reported in this Chapter (McCray and Wright)	Corrales et al. (2011)	Gabriel et al. (2010)	Landing (2014)
S Removal from Harvest (kg/ha/yr)	39	80	91	105
Min. S Removed Per Year (kg/yr)[2]	1,578,272	3,237,480	3,682,634	4,249,19
Min. S Removed Per Year (mt/yr)[4]	1,578	3,237	3,683	4,249
Max S Removed Per Year (kg/yr)[3]	3,582,677	7,349,080	8,359,579	9,645,66
Max. S Removed Per Year (mt/yr)	3,583	7,349	8,360	9,646

[1] (Baucum and Rice, 2009); [2] Harvest Rate x Min. Cane Area/Max Harvest Interval; [3] Harvest Rate x Max. Cane Area/Min Harvest Interval; [4] 1kg = 0.001 mt

PRIORITY QUESTIONS FROM WATER YEAR 2014

Paul Julian II[1], Ben Gu and Garth Redfield

This section addresses four key questions that the FDEP and the District encountered during WY2014 through either peer-reviewed or technical literature. Specifically, these issues revolve around biota-Hg interactions, predictability of the proposed S-Hg relationship, Hg trophic dynamics, and microbial ecology. Highlights of these priority questions are presented below.

1. Does water column sulfate influence fish tissue mercury concentrations in a predictable manner?

The relationship between water column SO_4^{2-} and Hg concentrations in fish tissue is discussed in *Fish Mercury and Surface Water Sulfate Relationships in the Everglades Protection Area* (Gabriel et al., 2014). This possible interaction, if shown to be quantitatively definable, could allow for the control of SO_4^{2-} concentrations to be a management tool for addressing the mercury problem in South Florida. The paper attempts to identify patterns consistent with the proposed sediment MeHg and surface water sulfate relationship using surface water SO_4^{2-} and fish tissue Hg concentrations from mosquitofish, sunfish, and largemouth bass collected within the EPA.

Upon careful review, the interpretation of the reported data unfortunately does not demonstrate any meaningful relationship as critiqued in (Julian et al., 2014b). More specifically, data interpretations do not capture important findings, and derived management guidance for S and Hg in the Everglades is not considered to be defensible by agency reviewers of the article. The graphical representation of the data is the basis for the arguments outlined (see **Figure 3B-26**) and, yet, that analysis is only qualitative, making it impossible to link established data patterns to the reported conclusions. Furthermore, a bell-shaped relationship is not evident in the original, untransformed data (not shown). This is relevant because it is suggested that the shape of the curve supports the conclusion that SO_4^{2-} is a driver of elevated fish THg concentrations. Plotting just the 25th and 75th percentiles of the data also must be carefully considered (**Figure 3B-26)**. This data manipulation has the potential to eliminate 50 percent of the variability of both fish THg and surface water SO_4^{2-} data. If all the data variability were displayed, then the data would span both axes of each plot in a random pattern (i.e., a bell-shaped relationship would not be evident from the complete data sets).

In Gabriel et al., (2014), the results and discussion acknowledges the Goldilocks concept, a suggested unimodal relationship between S and MeHg production that strikes a balance between sulfate limiting and hydrogen sulfide inhibiting Hg methylation. (Gilmour, 2011) documents aspects of this S-Hg relationship from numerous studies and highlights the responses to SO_4^{2-} additions as well as the very high variability of MeHg within sediments. However, that analysis does not directly address the variability inherent in translocating MeHg into the water column or transferring it through constantly changing trophic structures in wetlands. This complexity helps explain why (Julian et al., 2014a) reported that ecosystem-level Hg accumulation is not predictable from SO_4^{2-} concentrations in marsh surface waters like the Everglades. Gabriel et al., (2014) attempted to apply the unimodal concept to log-transformed data that appears to follow a bell-shaped relationship between THg in fish and SO_4^{2-} concentrations in the water column. For this case, Pollman, (2012) was the key source with an analysis of REMAP mosquitofish data.

Pollman found that the 90th percentile of the THg concentrations in fish followed a rough bell-shaped response curve and that peak THg levels were found between 1 and 10 mg/L of SO_4^{2-}. However, it was not acknowledged by Gabriel et al., (2014) that 90 percent of the data plotted followed no significant pattern for SO_4^{2-} and THg. Even if a bell-shaped response curve is assumed, then some sites could experience increasing fish THg with decreases in surface water SO_4^{2-} and this tradeoff would have to be considered for each part of the downstream system.

Gabriel et al., (2014) also considered implications of the possible mercury bioaccumulation relationship to Everglades' management and restoration. However, their findings suggest that there is evidence for the mercury problem across all sulfate concentrations and that factors influencing Hg methylation must be addressed. Specifically, the paper concludes that decreasing SO_4^{2-} concentrations to 1 mg/L, the CERP performance measure, or maintaining levels at >20 mg/L would significantly reduce the MeHg risk. Other researchers have also suggested implementing actions to reduce sulfate levels to 1 mg/L (Corrales et al., 2011; Orem et al., 2011). Their data simply do not support these actions as a means to reduce the mercury problem. The 1 mg/L target is an unrealistic and unattainable goal, even if the CERP performance measure (which has not been demonstrated scientifically) was a valid water quality criterion.

Overall, there is much variability and complexity in any relationship between water column sulfate and THg in fish tissue (Julian et al., 2014a). Gabriel et al. (2014) shows the empirical relationship between surface water SO_4^{2-} and fish THg in each fish species is highly variable at <20 mg/L and skewed to the right at >20 mg/L, and unquantifiable for SO_4^{2-} concentrations from <1 to 60 mg/L. This undefinable interaction is observed despite some indication from literature on porewater that a SO_4^{2-}/Hg relationship would be evident in long-term field data. There was high variability in the fish THg data to the extent that no predictive pattern was observed in the data, and there were several instances of relatively high fish THg levels in both high (>30 mg/L) and low (<1 mg/L) sulfate conditions. Importantly, the data in this and other recent studies document that too many factors influence the biogeochemistry of SO_4^{2-} and Hg in natural ecosystems to allow any consistent and identifiable areas of high and low fish THg across the spectrum of surface water SO_4^{2-} concentrations. Therefore, without the ability to quantitatively link surface water SO_4^{2-} and ambient MeHg and THg in fish tissue, an ecosystem-wide sulfur strategy as a management approach to reduce MeHg risk in the EPA is not warranted. In the meantime, based on available literature and field data from the EPA, efforts to control Hg inputs internationally must be redoubled to help address the Everglades mercury problem.

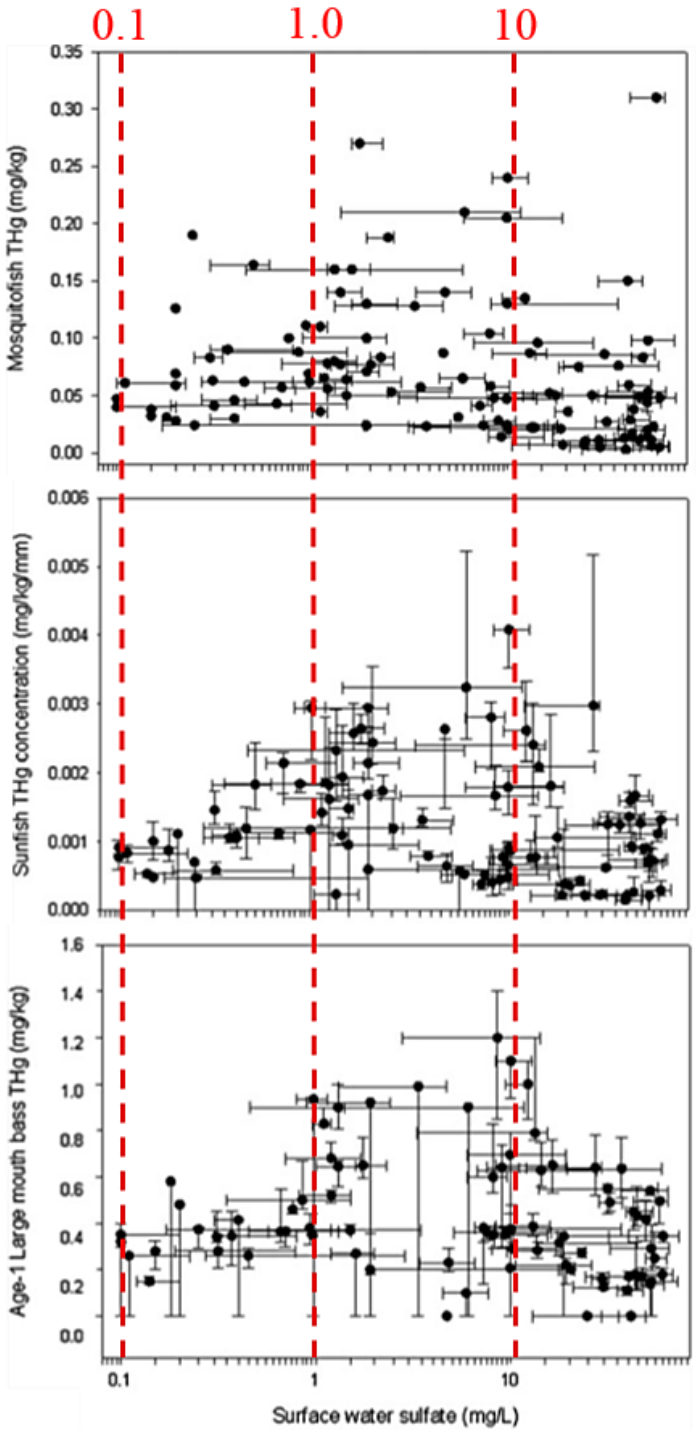

Figure 3B-26. Fish THg and SO_4^{2-} relationship including the 25th and 75th percentile and a log-transformed axis (adapted from Gabriel et al., 2014). Red-dashed line indicates SO_4^{2-} concentrations at 0.1, 1.0, and 10.0 mg/L.

2. Do other water quality parameters affect fish tissue mercury concentrations?

The bioaccumulation of Hg in aquatic biota is controlled by a suite of species-specific and environmental interconnected factors. Species-specific factors related to fish include trophic position, feeding selectivity, physiology, and age (Bodaly et al., 1993). Environmental factors within aquatic ecosystems include the microbial activity of MeHg-producing bacteria, water quality conditions, bioavailability of MeHg, and load into a given ecosystem or region (Bodaly et al., 1993; Gilmour et al., 1998; Gilmour and Henry, 1991; Orem et al., 2011). Also, in the South Florida region, (Julian and Gu, 2014) conduct an empirical analysis of Hg bioaccumulation and water quality constituents in the EPA.

Evidence suggests that several water column chemical parameters (e.g., alkalinity, chloride, pH and others) either influences fish physiology or alters the nature of Hg so that it can be absorbed easier (Lange et al., 1993; Spry and Wiener, 1991). Within aquatic ecosystems, alkalinity also can play a significant role in the bioavailability and toxicity of cationic metals, including Hg (Lange et al., 1993; Spry and Wiener, 1991). As observed in other studies, fish have the ability to accumulate Hg directly from the water via gill membranes, which is more efficient at low calcium concentrations or when chloride-Hg complexes are formed (Gutknecht, 1981; Rodgers and Beamish, 1983; Zheng et al., 2013). Alkaline water conditions have been known to increase Hg tissue concentrations in fish species due to changes in physiological and ecological factors including fish metabolism, dietary composition, and prey species composition (Rodgers et al., 1987). There are other interactive effects of water quality conditions can also influence fish tissue Hg concentrations. For example, several studies have demonstrated that H+ ions compete with Hg(II) for binding sites located within dissolved organic matter under low pH conditions (Ravichandran, 2004; Skyllberg, 2008) and thereby increase the availability of Hg for methylation.

Water quality conditions within each region of the EPA are unique (Julian et al., 2013). (Julian and Gu, 2014) performed a multiple regression analysis on selected water quality parameters and bass tissue THg in the EPA (normalized by body weight). This analysis indicates that Hg accumulation depends to some extent on several water column characteristics, including alkalinity, pH, sulfate, and specific conductance (**Figure 3B-27**). However, this dependency is region-specific as indicated by the large variations in parameter estimates among regions. For instance, there was a significant relationship between alkalinity and tissue THg concentrations within WCA-1, WCA-3, and ENP, but this relationship was not significant in WCA-2. Meanwhile, the relationship between pH sulfate and THg tissue concentrations were significant for the entire EPA. Specific conductance was not significant for any area except ENP.

The analysis performed by Julian and Gu, (2014) is constrained by limited data and distances between fish collection site and water quality monitoring locations. However, it does demonstrate that (1) SO_4^{2-} is one of the water quality constituents that have some modest influence on Hg accumulation in bass, and (2) there were elevated bass tissue THg concentrations at both extremely low (<1 mg/L) and high (>~30 mg/L) SO_4^{2-} concentrations in the Everglades marsh. The multiple regression models resulted in low R^2 estimates, signifying low predictability and high variability among water quality parameters and fish Hg levels. This suggests that multiple factors may result positively or negatively on Hg accumulation in fish. The low R^2 values also imply the complexity of the Everglades ecosystem, underscoring that no one-variable empirical model can capture the complexities of the Everglades Hg cycle. Overall, these data provide no evidence that reducing SO_4^{2-} concentrations can be expected to lower Hg bioaccumulation in fish.

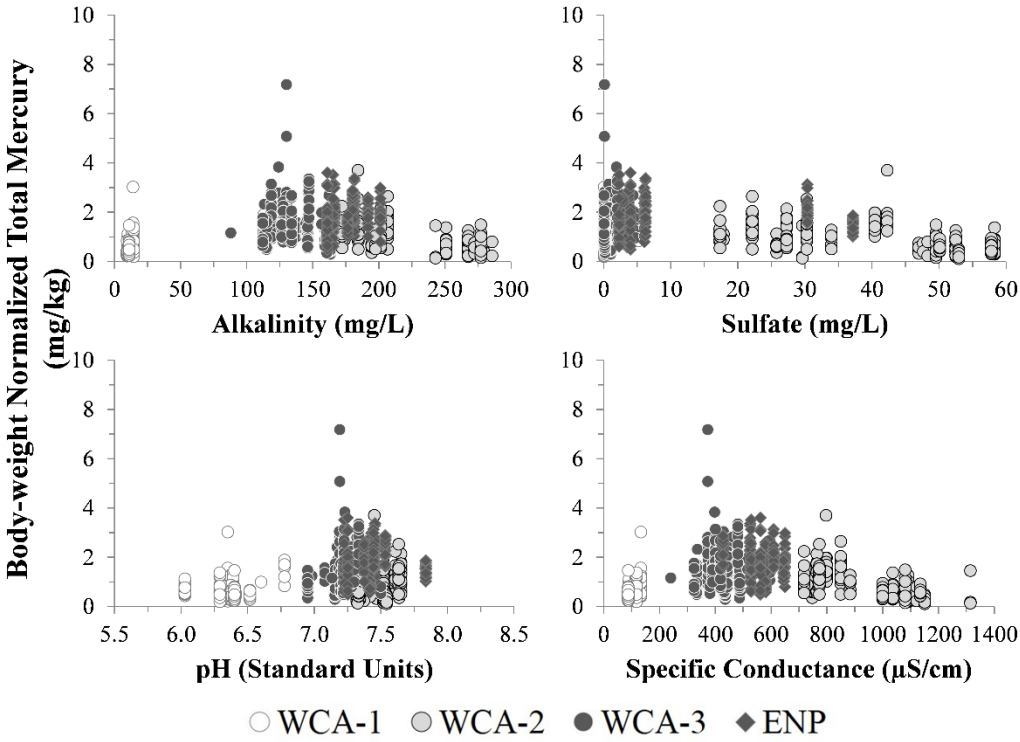

Figure 3B-27. Region-specific Everglades largemouth bass tissue THg concentration and surface water quality relationships for data from 2005–2013 (Julian and Gu, 2014). Bass data were normalized by body weight and water quality parameters are annual (365 days) mean concentrations prior to fish collection.

3. Are sulfate-reducing bacteria the sole mercury methylators or are there other bacteria groups involved?

Generally, it is accepted that sulfur plays a role in the S-Hg biogeochemical cycle through sulfate-reducing bacteria (SRB). Biogeochemical cycling of Hg within the Everglades is confounded by many chemical (dissolved organic carbon, pH, chloride, dissolved oxygen, etc.) and non-chemical variables (hydrology, food web structure, etc.). Highly variable methylation rates and consumer (i.e., fish species) Hg accumulation has been experienced throughout the Everglades system, with some locations with relatively low S concentrations experiencing high consumer Hg accumulation and vice versa (Julian et al., 2014a). It is possible that microbial population dynamics and community composition could explain some of this variability if different bacterial assemblages have unique methylation abilities in these wetlands.

In most ecosystems including the Everglades, SRB have been identified as the primary Hg methylators (Liu et al., 2012). While not all SRB species methylate mercury, species that are responsible include bacteria from the families Desulfobacteriaceca and Delsulfovibrionaceace, in the subclass Deltaprotobacteria (Gilmour, 2011; Hu et al., 2013; Liu et al., 2012). For those SRB that do methylate Hg, the exact mechanism(s) involved are still not fully understood (Hu et al., 2013; Parks et al., 2013).

In recent years, SRB have been suspected of or implicated in Hg methylation in the Everglades. Yet, past molecular techniques for monitoring the Hg methylating communities have been limited to a more indirect relationship between specific phylogenetic markers (specific genes) with MeHg formation (Schaefer et al., 2014). Unfortunately, very little rigorous genetic analysis has been undertaken within the Everglades ecosystem to identify particular phylogenetic affiliations among SRB. Nonetheless, it is evident that only particular microbes including SRB have the ability to methylate Hg based on advanced genetic analyses conducted in other areas (Gilmour, 2011; Hu et al., 2013).

Iron-reducing bacteria (Fe-RB) are also potential methylators in the Everglades. Much like SRB, FeRB are prokaryotic microorganisms that gain energy for cell synthesis and growth by coupling the oxidation of organic matter or, in some cases, molecular hydrogen (electron donors) to reduce ferric iron (Fe3+; electron acceptor). Kerin et al., (2006) determined through pure cultures that FeRB belonging to the class Deltaproteobacteria, and specifically the genera Geobacter and Desulfuromonas, produced MeHg at concentrations exceeding those of abiotic controls when grown with ferric iron (Fe^{3+}) as the terminal electron-accepting process.

The exact biochemical mechanism involved with the FeRB Hg methylation pathway also is not fully understood. Presumably FeRB can utilize the acetyl-CoA pathway similar to SRB discussed above. Similar to SRB, not all FeRB methylate Hg, as evidenced by the lack of methylation by particular FeRB species (Kerin et al., 2006). MeHg can be produced by FeRB under various terminal electron accepting conditions and ferric iron (Fe^{3+}) phases (i.e., solid or dissolved) (Fleming et al., 2006; Kerin et al., 2006; Yu et al., 2012). Analysis of river sediments by Yu et al., (2012) including genetic analysis indicated that both Fe and SO_4^{2-} reducers were active. It was further concluded that SRB and FeRB coexist where SRB are likely restricted to the upper layers of the sediment due to its dependence on soluble electron acceptors and the presence of Hg. Building on the work of Fleming et al., (2006), Yu et al., (2012) provided supporting evidence to the hypothesis that the extent of the Hg methylation zone in sediments is extended due to the ability of FeRB to utilize solid Fe^{3+} and adsorbed Hg. In an environment where electron donors are not limited, it would be possible that these processes (SO_4^{2-} reduction and Fe reduction) could co-exist spatially throughout the soil/sediment column.

Microbes known as methane-producing bacteria (MPB) may also be active in South Florida wetlands. MPB form large quantities of methane as a by-product of their energy metabolism. In

natural environments, MPB utilize organic rich soils, while in pure culture they utilize substrates such as hydrogen (H_2) and very simple carbon compounds (e.g., carbon dioxide, formate, and acetate). Based on pure culture experiments, Yu et al., (2012) demonstrated that MPB have the ability to methylate Hg at comparable methylation rates to FeRB and SRB, and higher MeHg yields relative to SRB or FeRB were also observed. Gilmour et al., (2013) demonstrated that both methylating and non-methylating MPB exist by experimentally (in-vitro) derived methylation rates, which indicate that MeHg yields from MPB are relatively higher than those observed by known MeHg producing SRB and FeRB. However, very little information regarding environmental (in-situ) methylation rates are available. It should be noted that methanogenic respiration yields the least energy relative to the common electron acceptor processes (i.e., Fe and SO_4^{2-} reduction) and occurs in sediments/soils that are highly reduced, such as regions with very low dissolved oxygen concentrations (i.e., hypoxic conditions) and low SO_4^{2-} concentrations (Reddy and DeLaune, 2008; Sturman et al., 2008).

Recently, Hamelin et al., (2011) observed MeHg production in a mineral-poor (sulfate and iron-limited) environment within periphyton communities from a shallow fluvial lake (although not in Florida). It was determined through a metabolic inhibition study and subsequent genetic analysis that MPB were the dominant microbial guild responsible for MeHg production. These findings suggest that MPB rather than SRB were likely the dominate methylators in the periphyton communities. This possibility could explain some of the variability in Hg/MeHg dynamics within the Everglades ecosystem due to the prevalence of periphyton communities. Methanogenesis requires highly reduced anoxic conditions dependent on the structure, thickness, and overall productivity of the periphyton complex, as well as the diversity of microbes present in these conditions within periphyton.

In conclusion, within the Everglades ecosystem, SRB are thought to be the dominate microbe guild that facilitates microbial Hg methylation. However, the relationship between MeHg and S is highly unpredictable within the Everglades ecosystem and other microbe guilds may be responsible for Hg methylation.

4. Can background inputs of sulfate maintain the production of methylmercury?

As previously discussed, several studies have indicated that reductions in marsh MeHg concentrations (sediment/soil, consumers, etc.) can be achieved by controlling SO_4^{2-} inputs to the EPA. However, consumers (fish) at the southern end of the system (ENP) continue to experience very high tissue Hg concentrations despite the relatively low water column SO_4^{2-} concentrations. Consistent with Dierberg et al., (2014), this relationship suggests that very low, possibly background concentrations of SO_4^{2-} can promote environmentally significant concentrations of MeHg. As such, a controlled laboratory study was conducted to determine if background SO_4^{2-} concentrations can stimulate MeHg production (see Appendix 3B-1 of this volume) (Dierberg et al., 2014). This study included dosing with both SO_4^{2-} and Hg at various concentrations.

Study results showed that relatively low SO_4^{2-} additions (0.5 to 1.0 mg/L) significantly increased water column MeHg concentrations. This indicates that non-abatable sources of SO_4^{2-} could support meaningful MeHg production, especially in the presence of bioavailable inorganic Hg. The effect of Hg dosing on MeHg production was also significant, with its production being 4.5 to 5 times higher in Hg-spiked vessels compared to those of the same SO_4^{2-} dose without Hg (**Figure 3B-28**). While these results were hypothesized based on studies reported in the peer-reviewed and technical literature, it is suggested that MeHg production is likely dependent on Hg availability, as indicated by the net Hg methylation. Consequently, study findings indicate that controlling the bioavailability of inorganic Hg in the Everglades through source reduction could play a crucial role in mitigating MeHg production and accumulation, which is the premise of Florida's Total Maximum Daily Load for mercury that was implemented in WY2013.

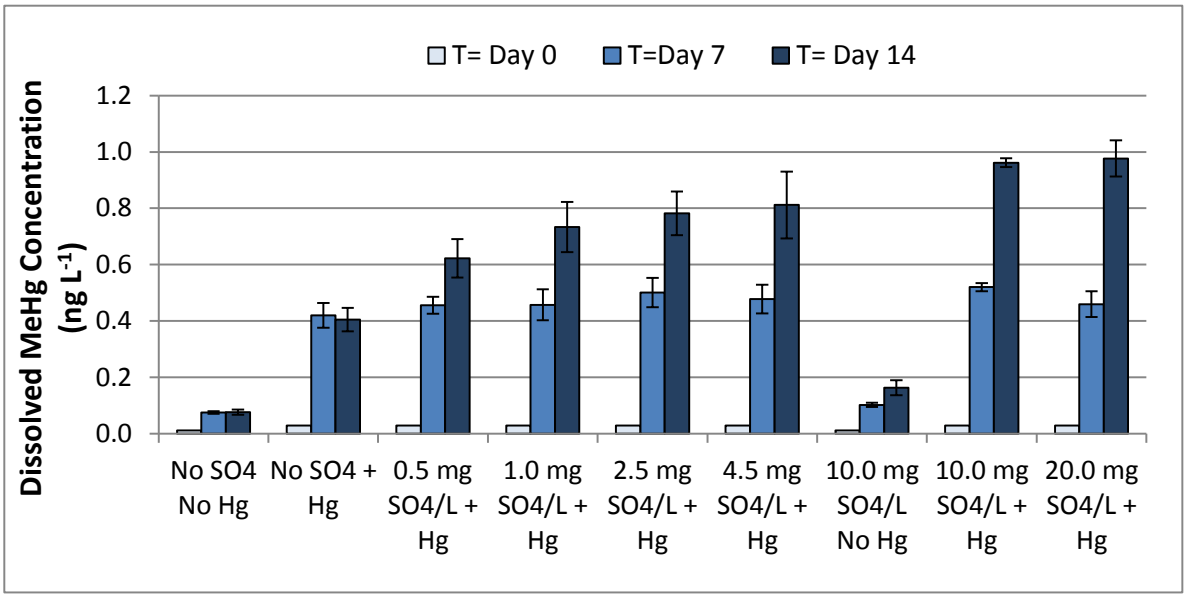

Figure 3B-28. Mean (± SE; n=3) for days 7 and 14 and initial (n=1). Dissolved MeHg concentrations at T-0 and days 7 and 14 in SO_4^{2-} and Hg amended and unamended WCA-3A surface water and soil.

CURRENT AND FUTURE RESEARCH

To address the above questions on sulfur and Hg relationship, predictability, Hg trophic dynamics, and microbial ecology, the FDEP and the District have taken several steps to provide more insights of mercury contamination in the Everglades surface water and wildlife as follows:

- A commentary on a paper published by Gabriel et al. (2014) was developed to provide alternative interpretations on monitoring data between fish mercury levels and sulfate concentrations, and mercury management in the Everglades.
- A mercury and environmental data analysis was conducted to assess biotic and abiotic factors affecting THg accumulation in largemouth bass from the Everglades marshes.
- The agencies are supporting a multiyear research project, which is focusing on factors affecting mercury methylation and accumulation in the Everglades marshes. Key topics include minimal levels of sulfate supporting elevated MeHg production, microbial community capable of Hg methylation, mercury transfers in flora and biomagnification in fauna, impacts of sediment dryout-reflooding on MeHg rate, and roles of dissolved organic matter in mercury methylation.
- A webinar was held in 2013 to present and discuss research results on sulfur sources (EAA discharge, connate seawater, atmospheric deposition, and ground water contribution, as well as soil oxidation), storage, loads and mass balance in the EAA, with various representatives from the FDEP, District and federal agencies, as well as consultants and academic institutions. Future work is needed to gain more understanding of sulfur storage, oxidation rates, and transports within the EAA, and the role of connate seawater and soil oxidation on supporting mercury methylation in South Florida.
- Future research projects are being developed to continue to address Hg within the Everglades ecosystem.

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