Mercury Trends in Multiple Fish Species in the Everglades Protection Area

Major Paper
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Spring 2011
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Introduction

Mercury in the South Florida aquatic ecosystem originates from local, regional and global sources (Axelrad et al., 2011). Many anthropogenic-based sources include emissions from medical waste incinerators and coal-fired and chlor-alkali facilities. When mercury-containing materials are burned or incinerated, mercury is released in gaseous and particulate forms that eventually deposit onto water bodies and the terrestrial surface. In wetlands, sulfate-reducing bacteria (SRB) are the primary organisms that convert inorganic mercury to methyl-mercury (MeHg). MeHg, the most toxic form of the element, bioaccumulates in the food chain and threatens the health of wildlife and humans (Figure 1).

In the late 1980s and early 1990s, various states and the federal government imposed limits on mercury emissions from medical and municipal waste incinerators in an effort to reduce mercury deposition to water bodies within the continental United States, including the Everglades. Studies have produced convincing evidence that controls on emissions from waste incinerators, combined with a reduction in the use of mercury in household items, have resulted in a sharp decline of mercury levels in the Everglades (Atkeson et al., 2005). Research indicates that mercury dropped sharply after 1994, reflecting the delayed effect of emissions regulations (Atkeson et al., 2005; Gabriel et al., 2009). However, it is not clear whether the drop of mercury in fish is due directly to reductions in atmospheric mercury deposition and/or limiting biogeochemical processes in wetlands.

Objective

The objective of this project is to evaluate temporal trends of mercury in multiple fish species representing three distinct trophic levels within the Everglades Protection Area (EPA) (Figure 2). This information will be used to validate the downward trend in mercury (as reported in Axelrad et al. 2011) that has been observed since limits on incineration emissions were imposed. To support these analyses, an additional investigation will be performed to assess the cause(s) for the observed trends in fish mercury concentrations. This analysis includes evaluating the relationship between mercury in fish and surface water sulfate concentrations over spatial and temporal scales.

Hypothesis

Mercury concentrations have decreased in all fish species since the beginning of the period of record (POR). Sulfate concentrations in surface water are spatially correlated with mercury in fish.

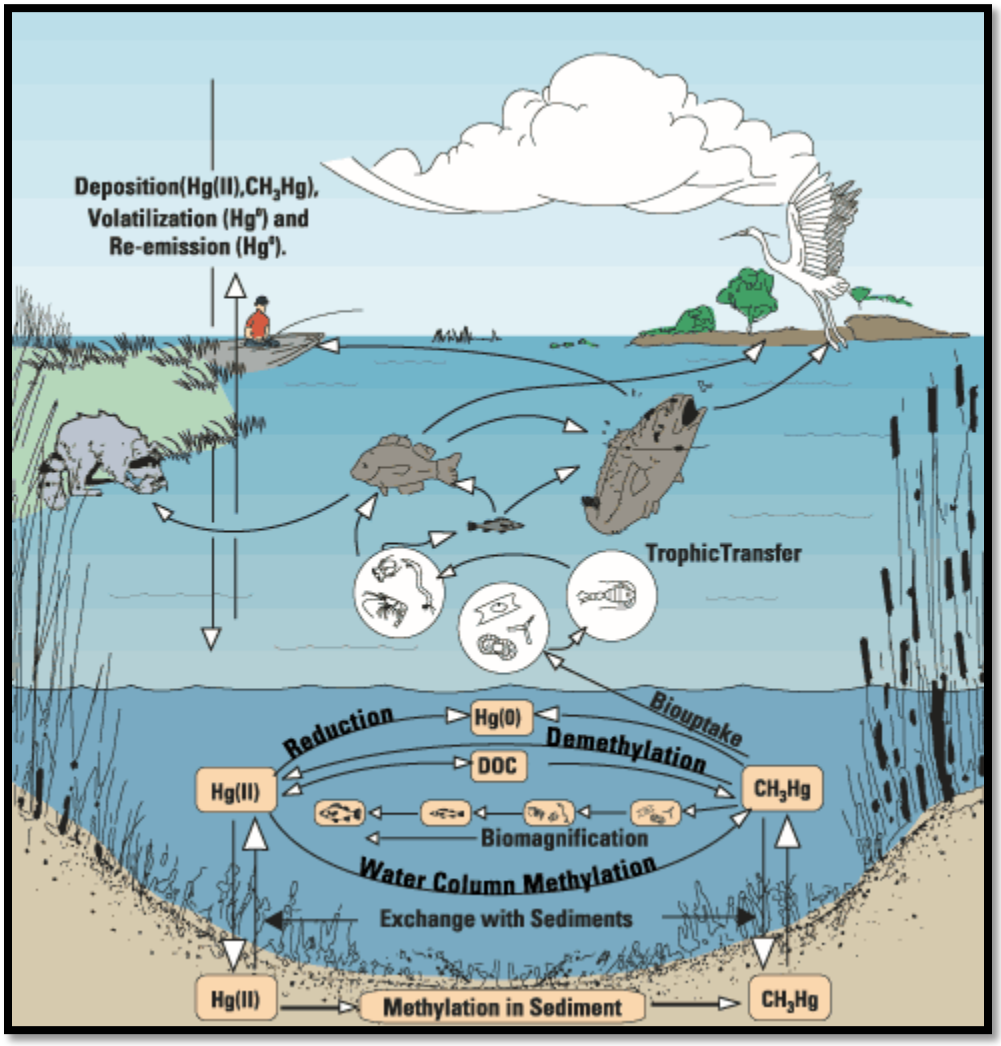


Figure 1. Mercury Cycling in an Aquatic Ecosystem (figure from USGS, 2011)

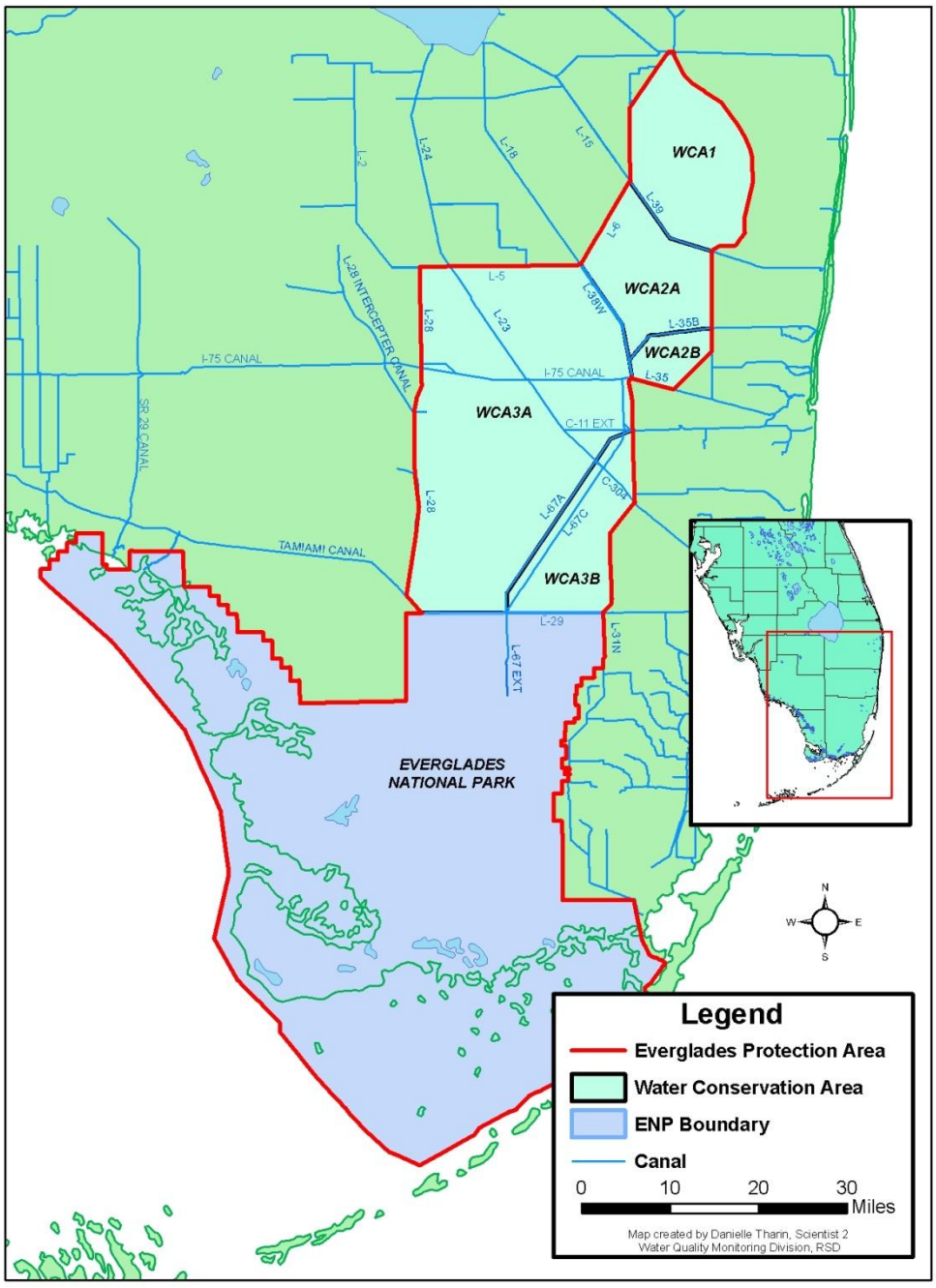


Figure 2. The Everglades Protection Area consists of Water Conservation Areas 1, 2A, 2B, 3A and 3B, and Everglades National Park

Data Description

This project focuses on data collected from 1998 to 2010 by the South Florida Water Management District (District). As a condition of its operating permits, the District is required to monitor mercury in fish tissue and sulfate in surface water at various locations throughout the EPA. All data used for this project was obtained from the District's DBHydro database. Surface water sulfate and mercury in fish monitoring locations and quantities have varied considerably over time. In addition, mercury and sulfate monitoring has not always occurred concurrently at common stations. Mercury and sulfate monitoring data were paired for correlative analyses based on spatial and temporal proximity (Table 1). Hydrologic relevance was also a consideration for pairing mercury and sulfate monitoring locations.

THg in Fish Monitoring Stations	Surface Water Sulfate Monitoring Stations
LOXF4	LOX11
CA2NF	2AN1
WCA2F1	WCA2F1
WCA2U3	CA215/U3
HOLYBC	G372
ROTENC	ROTA4
CA33ALT	CA33
CA35ALT	CA38
CA3F1	S140
CA315	CA311
CA3F2	S12A
L67F1	S12D

Table 1. Table showing how THg in fish and surface water sulfate monitoring locations were paired for correlative analyses.

Summary of the Monitoring Program

Preyfish

Using a dip net, a grab sample of 100 - 250 mosquitofish (*Gambusia* spp.) are collected at each monitoring station (Figure 3) on an annual frequency. Mosquitofish are selected as a representative indicator of short-term, localized changes in water quality because of their small range, short life span, and widespread occurrence in 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 may live up to 1.5 years); the life span of males is shorter than females (Haake and Dean, 1983; Haynes and Cashner, 1995; Cabral and Marques, 1999). After collection, the mosquitofish are homogenized and each sample is analyzed for total mercury (THg).

More than 85 percent of the mercury found in the muscle tissue of fish is in the methylated form (Grieb et al., 1990; Bloom, 1992). Therefore, the analysis of fish tissue for THg, which is a more straightforward and less costly procedure than the analysis for MeHg, can be interpreted as being equivalent to the analysis of MeHg.

Secondary Predator Fish

Up to 20 sunfish (*Lepomis* spp.) are also collected annually at the same interior marsh sites using electroshocking techniques. Sunfish are thought to have an average life span of four to seven years in the wild. Each whole fish is analyzed for THg. Sunfish are prevalent in the Everglades and are the preferred prey for a number of fish-eating species; therefore, this species was selected as an indicator of mercury exposure for wading birds and other fish-eating wildlife.

Top-Predator Fish

Using electroshocking techniques, up to 20 largemouth bass (*Micropterus salmoides*) (LMB) are also collected annually at the same interior marsh sites; the fillets are analyzed for THg.

Largemouth bass are long-lived; therefore, LMB were selected as an indicator of potential human exposure to mercury.

Tissue concentrations in each of these three monitored fish species reflect ambient MeHg levels; i.e., their exposure is a function of a combination of factors, including body size, age, rate of population turnover, and trophic position.

Mosquitofish should respond rapidly to changing ambient MeHg concentrations due to their small size, lower trophic status, short life span, and rapid population turnover. Conversely, sunfish and LMB should take a greater amount of time to respond, in terms of tissue concentrations, to changes in ambient MeHg availability. Most importantly, sunfish and LMB represent exposure at higher trophic levels with a requisite time lag for trophic exchange. The key is to use these species-related differences to better assess MeHg availability within the system.

Surface Water

On a quarterly frequency, 125-milliliter filtered (0.45 μm) grab samples of water are collected at water management structures and interior marsh stations and analyzed for sulfate (Figure 4).

MERCURY IN FISH MONITORING LOCATIONS

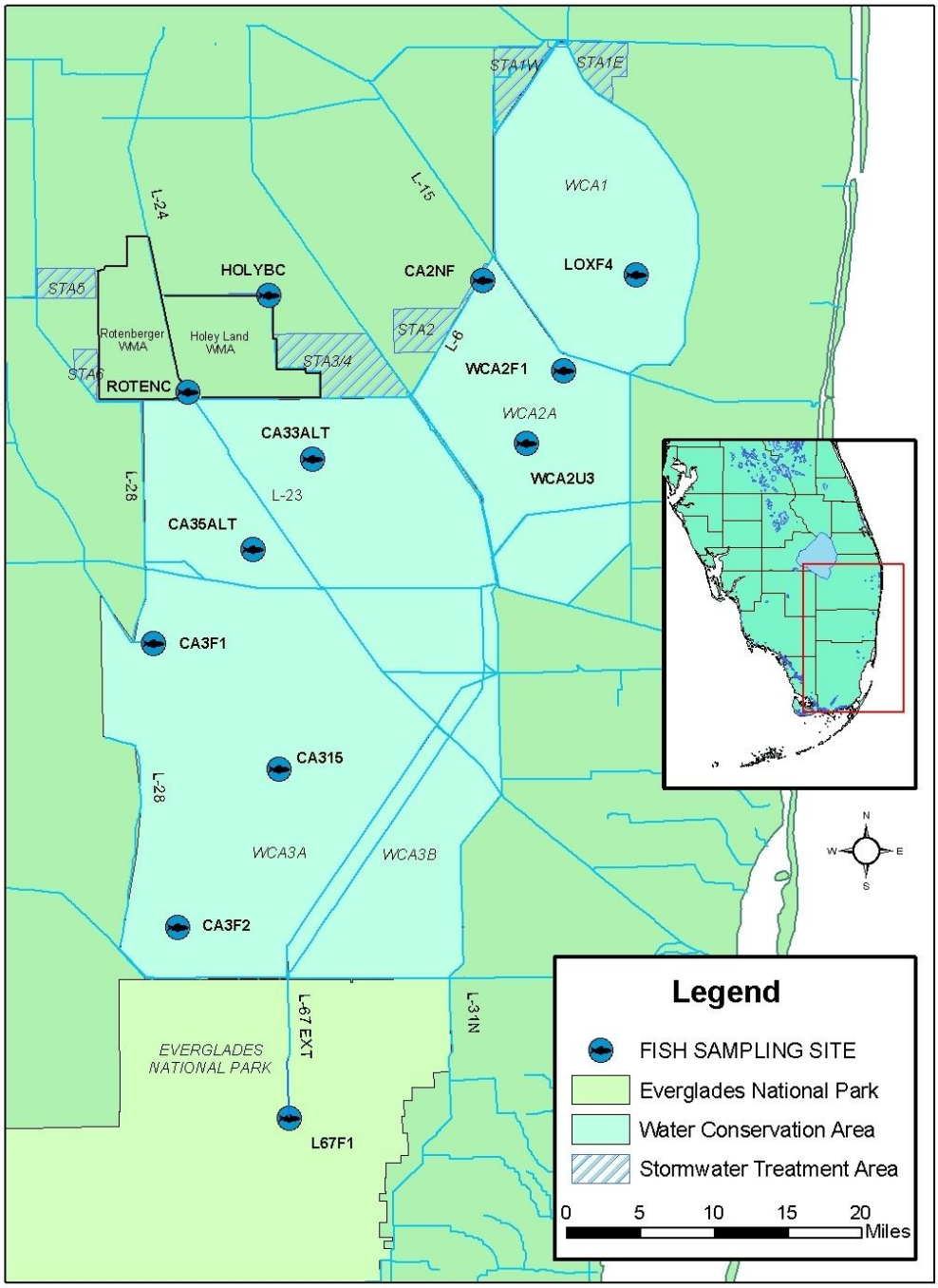


Figure 3. Collection sites for monitoring THg levels in mosquitofish (*Gambusia* spp.), sunfish (*Lepomis* spp.), and largemouth bass (*Micropterus salmoides*).

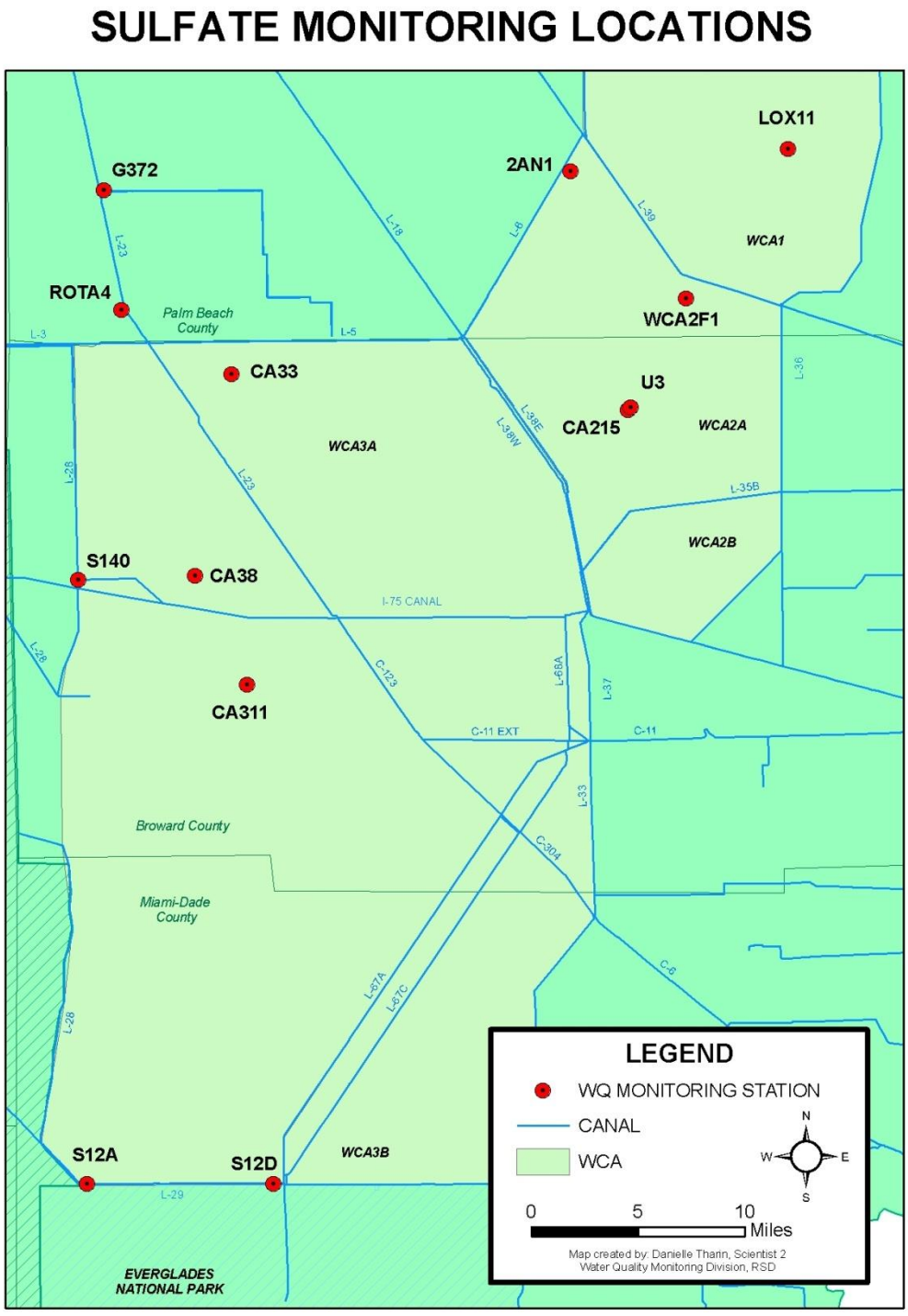


Figure 4. Surface water sulfate monitoring locations.

Statistical Analysis

Data heteroscedasticity was evaluated using the Shapiro-Wilkinson normality test. Pearson correlation was used for parametric data and Spearman correlation for non-parametric data. Statistical significance was considered ≥95% confidence. Interpretability of residue mercury levels in animals can be problematic due to the confounding influences of age or species. Therefore, for comparative purposes, fish data were standardized by age and length where appropriate.

Results and Discussion

Temporal Trends of Mercury in Fish in the EPA

Axelrad et al. (2011) report a 62 percent decline in mercury levels in largemouth bass across the EPA following government imposed limits on mercury emissions from medical and municipal waste incinerators, with annual median THg concentrations frequently exceeding 1 mg/kg prior to 1995 (Figure 5). Results from the present study validate this reported decline of mercury in largemouth bass with a median annual concentration exceeding 1 mg/kg only two times since 1998. These exceedances were observed at station L67F1 in 1999 (1.1 mg/kg) and 2003 (1.2 mg/kg).

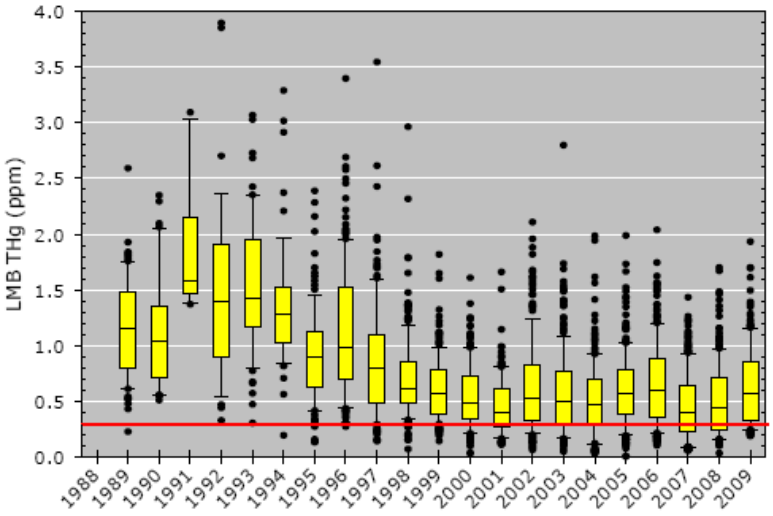


Figure 5. Annual pooled summaries of mercury concentrations in largemouth bass in the EPA 1989–2009 (Axelrad et al., 2011)

Axelrad et al. (2011) report median concentrations in largemouth bass stabilized by 1998 with little variation since that year. Results from this study reveal a continued decline in mercury levels since 1998, however, few of those declines are statistically significant. Over the entire POR, Spearman correlation analyses performed using annual median THg concentrations indicate a decline in mosquitofish THg levels at eleven stations, with four of those stations showing statistically significant decreases: LOXF4 (ρ=-0.847, p=<0.001, n=11 years), CA2NF (ρ=-0.758, p= 0.001, n=10 years), CA3F2 (ρ=-0.866, p=<0.001, n=12 years) (Figure 6), and L67F1 (ρ=-0.606, p=0.033, n=12 years). THg declines were observed in sunfish at eight stations. Only one of those declines, station CA3F2, was statistically significant (ρ=-0.636, p=0.024, n=12 years) (Figure 7). THg declines in largemouth bass were observed at seven stations, but none were statistically significant. A few stations reveal consistently low (e.g., WCA2F1 and LOXF4) or high (L67F1) mercury levels; however, there does not appear to be any definitive spatial trend or concentration gradient.

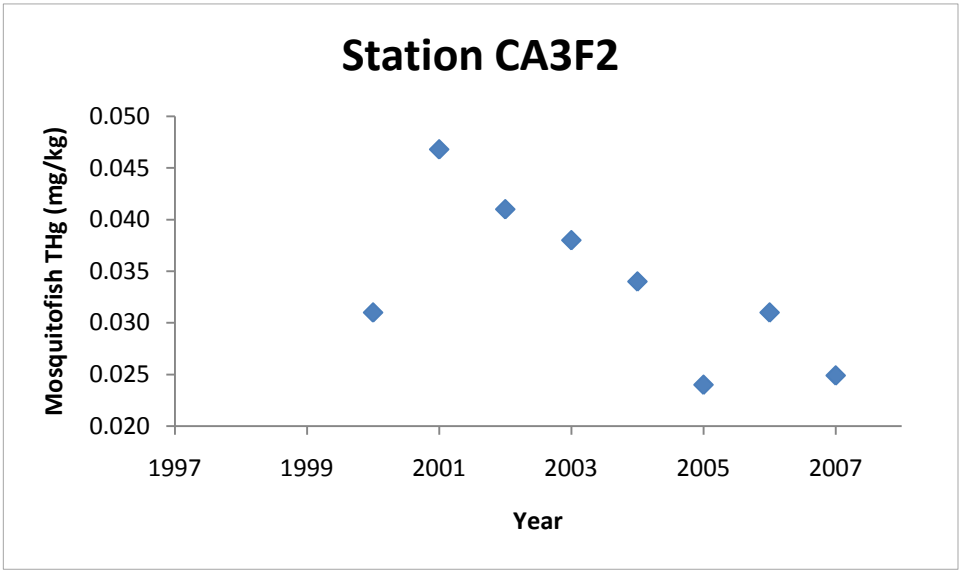


Figure 6. Annual median THg concentrations in mosquitofish at station CA3F2

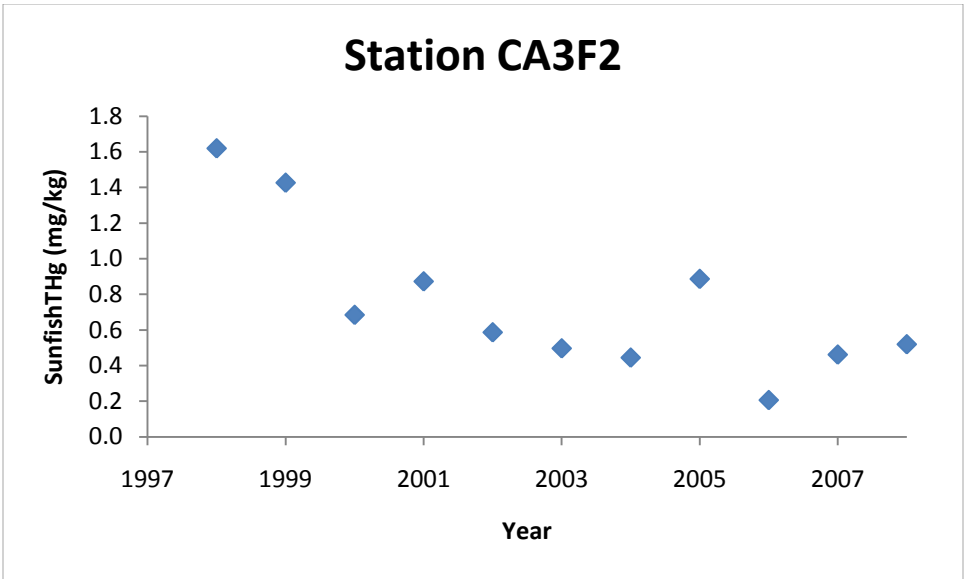
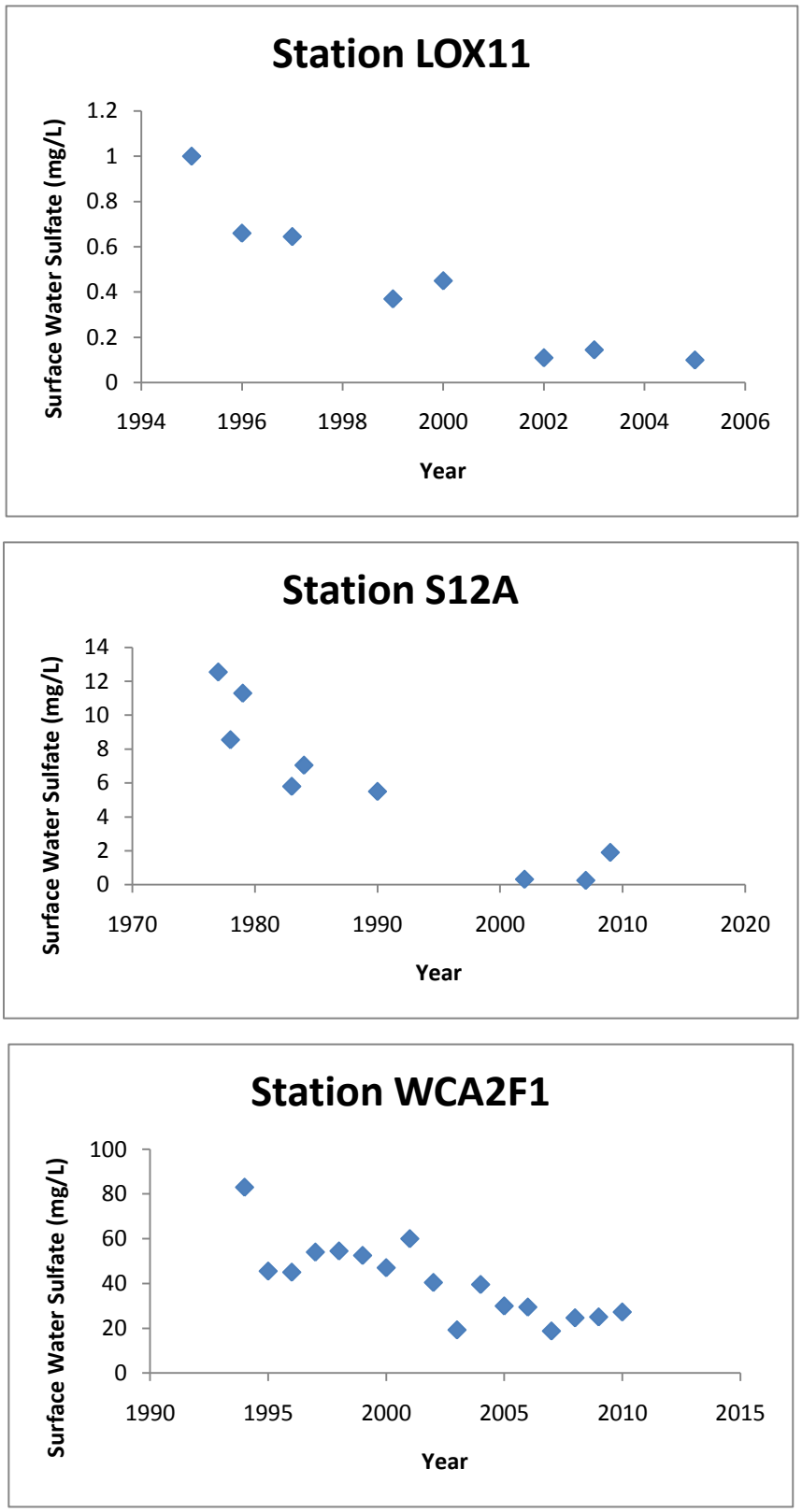


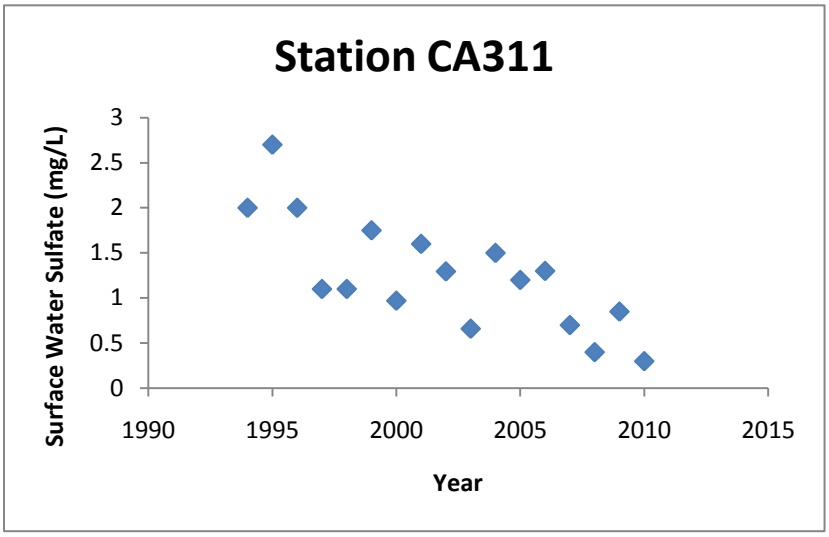
Figure 7. Annual median THg concentrations in sunfish at station CA3F2

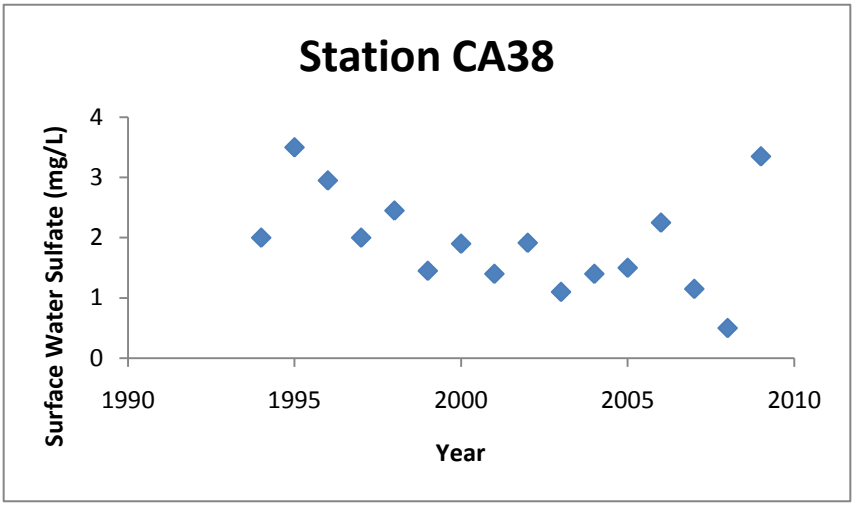
USEPA (2001 and 2007), Kalla et al. (2010), and Krabbenhoft et al. (2010) attribute the decline in mercury bioaccumulation not only to reduced atmospheric inputs of mercury, but also to declines in sulfate concentrations in the EPA during the late 1990s. Results from the current study show a continued decline in sulfate concentrations at seven of twelve stations examined: LOX11 (ρ=-0.889, p=<0.001, n=13) (Figure 8), WCA2F1 (ρ=-0.804, p=<0.001, n=17) (Figure 9), CA215 (ρ=-0.611, p=0.015, n=15), CA33 (ρ=-0.796, p=<0.001, n=22), CA38 (ρ=-0.535, p=0.026, n=17) (Figure 10), CA311 (ρ=-0.727, p=<0.001, n=17) (Figure 11), and S12A (ρ=-0.558, p=0.006, n=23) (Figure 12).

Only two of the twelve paired THg in fish and surface water sulfate monitoring locations showed a statistically significant decline in both THg and sulfate: LOXF4/LOX11 and CA3F2/S12A.



Figures 8 - 10. Annual median sulfate concentrations at various locations within the EPA.





Figures 11 and 12. Annual median sulfate concentrations at various locations within the EPA.

In aquatic systems, methylation of inorganic mercury is mediated largely by SRB (Gilmour et al., 1992; Gilmour et al., 1998; Jeremiason et al., 2006). Laboratory and field experiments show that sulfate stimulates SRB activity and methylmercury production. Declining sulfate concentrations have likely contributed to the rapid declines in MeHg production and concomitant declines in fish THg concentrations. However, other factors affecting temporal and spatial patterns of MeHg production and bioaccumulation in fish are also likely important in explaining the variations in mercury bioaccumulation observed since 1998.

Relationship Between Mercury in Fish and Surface Water Sulfate Concentrations

Spearman correlation analyses indicate that for the POR, there is no significant linear relationship between THg in fish and surface water sulfate concentrations. This is true for each of the three trophic levels of fish examined: mosquitofish (ρ= -0.0882, p= 0.764, n=13), sunfish (ρ= -0.14, p= 0.629, n=13), and largemouth bass (ρ= -0.118, p= 0.682, n=13). However, plots developed with annual median concentrations of THg and sulfate for each monitoring station suggest a nonlinear relationship exists (Figure 13).

Results of this study indicate that mercury bioaccumulation increases with sulfate concentrations up to 10 mg/L (Figures 13 and 14). This supports laboratory experiments that show methylmercury production increases with sulfate concentrations up to 10 mg/L and declines when porewater sulfide exceeds 0.6 mg/L (Gilmour et al., 1992). Sulfate stimulates SRB activity and methylmercury production, but as it is reduced to sulfide, the sulfide accumulates in porewater and binds with inorganic mercury limiting what fractions are available for methylation (Cleckner et al. 1998). This may explain the observed decrease in mercury bioaccumulation when surface water sulfate exceeds 10 mg/L. The dual effect of sulfur on methylation results in maximum MeHg production, and ultimately bioaccumulation, in so-called "Goldilocks" zones where sulfate and sulfide levels are just right for mercury methylation.

Mercury concentrations in each of the three trophic levels of fish examined show a subsequent upward trend when sulfate concentrations exceed 20 mg/L. This subsequent increase was not reported by Gilmour et al. (1992) or Pollman (2008) and may hint to a complex relationship where the magnitude of methylmercury accumulation within the Everglades varies as consequence of biogeochemical factors other than sulfur. Alternatively, it may simply be an artifact of the data; this subsequent increase disappears in plots showing the 25^{th} and 75^{th} percentiles (Figure 14). Polynomial regression equations developed for each of the three trophic levels of fish examined suggest that only 4-8 percent of the variation of THg in fish is due to surface water sulfate concentration. The low R^2 values indicate that these regression equations would not serve as strong tools for predicting THg bioaccumulation as a function of surface water sulfate concentrations.

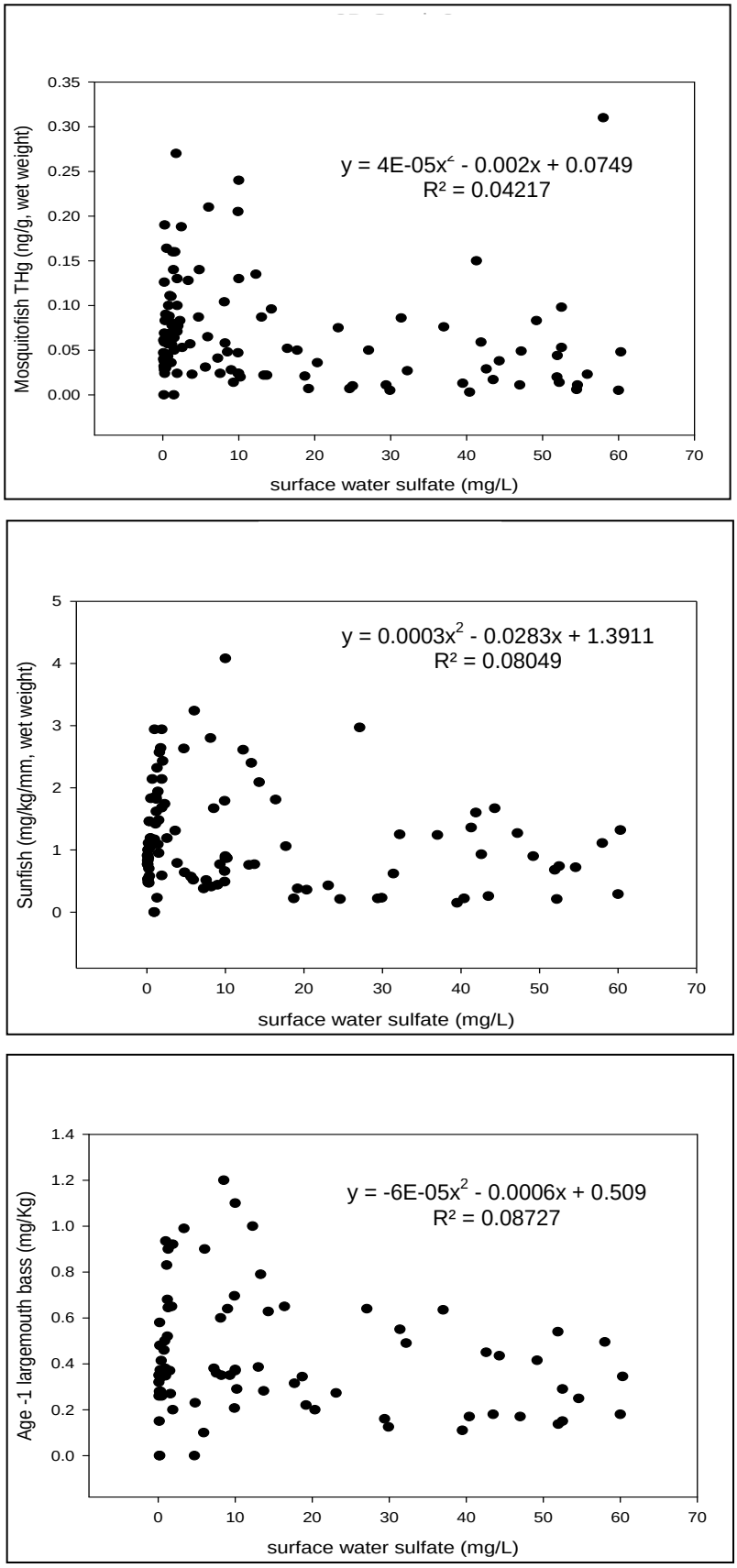
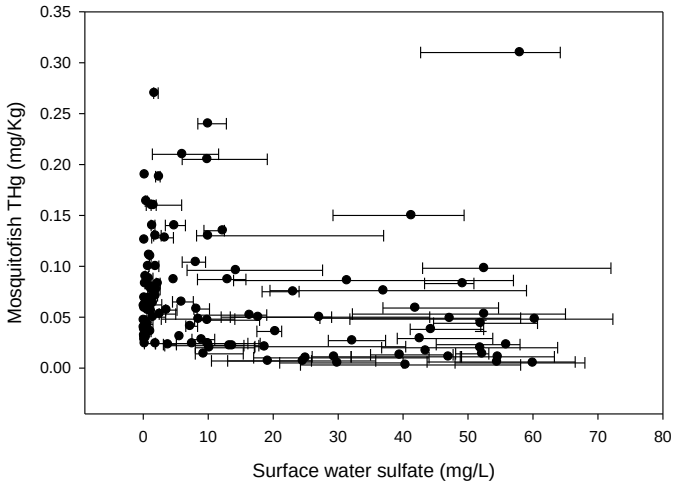
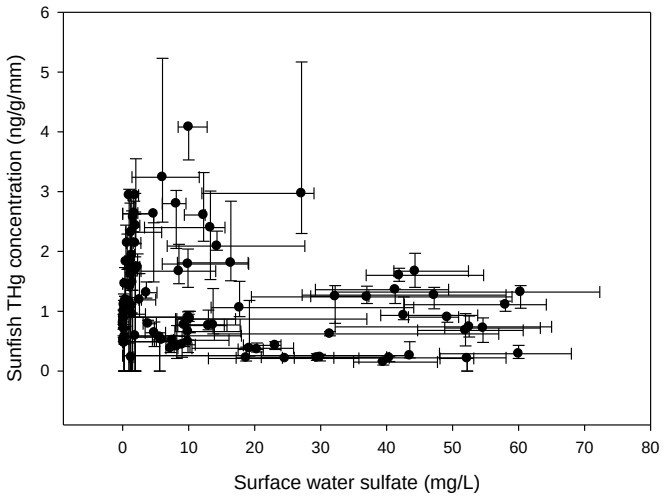


Figure 13. Mercury concentrations in mosquitofish, sunfish, and largemouth bass in the EPA as a function of surface water sulfate cc





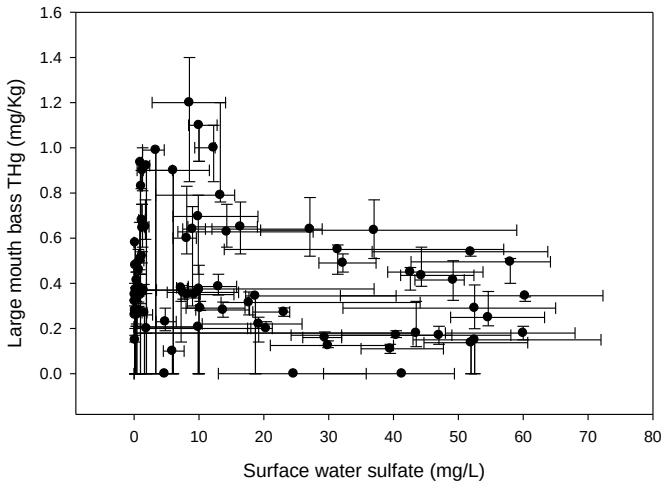


Figure 14. Plots showing 25th and 75th percentile (*whiskers*) concentrations of mercury in fish as a function of surface water sulfate concentrations.

While freshwater wetlands typically have low sulfate concentrations (Wetzel, 2001), surface water sulfate concentrations in the Everglades are high due to major inputs from the Everglades Agricultural Area (EAA) (Orem, 2004). Sulfate levels in marshes closest to the EAA often exceed 100 times historical levels (Bates et al., 2002; Gilmour et al., 2007a; Weaver et al., 2007). Sources of sulfur to EAA canals include wet and dry atmospheric sulfur deposition (though atmospheric deposition is a minor input), agricultural application, and soil oxidation in the EAA (Gabriel et al., 2010a). Soil oxidation occurs when soils are drained (e.g., drainage that is anthropogenically induced for agricultural purposes or occurs naturally during drought) or as a result of fire. The USGS and the Smithsonian Institution have jointly examined the impacts of dry/rewet cycles on the biogeochemistry of the Everglades, in both field and laboratory studies (Krabbenhoft and Fink, 2001; Gilmour et al., 2004). Results of these studies show that drought or fire followed by rewet causes: (1) oxidation of organic soils, converting reduced sulfur in sediments (organic sulfur and metal sulfides) to sulfate, (2) remobilization of this sulfate into the water column following rewetting, and (3) stimulation of microbial sulfate reduction and MeHg production from the remobilized sulfate.

Field surveys have shown that sulfate stimulation of MeHg production and sulfide inhibition of MeHg production explain the variations of MeHg observed in soils and fish across the Everglades ecosystem (Gilmour et al., 1998; Benoit et al., 2003; Gilmour et al., 2007b). These variations may also result from other biogeochemical factors that influence mercury availability, methylation, and bioaccumulation.

Other Biogeochemical Factors Affecting Mercury Methylation

Mercury methylation is extraordinarily effective in the Everglades not only due to the availability of sulfate, but also the large pool of readily available dissolved organic matter (DOC), and significant mercury source input from atmospheric deposition (Gilmour and Krabbenhoft, 2001; Renner, 2001; Bates et al., 2002). These constituents, as well as pH, have a strong influence on mercury availability and methylation in aquatic systems. Increasing the acidity of the water and/or the DOC content enhances the mobility and availability of mercury in the environment, making it more likely to enter the food chain. Hydrophobic acids contained in

DOC significantly increase the overall solubility and thus mobility of mercury (Ravichandran et al., 1998). Another factor contributing to the high levels of MeHg in the Everglades is the large expanse of wetland area with anoxic soils that support microbial methylation of mercury.

Conclusion

Results from this study 1) validate the reported decline of mercury in the Everglades following government imposed limits on mercury emissions from medical and municipal waste incinerators and 2) suggest mercury concentrations in Everglades fish has a nonlinear relationship with surface water sulfate concentrations.

Mercury concentrations in fish declined at only a few of the twelve stations examined for the POR (i.e., four stations for mosquitofish, one station for sunfish, and zero stations for largemouth bass). The POR for this study begins in 1998, precisely when mercury concentrations in fish stabilized within the EPA. Nonetheless, this study does confirm that mercury concentrations in largemouth bass are significantly less now than they were prior to and shortly after the implementation (late 1980s – early 1990s) of government imposed limits on mercury emissions.

Sulfate concentrations in surface water have a nonlinear spatial correlation with mercury in fish. This is true for each of three trophic levels of fish examined. Plots revealed characteristic increases in mercury bioaccumulation as surface water sulfate concentrations approached 10 mg/L. This increase was followed by a steady decline in mercury bioaccumulation, reflecting the dual effect of sulfur on MeHg production in "Goldilocks" zones where sulfate and sulfide levels are just right for mercury methylation and, ultimately, bioaccumulation. Mercury concentrations in each of the three trophic levels of fish examined showed a subsequent upward trend when sulfate concentrations exceeded 20 mg/L. This subsequent increase may be a reflection of a complex relationship where the magnitude of methylmercury accumulation within the Everglades varies as consequence of biogeochemical factors other than sulfur. Plots also revealed that sulfate accounts for 4-8% of the variability in THg concentrations in fish.

Although long-term monitoring of mercury concentrations in fish conducted in the EPA has shown significant declines in mercury bioaccumulation, hot spots exist and humans and fish-eating wildlife continue to be at risk due to mercury exposure (Gabriel et al., 2010a and Rumbold et al., 2008). In order to decrease the risk of exposure to toxic MeHg, factors promoting methylation need to be addressed. Reductions in the amount of wetland area are obviously not consistent with restoration goals, although minimizing the occurrence of dry/rewet cycles could reduce spikes in MeHg production. Reducing DOC could reduce the bioavailability of mercury for methylation, but reducing DOC is not realistic in a peat-forming environment like the Everglades. Since controls on emissions from waste incinerators were imposed, local sources of mercury emissions have declined by approximately 90% (Atkeson, 2005). Because most of the remaining atmospheric mercury deposition on the Everglades is from long-range atmospheric transport originating outside the United States, further reductions in atmospheric input of mercury to the Everglades would require international cooperation. This leaves the control of sulfate inputs as the most feasible option for reducing MeHg production and bioaccumulation in the Everglades.

To effectively manage issues linked to elevated mercury and sulfur concentrations, dedicated research is needed to determine the causes of mercury hot spots and the sources, fate, and transport of sulfur in the Everglades (Gabriel et al., 2010b). Results of future research may warrant changes to water management operations and expand the current scope of water quality improvement efforts.

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