Appendix 3B-1: Everglades Mercury Hotspot Study: Preliminary Analysis

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Mercury (Hg) contamination of freshwater fisheries has been recognized as a significant ecological problem (Lange et al. 1993). Within the Everglades ecosystem, elevated Hg concentrations were first observed within biota, including fish species collected within Everglades National Park (ENP) during the early 1970s (Ogden et al. 1973). Further evidence of widespread elevated Hg concentrations within fish species was obtained during the late 1980s, which promoted a state fish consumption advisory for selected species and locations within the Greater Everglades (Rumbold et al. 2001). Elevated Hg concentrations in Everglades biota remains a concern at present (Julian et al. 2015). Elevated Hg concentrations in fish also are of concern because of their potential threat to the health of humans and other piscivorous mammals and birds that consume Hg-laden fish (Wiener et al. 2003). However, the bioaccumulation of Hg in aquatic biota is controlled by a suite of interconnected species-specific and environmental variables, including site conditions (i.e., water and soil quality), trophic position, and habitat (Julian and Gu 2015, Lange et al. 1993, Spry and Wiener 1991).

This appendix provides an overview of the Everglades Mercury Hotspot Study, in which study data were collected during Calendar Years 2010–2013 by Tetra Tech Inc., under contract (Contract Number 4600002003) to the South Florida Water Management District (SFWMD or District). The primary objective of this study was to evaluate biogeochemical and Hg parameters in surface water, porewater, sediment, periphyton, and mosquitofish to potentially identify mechanisms or processes influencing differences in total mercury (THg) concentrations in fish between those areas identified as hotspots and non-hotspots.[3]

METHODS

STUDY AREA

Monitoring locations were located within Water Conservation Area (WCA-) 3A, WCA-2A, and Stormwater Treatment Area (STA-) 1 West (STA-1W; **Figure 1**). WCA-2 and WCA-3 are located within the Everglades Protection Area (EPA), a complex system of marsh areas, canals, and levees with inflow and outflow water control structures that covers approximately 2.5 million acres of former contiguous Everglades' marsh (Light and Dineen 1994). STA-1W, located just

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[2] The authors acknowledge Julianne LaRock and Garth Redfield (SFWMD) for reviewing drafts of this report.

[3] Hotspots are defined as those areas with persistently high THg concentrations [i.e., THg levels exceeding the U.S. Environmental Protection Agency (USEPA) criterion for the protection of trophic level (TL) 3 or 4 fish], and non-hotspots represent those areas with relatively low THg concentrations (i.e., THg levels in the range of one-half of the USEPA criterion for the protection of TL3 or TL4 fish); TL3 fish include mosquitofish (*Gambusia holbrooki*) and sunfish (*Lepomis* spp.) and TL fish include largemouth bass (*Micropterus salmoides*).

northwest of the EPA, is a constructed wetland covering approximately 6,544 acres of effective treatment area in former agricultural land constructed for the primary purpose of removing nutrients from the water column before entering the EPA. STA-1W is currently arranged into three flow-ways with eight treatment cells, which consist of mixed emergent, submergent, and floating aquatic vegetation (see Chapter 5B of this volume).

Sampling locations were parsed into hotspot and non-hotspot locations. Hotspots are defined as those areas with persistently high THg concentrations [i.e., THg concentrations exceeding the United States Environmental Protection Agency (USEPA) criterion for the protection of trophic level 3 (TL3) or trophic level 4 (TL4) fish], and non-hotspots represent those areas with relatively low THg concentrations (i.e., THg concentrations in the range of one-half of the USEPA criterion for the protection of TL3 or TL4 fish). TL3 fish include mosquitofish (*Gambusia holbrooki*) and sunfish *(Lepomis* spp.). TL4 fish include largemouth bass (*Micropterus salmoides*), as reported in Chapter 3B of this volume.

SOURCE OF DATA AND DATA HANDLING

All data for this study were collected by Tetra Tech, Inc. at four sites, one located within STA-1W (ENR302) and three located within the EPA (**Figure 1**) between late 2010 and early 2013. These sites were separated into suspected hotspot (i.e., WCA2U3 and WCA315) and non-hotspot stations (i.e., ENR302 and WCA2F1). A total of seven sampling trips were conducted during this sampling period. A very large suite of parameters was collected and analyzed as a part of this effort. All parameters and matrices collected as part of this study are presented in **Table 1**. All samples were collected in accordance with the District's sampling protocols (SFWMD 2009, 2010, 2011), and chemical analyses were performed consistent with USEPA- and Florida Department of Environmental Protection (FDEP)-approved methods.

Surface water samples were collected via grab sample, either by direct collection into sample bottles or by portable peristaltic pumps where site disturbance was problematic. Porewater samples were collected via sipper probe at a depth of 6 to 10 centimeters (cm) below the sediment surface consistent with the District's sampling protocol. Soil samples were collected at a depth of 0 to 10 cm using a cellulose acetate butyrate core tube. Duplicate soil samples were collected and composited.

All data were screened based on laboratory qualifier codes consistent with the FDEP's Quality Assurance Rule [Chapter 62-160, Florida Administrative Code (F.A.C.)]. Any datum associated with a fatal qualifier indicating a potential data quality problem was removed from the analysis. For purposes of data analysis and summary statistics, data reported as less than the method detection limit (MDL) were assigned a value equal to the MDL, unless otherwise noted. Percent methyl mercury (MeHg) values were calculated as the percentage of MeHg to THg concentrations within each compartment. Due to very few surface water MeHg samples, the mosquitofish bioconcentration factor was calculated as the ratio between mosquitofish THg and surface water THg and denoted as BCF_{THg}.

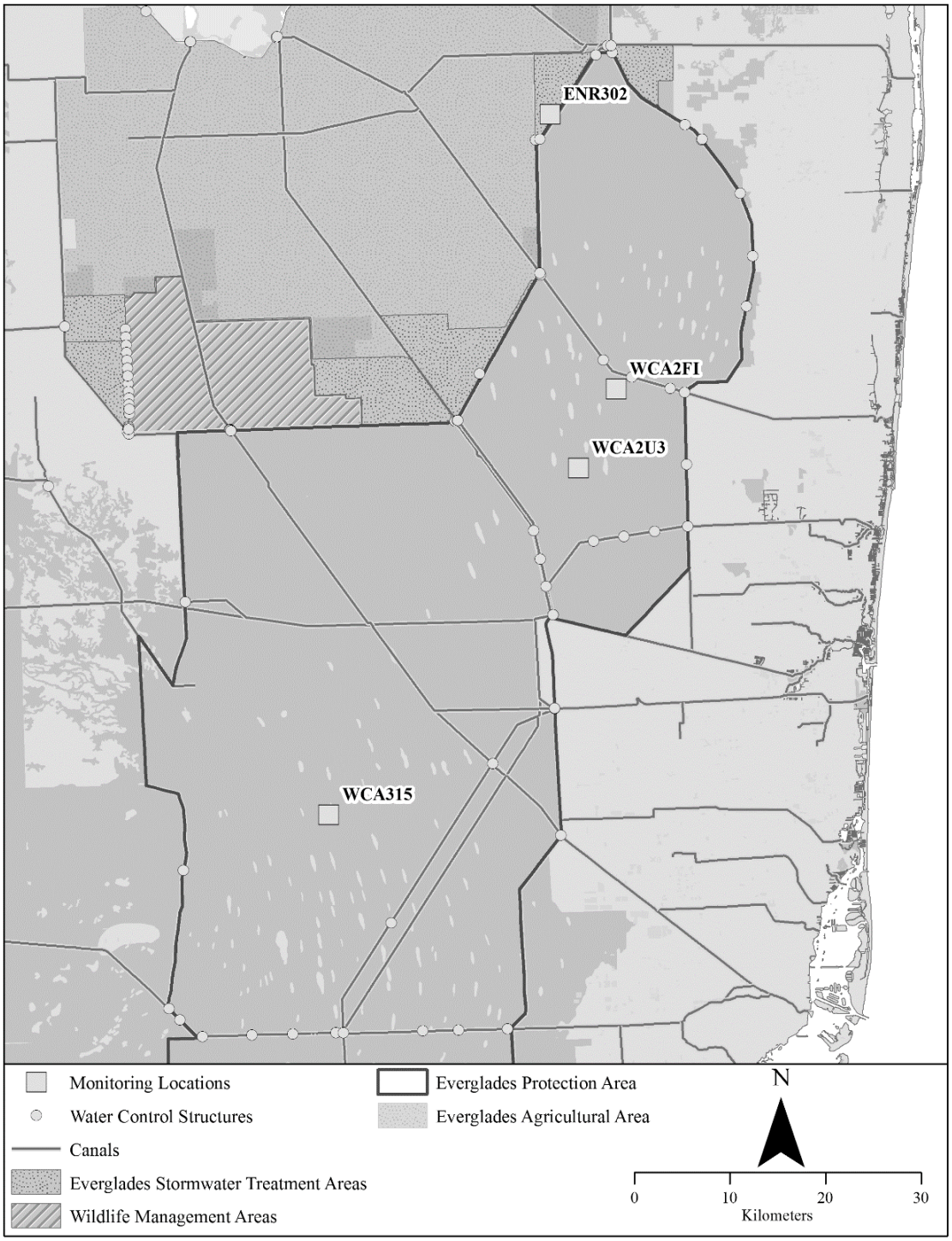

Figure 1. Monitoring locations relative to the EPA and surrounding features including the Everglades STAs and Wildlife Management Areas.

Table 1. Summary of parameters, matrices, and analytical methods used for this study. Additional parameters were collected but not used in this analysis. [ASTM = American Society for Testing and Materials; EPA – Environmental Protection Agency; SM – Standard Methods]

Matrix	Parameter	Analytical Method
Surface Water	Dissolved Iron	EPA 1638 Modified
	Dissolved Sulfate	SM 4110 B
	Dissolved Organic Carbon	SM 5310B
	Total Organic Carbon	SM 5310B
	Total Mercury	EPA 1631E
	Methylmercury	EPA 1630
	Total Phosphorus	SM 4500-P-F
Porewater	Dissolved Iron	EPA 1638 Modified
	Dissolved Sulfate	SM 4110 B
	Sulfide	EPA 9215
	Dissolved Organic Carbon	SM 5310B
	Total Mercury	EPA 1631E
	Methylmercury	EPA 1630
Soil	Total Sulfur	ASTM E-1915
	Total Organic Carbon	SM 5310B
	Calcium	EPA 1638 Modified
	Total Oxidizable Sulfur	SM 4110B
	Total Mercury	EPA 1631 Appendix
	Methylmercury	EPA 1630 Modified
Fish tissue	Total Mercury	EPA 1631 Appendix
	Methylmercury	EPA 1630 Modified
Periphyton	Total Mercury	EPA 1631 Appendix
	Methylmercury	EPA 1630 Modified

DATA ANALYSIS

Mosquitofish THg and MeHg were analyzed using linear regression. Linear model assumptions were tested for all regression models using the linear model global validation package in R (gvlma) (Peña and Slate 2006). All Spearman rank sign correlation analyses were conducted using a two-sided comparison (determines if r is not equal to zero) unless otherwise noted. Spearman ranked sign correlation analysis was used to compare THg and MeHg in periphyton samples. Percent MeHg in periphyton, soil, surface water, and porewater was compared among stations and station designation (i.e., hotspot versus non-hotspot) using the Kruskal-Wallis rank sum test. To determine differences between different monitoring stations, Dunn's test of multiple comparisons using rank sum was used to compare surface water percent MeHg. Spearman ranked sign correlation analysis was performed between percent surface water MeHg and surface water parameters including dissolved iron (Fe), dissolved organic carbon (DOC), sulfate (SO_4^{2-}), total organic carbon (TOC), and porewater percent MeHg. Spearman ranked sign correlation analysis was performed between percent porewater MeHg and surface water parameters including dissolved Fe, DOC, SO_4^{2-}, and sulfide (S^{2-}). Porewater S^{2-} was compared to porewater SO_4^{2-}, and DOC using Spearman ranked sign correlation. To determine differences among monitoring stations, Dunn's test of multiple comparisons using rank sum was used to compare porewater S^{2-} concentrations among individual monitoring stations. Spearman ranked sign correlation analysis was performed between percent soil MeHg and soil total sulfur (TS), total oxidizable sulfur (TOS), calcium (Ca), and TOC. Spearman ranked sign correlation analysis was used to compare soil MeHg concentrations to porewater S^{2-}, dissolved Fe, DOC, and SO_4^{2-}. BAF_{THg} was compared to surface water DOC, dissolved Fe, total phosphorus (TP), SO_4^{2-}, periphyton MeHg, soil percent MeHg, and porewater S^{2-} using Spearman ranked sign correlation analysis. All statistical analyses performed on study data are summarized in **Table 2**.

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

Table 2. Summary of statistical analyses performed on study data from 2010 to 2013. [Note: SW – surface water; PW – porewater;]

Statistical Test	Response	Predictor
Linear Regression	Tissue MeHg	Tissue THg

Statistical Test	Variable 1	Variable 2
Correlation (Spearman)	% SW MeHg	SW Dissolved Fe
		SW DOC
		SW Sulfate
		SW TOC
	% PW MeHg	PW Dissolved Fe
		PW DOC
		PW Sulfate
		PW Sulfide
	PW Sulfide	PW Sulfate
		PW DOC
	% Soil MeHg	Soil Total Sulfur
		Soil Total Oxidizable Sulfur
		Soil Calcium
		Soil TOC
	Soil MeHg	PW Sulfide
		PW Dissolved Fe
		PW DOC
		PW Sulfate
	Mosquitofish BAF_{THg}	SW DOC
		SW Dissolved Fe
		SW TP
		SW Sulfate
		Periphyton MeHg
		% Soil MeHg
		PW Sulfide

Statistical Test	Variable	Grouping
Kruskal-Wallis	% SW MeHg	Station Designation (i.e., Hot Spot/Non-Hotspot)
	% PW MeHg	
	% Soil MeHg	
	% Mosquitofish MeHg	
	% Periphyton MeHg	
Dunn's test	% SW MeHg	Individual Monitoring Stations
	% PW MeHg	
	% Soil MeHg	
	% Mosquitofish MeHg	
	% Periphyton MeHg	

RESULTS AND DISCUSSION

MERCURY DISTRIBUTION

In mosquitofish collected during this study, THg concentrations in ranged from 8.45 to 312.0 nanograms per gram (ng/g), while MeHg concentrations ranged from 9.00 to 241 ng/g (**Figure 2**). Mosquitofish THg and MeHg data did fit the assumptions of a linear model (Global Statistic: 6.9, $\rho = 0.14$) and the relationship was statistically significant ($R^2 = 0.98$, $F(1,6) = 344.6$, $\rho < 0.001$), with THg representing 99 percent (linear model: THg = 0.99(MeHg) - 3.34) of MeHg in mosquitofish samples (**Figure 3**). These results are consistent with other studies, in which greater than 85 percent of the THg found in fish is in the form of MeHg (Bloom 1992, Grieb et al. 1990). Based on these results, when comparing mosquitofish THg concentrations with the USEPA MeHg criterion of 77 ng/g for the protection of piscivorous wildlife (USEPA 1997), 47 percent of the data exceeded the criterion at study sites WCA2U3 and WCA315 (i.e., suspected hotspot areas). This exceedance rate is higher than historical annual exceedance rate of 29 percent from 14 stations within the EPA (see Chapter 3B of this volume).

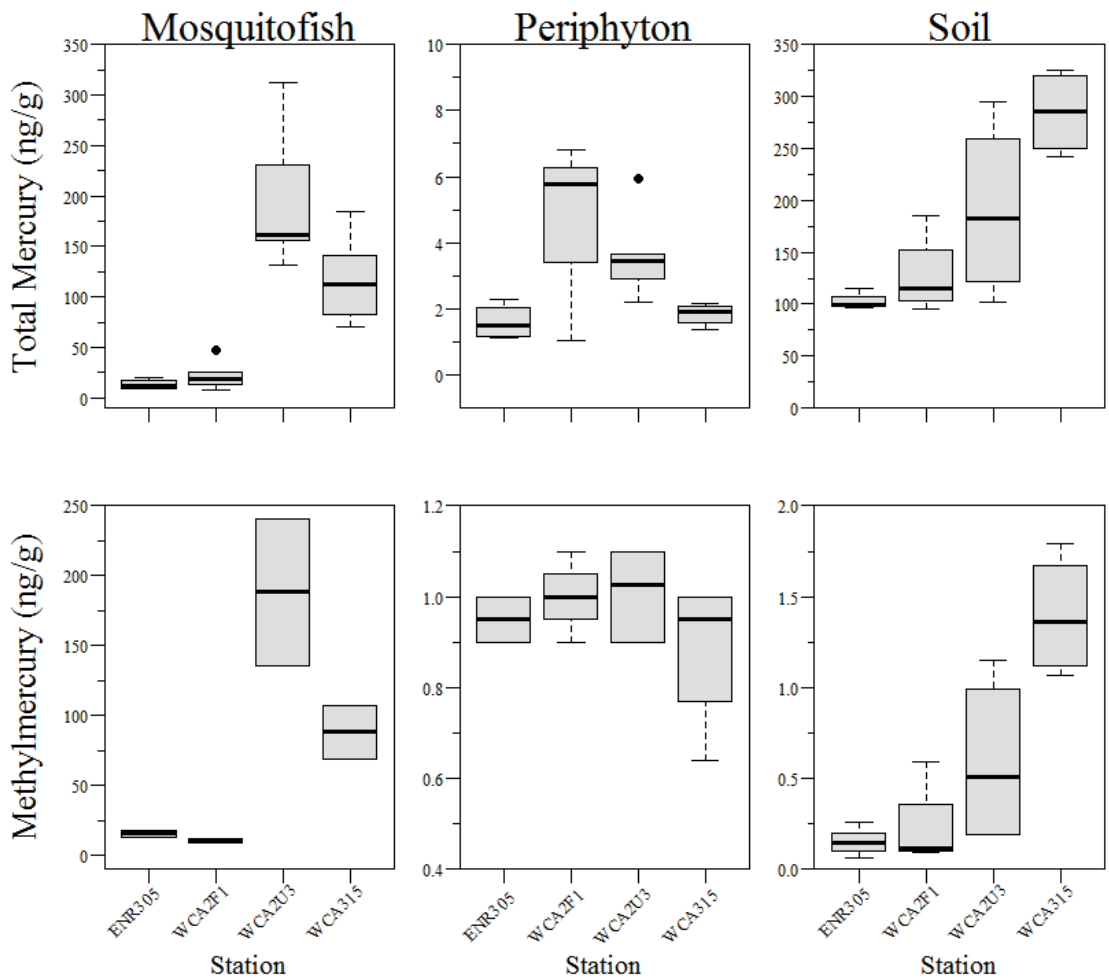

Figure 2. THg and MeHg concentrations for mosquitofish, periphyton, and soil from samples collected at monitoring locations within the EPA and STA-1W between 2010 and 2013. [Note: the change in y-axis values for each ecosystem compartment.]

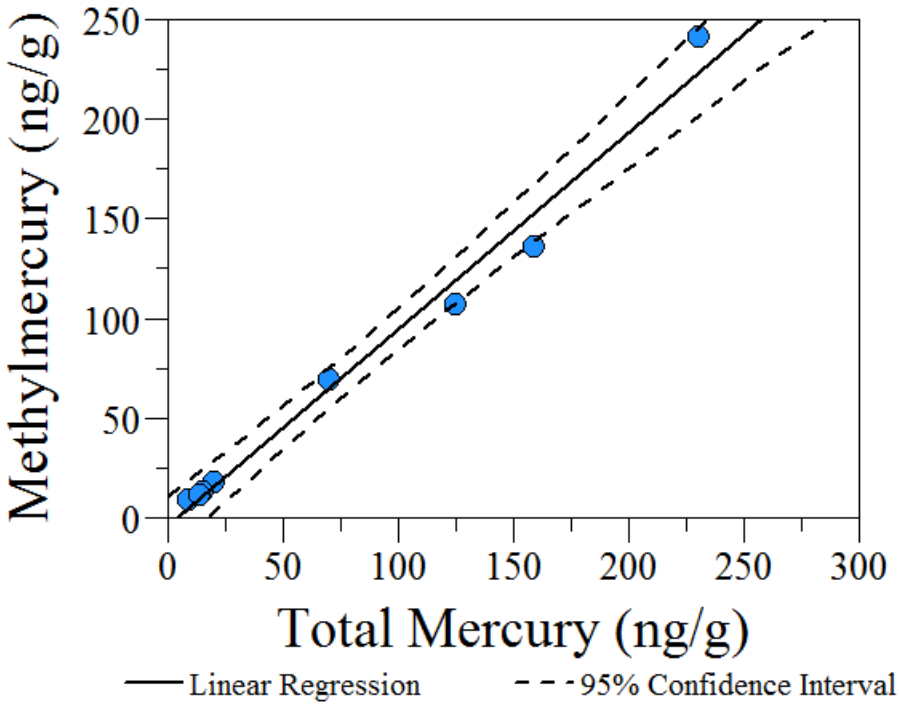

Figure 3. Mosquitofish THg and MeHg linear regression from samples collected at monitoring locations within the EPA and STA-1W between 2010 and 2013. Linear Model: THg = 0.99(MeHg)-3.34 (R^2 = 0.98, F(1,6) = 344.6, $\rho < 0.001$).

Hg concentrations within periphyton communities were several orders of magnitude lower than Hg concentrations observed in mosquitofish throughout this study. THg concentrations ranged from 1.06 to 6.81 ng/g, while MeHg concentration ranged from 0.64 to 1.10 ng/g (**Figure 2**). Periphyton THg and MeHg data did not fit the assumptions of a linear model (Global Statistic: 16.4, $\rho < 0.01$); therefore, Hg concentrations could not be compared using a linear regression. However, the Spearman rank correlation analysis determined that periphyton THg and MeHg concentrations were positively correlated ($\rho = 0.49$, $\rho < 0.05$). On a sample-by-sample basis, the percent of MeHg in periphyton ranged from 15 to 85 percent, with no consistent relationship between stations ($\chi^2 = 6.29$, df = 3, $\rho = 0.10$) or station designations ($\chi^2 = 2.30$, df = 1, $\rho = 0.13$). Therefore, based on the data, periphyton can at times act as a source of MeHg to the trophic chain. Furthermore, several recent studies have determined the ability of periphyton communities, more specifically microbes such as sulfate reducing bacteria, methanogens, and other *Deltaproteobacteria* within the periphyton consortium, have the ability to methylate and de-methylate available Hg (Achá et al. 2012, Cleckner et al. 1999, Hamelin et al. 2011).

Generally, soil Hg concentrations followed a north-to-south gradient, with the highest concentration observed at the southernmost site, WCA315. THg concentrations ranged from 94.9 to 325 ng/g, while MeHg concentrations ranged from 0.06 to 1.79 ng/g in mosquitofish during this study (**Figure 2**). Furthermore, the percent of MeHg in soil ranged from 0.05 to 0.74 percent, with the highest percentage of MeHg observed at WCA315 (mean ± standard error: 0.50 ± 0.06 percent) and lowest at ENR305 (0.15 ± 0.04 percent), although there was no statistical difference among sampling locations ($\chi^2 = 5.91$, df = 3,$\rho = 0.12$) or station designations ($\chi^2 = 0.34$, df = 1, $\rho = 0.56$). These results indicate that the majority of the Hg in soil is in the form of THg rather than MeHg, thereby making soil a sink of THg as a result of atmospheric deposition (Cohen et al. 2009).

Throughout the study period, surface water THg concentrations ranged from 0.16 to 5.93 nanograms per liter (ng/L) (**Figure 4**), with all concentrations well below the Florida Class III surface water quality standard of 12 (ng/L) (Subsection 62-302.500, F.A.C.). Similar to the other ecosystem compartments (i.e. periphyton, soil, surface water, etc.), surface water MeHg concentrations were less than THg concentrations, ranging from 0.02 to 0.90 ng/L (**Figure 4**). These study concentrations are consistent with those THg and MeHg concentrations reported across the Everglades ecosystem (Cohen et al. 2009, Kalla et al. 2010, Scheidt and Kalla 2007). In surface waters, the percent MeHg ranged from 0.51 to 41.3 percent at site WCAF1 and was significantly different between monitoring stations ($\chi^2 = 9.65$, df = 3, $\rho < 0.05$). Surface water percent MeHg at WCA2U3 was significant different from ENR305 ($F = 2.72$, $\rho < 0.01$) and WCA2F1 ($F = 2.19$, $\rho < 0.05$), and WCA315 was significantly different from WCA2U3 ($F = -2.53$, $\rho < 0.01$). Surface water percent MeHg was not significantly different at all other station combinations. Furthermore, percent surface water MeHg did not significantly differ between station designations ($\chi^2 = 0.34$, df = 1, $\rho = 0.56$). This is expected since MeHg concentrations within surface waters are relatively short-lived due to uptake by biota, photodegradation, and demethylating dynamics (Liu et al. 2012, Oremland et al. 1991).

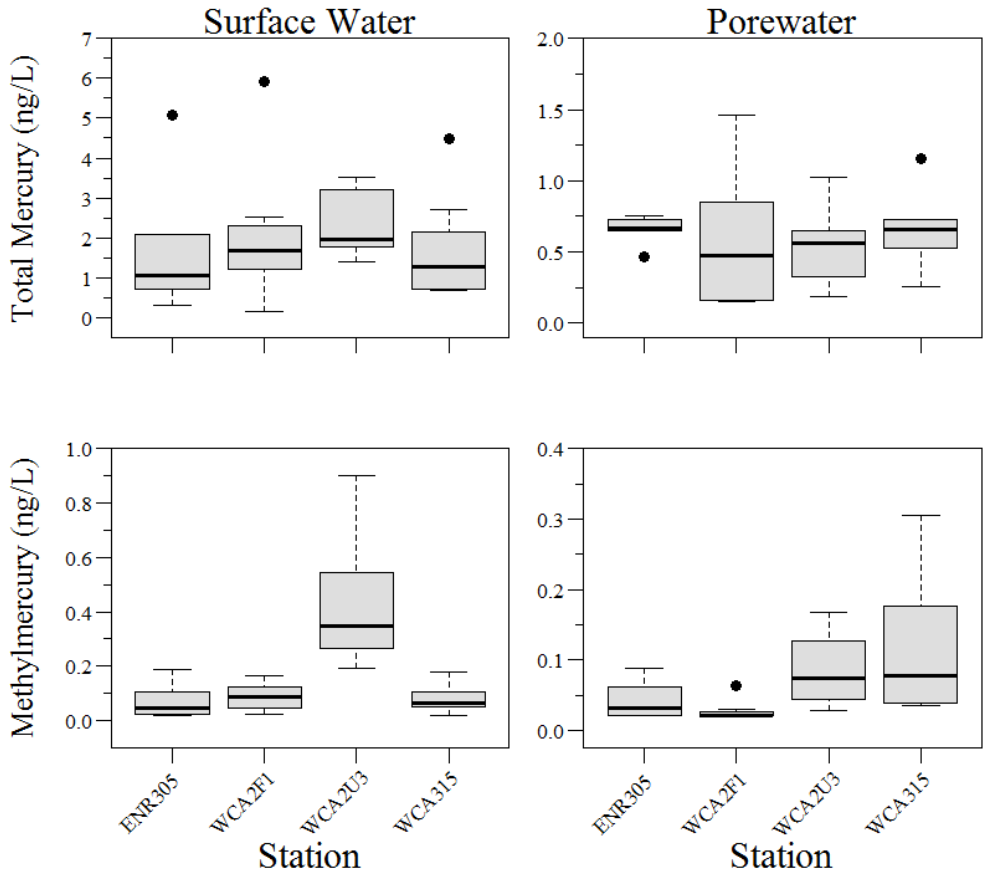

Figure 4. THg and MeHg concentrations for surface water and porewater from samples collected at monitoring locations within the EPA and STA-1W between 2010 and 2013. [Note the change in y-axis values for each ecosystem compartment.]

Porewater THg concentrations ranged from 0.15 to 1.46 ng/L, while porewater MeHg concentrations ranged from 0.02 to 0.31 ng/L (**Figure 4**). The percent MeHg in porewater ranged from 2.4 (WCA2F1) to 35.2 percent (WCA2U3), with these percentages significantly different between monitoring stations ($\chi^2 = 8.75$, df = 3, $\rho < 0.05$). Individually, porewater percent MeHg at hotspot stations were significantly different from non-hotspot stations, with WCA2U3 being significantly different than ENR305 (F = 2.13, $\rho < 0.05$) and WCAF1 (F = 2.13, $\rho < 0.05$), and WCA315 being significantly different from ENR305 (F = 2.05, $\rho < 0.05$) and WCA2F1 (F = 2.04, $\rho<0.05$). Percent porewater MeHg was not significantly different between hotspot (F=-0.09, $\rho = 0.45$) and non-hotspot stations (F = 0.11, $\rho = 0.46$). However, when stations were aggregated into station designations, there was no statistical difference of percent porewater MeHg ($\chi^2 = 0.002$, df = 1, $\rho = 0.96$), presumably due to extreme variations present between station designations (coefficient of variance: hotspot = 221.6%; non-hotspot = 136.5%). Among all ecosystem compartments, porewater Hg concentrations were the lowest, while soil concentrations were among the highest. This could be due to the organic-rich soil binding available Hg in the porewater (i.e., soil solution) to soil particles. Organic matter as soil organic matter or DOC is an effective lignand with the ability to bind Hg and potentially remove Hg from the porewater with soil particles acting a Hg sinks (Drexel et al. 2002, Khwaja et al. 2006), or within soil solution/surface Hg can be complexed with DOC (Skyllberg 2008). However, in high sulfur (S) soils, sulfide can outcompete other lignands for Hg complexation due to the thermodynamically favorable formation of Hg-S complexes (i.e., cinnabar, metacinnabar, etc.) (Benoit et al. 1999). Hg can also form complexes with Fe-containing minerals such as pyrite (FeS_2) and mackinawite (FeS), which have the potential to complex further with available (i.e., free) Hg (Liu et al. 2012, Skyllberg and Drott 2010).

MERCURY INTERACTIONS

Hg can be methylated through several different microbial pathways with microbes utilizing alternative electron acceptors to complete reduction reaction to obtain energy. As a result of these reduction reactions, some microbes have the ability to methylate Hg. The phylogenetic tree constructed by Gilmour et al. (2013) along with evidence from other studies (Gilmour et al. 2011, Parks et al. 2013, Yu et al. 2013, 2012) demonstrates the diverse Hg-methylating abilities of microbes. Microbial mediated Hg methylation is facilitated by various microbial pathways, and population/community dynamics are influenced by prevailing environmental conditions.

The production of MeHg is contingent upon several factors, including and most importantly, the bioavailability of Hg for methylation (Benoit et al. 1999), which can be influenced by geochemical process between porewater and the soil interface (as discussed above). Hg methylation is predominately a soils/soil process, with the loss of MeHg from soil being facilitated by flux from soil to the water column, bioaccumulation, microbial demethylation, or photodegradation (Gilmour et al. 1998, Li et al. 2010, Oremland et al. 1991). However, some studies have documented Hg methylation occurring within the water column. For instance, Eckley and Hintelmann (2006) determined that the Hg methylation rate is dependent upon the volume of the anoxic hypolimnia, duration of anoxic conditions, and DOC concentrations.

In this study, surface water percent MeHg was not significantly correlated with other surface water parameters including dissolved Fe (r = 0.001, $\rho = 1.00$), DOC (r = 0.10, $\rho = 0.63$), SO_4^{2-} (r = 0.06, $\rho = 0.78$), or TOC (r = 0.08, $\rho = 0.68$). However, surface water percent MeHg was positively correlated with porewater percent MeHg (r = 0.46, $\rho < 0.05$), possibly indicating that the source of surface water MeHg was flux from the soil solution (i.e., porewater) to the water column rather than water column methylation. Alternatively, Hg methylation could be occurring in the water at a lower rate than demethylation due to high light penetration through the water column or microbial processes (Seller et al. 1996).

Similar to surface water, based on Spearman rank sign correlation analyses, porewater percent MeHg was not significantly correlated with other porewater parameters including dissolved Fe ($r = 0.31$, $\rho = 0.15$), DOC ($r = -0.28$, $\rho = 0.18$), SO_4^{2-} ($r = -0.34$, $\rho = 0.12$) or S^{2-} ($r = -0.35$, $\rho = 0.10$). However porewater S^{2-} was positively correlated with porewater DOC concentrations ($r = 0.66$, $\rho < 0.001$) and porewater SO_4^{2-} concentrations ($r = 0.45$, $\rho < 0.05$) and negatively correlated with porewater Fe ($r = -0.68$, $\rho < 0.01$). These relationships could indicate dissimilar sulfate reduction rates and precipitation of S^{2-} with excessive porewater Fe^{2+} forming iron-sulfide minerals such as pyrite (FeS_2), mackinawite, or greigite (Fe_3S_2) (Burton et al. 2011, Rickard and Morse 2005). Furthermore, porewater S^{2-} concentrations were significantly different between hotspot and non-hotspot stations ($\chi^2 = 10.08$, df = 1,$\rho < 0.001$), with non-hotspot sites having elevated porewater S^{2-} concentrations relative to hotspot sites [non-hotspot: 1.02 ± 0.19 milligram per liter (mg/L); hotspot: 0.24 ± 0.07 mg/L).

Soil percent MeHg was not significantly correlated with TS ($r = -0.53$, $\rho = 0.16$) with soil TS values ranging from 0.67 to 1.6 percent S. Soil percent MeHg was not significantly correlated with soil TOC ($r = 0.38$, $\rho = 0.16$) with concentrations ranging from 373 to 496 grams per kilogram. However, soil percent MeHg was negatively correlated with soil Ca ($r = -0.56$, $\rho < 0.05$) and TOS ($r = -0.53$, $\rho < 0.05$). These results could indicate that soil Ca and TOS regulate MeHg production via the availability of THg for methylation. Ca, depending on pH, can influence the solubility of organic matter and inorganic mercury, Hg^{2+}, as well as compete for binding sites on organic matter with THg or MeHg. Yin et al. (1997) observed that higher calcium (II) (Ca^{2+}) concentrations in the soil solution decrease the concentration of dissolved organic matter at pH > 6 by either coagulation or complexation, in which Ca^{2+} serves as a bridge between solid surfaces and organic matter. Additionally, it has been suggested that Ca can also affect the solubility of thiol organic function groups at low pH conditions. Thiol groups have been reported to be binding sites on organic matter for MeHg (Hintelmann et al. 1997, Reimers and Krenkel 1974, Yin et al. 1997).

Soil MeHg concentrations were negatively correlated with porewater S^{2-} ($r = -0.73$, $\rho < 0.01$) and porewater DOC ($r = -0.67$, $\rho < 0.01$) concentrations, but conversely were positively correlated with porewater Fe concentrations ($r = 0.52$, $\rho < 0.05$); no correlations were found with porewater SO_4^{2-} concentrations ($r = -0.42$, $\rho = 0.12$). These findings suggest that there is a strong interaction between Fe and S in the regulation of soil MeHg concentrations, or through direct influence on the MeHg production process by regulating microbial reduction reactions by influencing the availability of electron donors (i.e., SO_4^{2-}, Fe, organic matter). Alternatively, the interaction among reduced S, Fe, and organic carbon could also influence the availability of THg for methylation. Based on these results, further investigation is needed to better understand Hg-S-Fe-DOC interactions as it relates to the overall Hg-MeHg cycle.

Study results also showed that BAF_{THg} was significantly correlated with several surface water, soil, and porewater parameters. BAF_{THg} was negatively correlated with surface DOC ($r = -0.45$, $\rho < 0.05$), surface water TP ($r = -0.75$, $\rho < 0.001$), surface water SO_4^{2-} (two-tailed test: $r = -0.41$, $\rho = 0.05$; one-tail-less than test: $r = -0.41$, $\rho < 0.05$) and porewater S^{2-} ($r = -0.71$, $\rho < 0.001$). In contrast, mosquitofish BAF_{THg} was positively correlated with soil percent MeHg ($r = 0.56$, $\rho < 0.05$) but not correlated with periphyton MeHg ($r = -0.18$, $\rho = 0.50$). While it has been suggested that a correlation with BAF_{THg} and surface water SO_4^{2-} is spurious (Pollman and Axelrad 2014a, 2014b), the trend in this study data is consistent with that presented by (Julian 2014, 2013) using a much larger and more spatially explicit data set. Dissolved organic matter, typically represented as DOC, interacts very strongly with Hg influencing speciation, solubility mobility, and toxicity. With respect to microbial methylation, DOC generally limits the amount of inorganic Hg available for uptake and methylation by microbes (Ravichandran 2004). Therefore, it would be expected that at higher DOC concentrations, the availability of MeHg for bioaccumulation would be lower. TP is a growth-limiting nutrient and it has been suggested that high TP essentially bio-dilutes Hg concentrations by allowing organisms to grow larger faster (Julian et al. 2015), which corresponds

to the negative correlation results. As soil percent MeHg was strongly correlated with BAF_{THg}, this suggests that mosquitofish may be feeding on benthic detrital material; however, additional trophic data is needed to better explain the nature of Hg accumulation in mosquitofish collected in this study.

Due to the spatial and temporal limitations of this study, more rigorous statistical analysis was not possible. This study utilized several combinations of parameters and matrices to conduct non-parametric correlation. While correlation is not causation, this study did result in some intriguing relationships, including correlations between soil Ca and TOS with percent MeHg and porewater Fe; DOC and S^{2-} with soil MeHg; mosquitofish BAF_{THg} with surface water DOC, TP and SO_4^{2-}; and porewater S^{2-} and soil percent MeHg. Further study is needed to explore the interaction of soil Ca other S species (i.e., oxidizable S, chromium-reducible S^{2-}, acid-volatile sulfides) and Fe with respect to MeHg formation and biotic uptake. Further analysis is also needed to determine the role of Ca in regulating THg and MeHg dynamics as well as other ions such as chloride. While water and soil quality relative to Hg dynamics is important, additional parameters (e.g., trophic status indicators) would provide more clarity in determining regulators of MeHg accumulation in biota.

LITERATURE CITED

Achá, D., C.A. Pabón and H. Hintelmann. 2012. Mercury methylation and hydrogen sulfide production among unexpected strains isolated from periphyton of two macrophytes of the Amazon. *FEMS Microbiology Ecology* 80:637-645.

Benoit, J.M., C.C. Gilmour, R.P. Mason and A. Heyes. 1999. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environmental Science & Technology* 33:951-957.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49:1010-1017.

Burton, E.D., R.T. Bush, S.G. Johnston, L.A. Sullivan and A.F. Keene. 2011. Sulfur biogeochemical cycling and novel Fe–S mineralization pathways in a tidally re-flooded wetland. *Geochimica et Cosmochimica Acta* 75:3434-3451.

Cleckner, L.B., C.C. Gilmour, J.P. Hurley and D.P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. *Limnology and Oceanography* 44:1815-1825.

Cohen, M.J., S. Lamsal, T.Z. Osborne, J.C.J. Bonzongo, S. Newman and K.R. Reddy. 2009. Soil total mercury concentrations across the Greater Everglades. *Soil Science Society of America Journal* 73:675.

Drexel, R.T., M. Haitzer, J.N. Ryan, G.R. Aiken and K.L. Nagy. 2002. Mercury (II) sorption to two Florida Everglades peats: Evidence for strong and weak binding and competition by dissolved organic matter released from the peat. *Environmental Science & Technology* 36:4058-4064.

Eckley, C.S. and H. Hintelmann. 2006. Determination of mercury methylation potentials in the water column of lakes across Canada. *Science of the Total Environment*, selected papers from the 7th International Conference on Mercury as a Global Pollutant, Ljubljana, Slovenia June 27–July 2, 2004, 368:111-125.

Gilmour, C.C., G.S. Riedel, M.C. Ederington, J.T. Bell, G.A. Gill and M.C. Stordal. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry* 40:327-345.

Gilmour, C.C., D.A. Elias, A.M. Kucken, S.D. Brown, A.V. Palumbo, C.W. Schadt and J.D. Wall. 2011. Sulfate-reducing bacterium desulfovibrio desulfuricans nd132 as a model for understanding bacterial mercury methylation. *Applied and Environmental Microbiology* 77:3938-3951.

Gilmour, C.., M. Podar, A.L. Bullock, A.M. Graham, S.D. Brown, A.C. Somenahally, A. Johs, R. Hurt, K.L. Bailey and D.A. Elias. 2013. Mercury methylation by novel microorganisms from new environments. *Environmental Science & Technology*: 47:11810-11820.

Grieb, T.M., G.L. Bowie, C.T. Driscoll, S.P. Gloss, C.L. Schofield and D.B. Porcella. 1990. Factors affecting mercury accumulation in fish in the upper michigan peninsula. *Environmental Toxicology and Chemistry* 9:919-930.

Hamelin, S., M. Amyot, T. Barkay, Y. Wang and D. Planas. 2011. Methanogens: Principal methylators of mercury in lake periphyton. *Environmental Science & Technology* 45:7693-7700.

Hintelmann, H., P.M. Welbourn and R.D. Evans. 1997. Measurement of Complexation of methylmercury (II) compounds by freshwater humic substances using equilibrium dialysis. *Environmental Science & Technology* 31:489-495.

Julian, P. 2013. Mercury bio-concentration factor in mosquito fish (*Gambusia* spp.) in the Florida Everglades. *Bulletin of Environmental Contamination and Toxicology* 90:329-332.

Julian, P. 2014. Reply to "Mercury Bioaccumulation and Bioaccumulation Factors for Everglades Mosquitofish as Related to Sulfate: A Re-Analysis of Julian II (2013)." *Bulletin of Environmental Contamination and Toxicology* 93:517-521.

Julian, P. and B. Gu. 2015. Mercury accumulation in largemouth bass (*Micropterus salmoides* Lacépède) within marsh ecosystems of the Florida Everglades, USA. *Ecotoxicology* 24:202-214.

Julian, P., B. Gu, G. Redfield, K. Weaver, T. Lange, P. Frederick, J.M. McCray, A.L. Wright, F.E. Dierberg, T.A. DeBusk, M. Jerauld, W.F. DeBusk, H.. Bae and A. Ogram. 2015. Chapter 3B: Mercury and Sulfur Environmental Assessment for the Everglades. In: *2015 South Florida Environmental Report – Volume I*. South Florida Water Management District, West Palm Beach, FL.

Kalla, P., C. Pollman, D. Scheidt and X. Yin. 2010. Mercury in the Greater Everglades: Changes in Bio-magnification over Time, and Relationships to Other Contaminants, Across the Landscape-R-EMAP 1995–2005. Presented at the Greater Everglades Ecosystem Restoration (GEER) Meeting, Naples, FL.

Khwaja, A.R., P.R. Bloom and P.L. Brezonik. 2006. Binding constants of divalent mercury (hg2+) in soil humic acids and soil organic matter. *Environmental Science & Technology* 40:844-849.

Lange, T.R., H.E. Royals and L.L. Connor. 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. *Transactions of the American Fisheries Society* 122:74-84.

Light, S.S. and J.W. Dineen. 1994. Water control in the everglades: A historical perspective. Pages 47–84 in: S. Davis and J. Ogden, J. (eds.), *Everglades: The Ecosystem and Its Restoration.* St. Lucie Press, Delray Beach, FL.

Liu, G., Y. Cai and N.J. O'Driscoll. 2012. Advances in Environmental Chemistry and Toxicology of Mercury. John Wiley & Sons, Hoboken, NJ.

Li, Y., Y. Mao, G. Liu, G. Tachiev, D. Roelant, X. Feng and Y. Cai. 2010. Degradation of methylmercury and its effects on mercury distribution and cycling in the Florida Everglades. *Environmental Science & Technology* 44:6661-6666.

Ogden, J.C., W.B. Robertson Jr, G.E. Davis and T.W. Schmidt. 1973. Pesticide, Polychlorionated Biphenols and Heavy Metals in Upper Food Chain Levels, Everglades National Park and Vicinity. National Park Service, Everglades National Park, Homestead, FL.

Oremland, R.S., C.W. Culbertson and M.R. Winfrey. 1991. Methylmercury decomposition in sediments and bacterial cultures: Involvement of methanogens and sulfate reducers in oxidative demethylation. *Applied and Environmental Microbiology* 57:130-137.

Parks, J.M., A. Johs, M. Podar, R. Bridou, R.A. Hurt, S.D. Smith, S.J. Tomanicek, Y. Qian, S.D. Brown, C.C. Brandt, A.V. Palumbo, J.C. Smith, J.D. Wall, D.A. Elias and L. Liang. 2013. The genetic basis for bacterial mercury methylation. *Science* 339:1332-1335.

Peña, E.A. and E.H. Slate. 2006. Global validation of linear model assumptions. *Journal of the American Statistical Association* 101:341-354.

Pollman, C.D. and D.M. Axelrad. 2014a. Mercury bioaccumulation and bioaccumulation factors for Everglades mosquitofish as related to sulfate: A re-analysis of Julian II (2013). *Bulletin of Environmental Contamination and Toxicology* 93:509-516.

Pollman, C.D. and D.M. Axelrad. 2014b. Mercury bioaccumulation factors and spurious correlations. *Science of the Total Environment* 496: vi–xii.

Ravichandran, M. 2004. Interactions between mercury and dissolved organic matter—a review. *Chemosphere* 55:319-331.

Reimers, R.S. and P.A. Krenkel. 1974. Kinetics of mercury adsorption and desorption in sediments. *Journal of the Water Pollution Control Federation* 46:352-365.

Rickard, D. and J.W. Morse. 2005. Acid volatile sulfide (AVS). *Marine Chemistry* 97:141-197.

Rumbold, D.G., S.L. Niemczyk, L.E. Fink, T. Chandrasekhar, B. Harkanson and K.A. Laine. 2001. Mercury in eggs and feathers of great egrets (*Ardea albus*) from the Florida Everglades. *Archives of Environmental Contamination and Toxicology* 41:501-507.

Scheidt, D. and P.I. Kalla. 2007. Everglades Ecosystem Assessment: Water management and Quality, Eutrophication, Mercury Contamination, Soil and Habitat: Monitoring for Adaptive Management: A R-EMAP Status Report. Technical Number EPA 904-R-07-001, United States Environmental Protection Agency, Athens, GA.

Seller, P., C.A. Kelly, J.W.M. Rudd and A.R. MacHutchon. 1996. Photodegradation of methylmercury in lakes. *Nature* 380:694-697.

SFWMD. 2009. Field Sampling Quality Manual. SFWMD-FIELD-QM-001-05, South Florida Water Management District, West Palm Beach, FL.

SFWMD. 2010. Field Sampling Quality Manual. SFWMD-FIELD-QM-001-06, South Florida Water Management District, West Palm Beach, FL.

SFWMD. 2011. Field Sampling Quality Manual. SFWMD-FIELD-QM-001-07, South Florida Water Management District, West Palm Beach, FL.

Skyllberg, U. 2008. Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: Illumination of controversies and implications for MeHg net production. *Journal of Geophysical Research* 113(G2), DOI 10.1029/2008JG000745.

Skyllberg, U. and A. Drott. 2010. Competition between disordered iron sulfide and natural organic matter associated thiols for mercury (II)—An EXAFS study. *Environmental Science & Technology* 44:1254-1259.

Spry, D.J. and J.G. Wiener. 1991. Metal bioavailability and toxicity to fish in low-alkalinity lakes: A critical review. *Environmental Pollution* 71:243-304.

USEPA. 1997. Mercury Study Report to Congress, Volume VI: An Ecological Assessment for Anthropogenic Mercury Emissions in the United States. EPA-452/R-97-008, United States Environmental Protection Agency, Washington DC.

Wiener, J.G., D.P. Krabbenhoft, G.H. Heniz and A.M. Scheuhammer. 2003. Ecotoxicology of mercury. Pages 409–463 in: D.J. Hoffman, B. Rattner, G.A. Burton, and J, Cairns (eds.), *Handbook of Ecotoxicology*, CRC Press, Boca Raton, FL.

Yin, Y., H.E. Allen, C.-P. Huang and P.F. Sanders. 1997. Effects of pH, chloride and Calcium (II) on adsorption of monomethylmercury by soils. *Environmental Toxicology and Chemistry* 16:2457-2462.

Yu, R.-Q., J.R. Flanders, E.E. Mack, R. Turner, M.B. Mirza and T. Barkay. 2012. Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in freshwater river sediments. *Environmental Science & Technology* 46:2684-2691.

Yu, R.-Q., J.R. Reinfelder, M.E. Hines and T. Barkay. 2013. Mercury methylation by the methanogen *Methanospirillum hungatei*. *Applied and Environmental Microbiology* 79: 6325-6330.