# Mercury Stoichiometric Relationships in a Subtropical Peatland

**Paul Julian II · Binhe Gu · Alan L. Wright**

**Abstract** Spatially variable areas, or hotspots, of elevated mercury (Hg) concentrations in soil, water, and wildlife occur throughout the Everglades wetland ecosystem. This study investigates the stoichiometric controls of Hg relative to soil, water, and biotic components. Surface water, porewater, soil, periphyton, and *Gambusia* spp. (mosquitofish) were collected from hotspots and non-spot stations and analyzed for various parameters, including total mercury (THg), organic carbon (OC), total carbon (TC), total phosphorus (TP), and total nitrogen (TN) between late 2010 and early 2013. Soil nutrient ratios were significantly different between hotspot and non-hotspot stations, indicating a difference in trophic status and position along the decay continuum or differences in limiting nutrients. Overall, soil total Hg concentrations were negatively correlated with soil TC/TN, while soil TC/TP and soil TN/TP molar ratios and soil THg were negatively correlated at hotspot stations. Meanwhile, mosquitofish THg was negatively correlated with soil TC/TN molar ratio and positively correlated with soil TC/TP and TN/TP molar ratios, suggesting trophic truncation. Soil, surface water, and porewater THg, TC, and OC interactions resulted in significant differences between hotspot and non-hotspot stations and between molar ratios of C, N, and P. Periphyton-surface water THg/OC homeostasis and soil nutrient ratios significantly explained mosquitofish THg concentrations, further indicating a trophic influence on mosquitofish THg and potential hotspot dynamics. Several factors and processes including bottom-up trophic interaction and vegetation influence on Hg accumulation dynamics and food-chain length explain the development and persistence of Hg hotspot formation within the Everglades system.

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## 1 Introduction

Mercury (Hg) accumulation in biota, including fish species, has been problematic throughout the world for several decades. Elevated Hg in fish and other biota was first observed within the Everglades in the early 1970s (Ogden et al. 1973). Spatially variable elevated Hg concentrations continue to be observed in fish within the Everglades ecosystem (Julian et al. 2016). The primary hypothesis for variable methyl-Hg accumulation

in ecosystem components within the Everglades is that variable Hg methylation rates caused by sulfate-reducing bacteria are stimulated by sulfate and inhibited by sulfide accumulation (Gilmour et al. 1998; Benoit et al. 2003; Orem et al. 2011). However, more recent hypotheses include the interaction of organic matter (Aiken et al. 2011b), alternative microbial guilds influencing Hg methylation (Bae et al. 2014; Julian et al. 2015a), hydrologic cycling stimulating Hg methylation (Jerauld et al. 2015), and other water quality parameters such as iron (Julian et al. 2016). Regardless, once Hg is methylated, uptake by biota is rapid (Loftus 2000) and Hg then enters the trophic continuum and is regulated by changes in the trophic cascade.

The trophic continuum is defined as an assemblage of organisms where energy is transferred from the smallest to the largest primarily through predation (except at the base; i.e., detritivores) from their local environment (Cousins 1980; France et al. 1998). Trophic position or trophic levels along the trophic continuum are now often estimated using nitrogen (N) and carbon (C) stable isotope ($\delta^{15}N$ and $\delta^{13}C$, respectively) values of the organism relative to other organisms along the continuum (Deniro and Epstein 1981; Schoeninger and DeNiro 1984; Post 2002; Davenport and Bax 2002). Investigations in the Florida Everglades have revealed high Hg concentrations in biota, especially in interior portions of Everglades far removed from any potential surface water sources of Hg. The primary source of Hg to the Everglades foodweb is via atmospheric deposition (Julian et al. 2014). Several studies have demonstrated that Hg is strongly associated with biotia's $\delta^{15}N$ values, indicating Hg biomagnification (Kidd et al. 1995; Jarman et al. 1996; Atwell et al. 1998). Positive correlation between trophic position and total Hg tissue concentrations provides identification of trophic end-members and a pathway of bioaccumulated Hg (Lange 2015; Rumbold 2015). The trophic cascade theory states that in aquatic systems, predation is a structuring force to the trophic continuum in that each trophic level of a foodweb is inversely and directly related to the next juxtaposed trophic level (Carpenter et al. 1985; Brett and Goldman 1996; Pace et al. 1999; Blanco et al. 2004). The trophic continuum concept is heavily rooted in ecological stoichiometry in which animals act as both nutrient and elemental sinks (Vanni 2002).

Stoichiometric relationships of an organism have implications for the nutritional value of resources, element trophic transfer, and cycling (Sterner and Hessen

1994; Elser and Urabe 1999). The most notable ecological stoichiometric relationship is the "Redfield Ratio" (Redfield 1958), which is a set of principles that relates the nutrient composition of marine plankton relative to the surrounding environment. Several studies have compared stoichiometric relationships between soil nutrients and soil microbial biomass (Cleveland and Liptzin 2007), and soil nutrients and vegetation (Langston and Spence 1995; Koerselman and Meuleman 1996). However, very few studies have investigated soil nutrient stoichiometry relative to mercury dynamics within the Everglades system (Vaithiyanathan et al. 1996; Demers et al. 2013).

Stoichiometric homeostasis refers to the degree to which an organism or community maintains its elemental ratios (i.e., C, N, and phosphorus [P]) when relative element resource availability changes (Sterner and Elser 2002). Sterner and Elser (2002) originally developed the concept of stoichiometric homeostasis in the context of ecosystem disturbances or perturbations. Whether organisms and ecosystems respond to changing conditions with respect to stoichiometric homeostasis depends upon several factors, including species-specific capacity for biomass nutrient storage, physiological plasticity, inter-specific competitive ability, and the relative availability of elements to organisms (Elser et al. 2010; Sistla et al. 2015). These factors ultimately dictate the stoichiometric flexibility of particular species and communities, which in-turn reflect ecosystem level stoichiometric flexibility. The stoichiometric homeostasis concept is of particular interest with respect to nutrient enrichment processes since nutrient homeostatic regulation has the potential to decouple the downstream transport of nutrients (Small et al. 2009). However, the concepts of stoichiometric homeostasis can also be applied to other elemental ratios such as THg and C. Plasticity of homeostatic regulation and stoichiometric relationships in response to changes in THg availability can be used to aid in the exploration of mechanisms involved with translocation, uptake, and transport of THg within an ecosystem.

In the past, Hg studies in the Everglades ecosystem focused on determining a link between sulfur (as sulfate) and Hg methylation and accumulation. However, recent studies have determined that while sulfate is important, other factors also contribute to Hg methylation and accumulation (Marvin-DiPasquale et al. 2014; Dierberg et al. 2015; Jerauld et al. 2015; Julian and Gu 2015). The objectives of this study were to confirm the occurrence

of suspected Hg accumulation hotspots; investigate Hg stoichiometry with respect to soil C, N, and P in a wetland ecosystem between areas identified as Hg accumulation hotspots and non-hotspots; and to examine Hg stoichiometry relative to Hg accumulation in periphyton and *Gambusia* spp. (mosquitofish). It is hypothesized that relative trophic conditions and decomposition of organic matter, as indicated by soil nutrient ratios, regulate soil Hg concentrations. We hypothesize that organic C availability strongly influences the availability and retention of Hg within periphyton and mosquitofish between areas identified as Hg hotspots and non-hotspots.

## 2 Methods

### 2.1 Study Area

This study was conducted in the northern portion of the Everglades Protection Area (EPA) at monitoring locations within Water Conservation Area (WCA) 2, 3 and Stormwater Treatment Area 1W (STA-1W) with a historic period of record related to soil and biota THg concentrations. The EPA, WCAs 1, 2, 3 and Everglades National Park, is a complex system of marsh areas with canals, levees, and water control structures that covers approximately 1.01 million hectares (ha) of former contiguous Everglades marsh (Light and Dineen 1994). Interior areas of this wetland still approximate the historic hydrologic (long hydroperiod) and water quality (low nutrients) conditions with *Cladium jamaicense* Crantz (sawgrass) sloughs and tree islands while areas near canal inflows have been gradually transformed to dense cattail *Typha* spp. (cattail) with elevated nutrients and variable hydrology (McCormick et al. 2009). STA-1W is located just north-west of the EPA and is a constructed wetland covering approximately 1620 ha of former agricultural land constructed for the primary purpose of removing nutrients from the water column before entering the EPA. STA-1W is currently composed of a series of cells containing a mix of emergent, submergent, and floating aquatic vegetation (Chen et al. 2015).

### 2.2 Source of Data and Data Handling

Data were collected from four stations within the Everglades ecosystem: one located within Stormwater Treatment Area-1W (ENR302) and three located within the EPA (Fig. 1), between late 2010 and early 2013. These stations were selected to represent two distinct regions within the Everglades ecosystem, as stations WCA2U3 and WCA315 represent suspected hotspots and stations ENR302 and WCA2F1 represent non-hotspots. Hotspots are defined as those areas with persistently high fish tissue THg concentrations exceeding the US Environmental Protection Agency (USEPA) criterion (trophic level 3 criterion, 77 ng $g^{-1}$; and trophic level 4 criterion, 346 ng $g^{-1}$) (USEPA 1997, 2001), and non-hotspots represent areas with relatively low fish THg concentrations based on long-term monitoring data (Julian et al. 2015a). Hotspot sites (ENR302 and WCA2F1) are characterized by dominate cattail, relatively high soil TP concentration and elevated surface water TP concentration when compared to non-hotspot stations (WCA2U3 and WCA315) which are characterized by dominate sawgrass and open slough stations with low TP soil and water column concentrations. A total of seven sampling events were conducted during the approximate 4-year period. Parameters, matrices, and analytical methods utilized in this study are identified in Table 1. All chemical analyses were performed consistent with USEPA approved methods (Table 1).

Surface water samples were collected via grab sample either by direct collection into sample bottles or portable peristaltic pump. Porewater samples were collected via sipper probe at a depth of 6 to 10 cm below the soil/sediment surface. Surface water and porewater samples were collected within a 50-m radius of the site in a manner to reduce disturbance of the water column and collect a representative sample. Soil samples were collected at a depth of 0–10 cm using a butyrate coring tube within a 25-m radius of each site, per sampling event. Duplicate soil samples were collected, sectioned, and composited for chemical analysis. Triplicate samples of periphyton were collected within a 25-m radius of the sampling station to form one composite sample where periphyton was present. A minimum of 100 mosquitofish were sampled by dip-net and composited for each sampling location within a 50-m radius of the sampling station. All sampling methods were consistent with South Florida Water Management District sampling protocols (SFWMD 2010).

Data were screened based on laboratory qualifier codes consistent with Florida Department of Environmental Protection's quality assurance rule (Florida Department of Environmental Protection 1991). Any
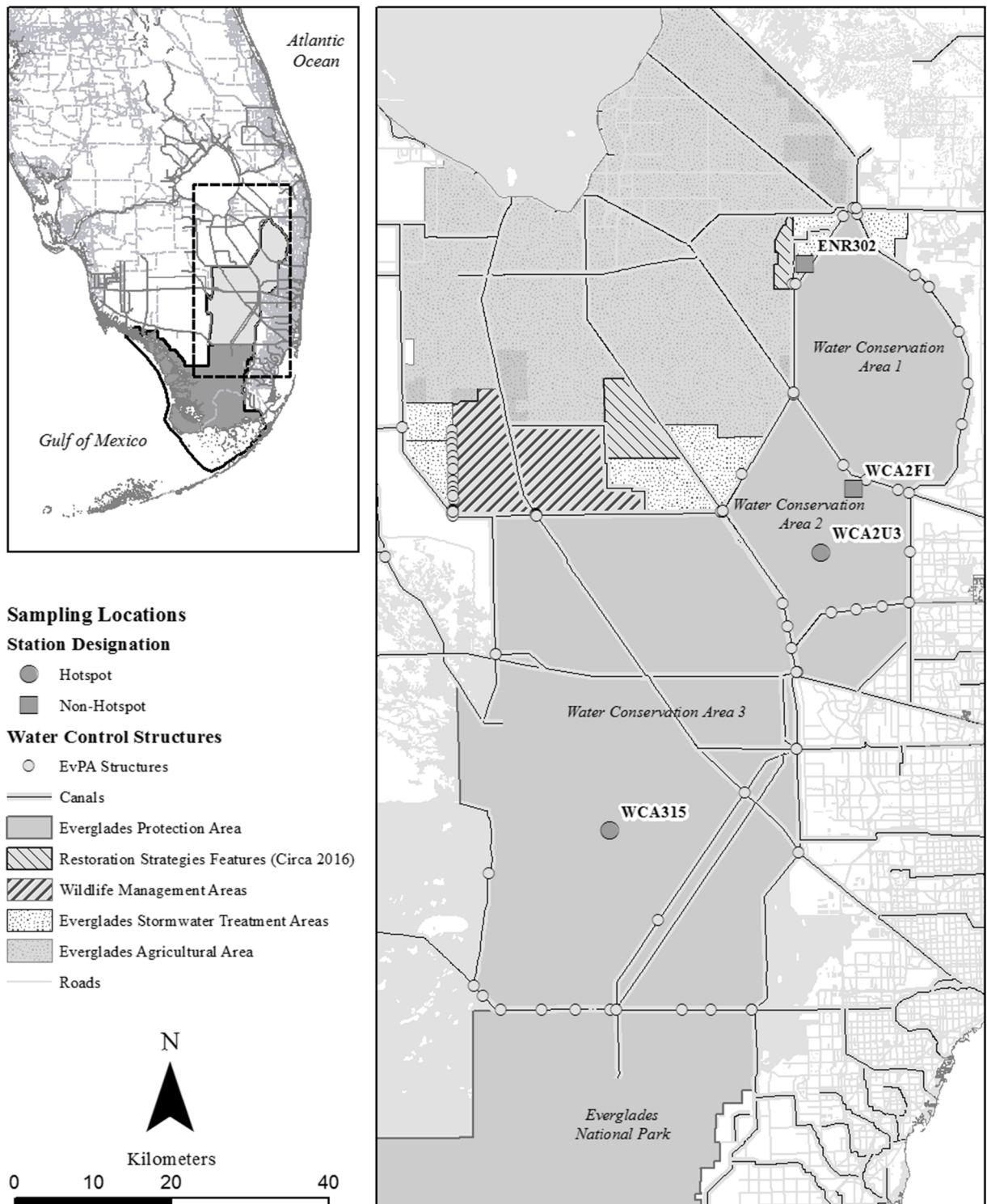
**Fig. 1** Map of monitoring locations relative to the Everglades Protection Area and surrounding features including the Everglades Stormwater Treatment Areas and Water Conservation Areas. Station ENR302 and WCA2F1 have been designated as non-hotspot stations while WCA2U3 and WCA315 are designated hotspot stations. All station designations were defined using historical monitoring data (Axelrad et al. 2008)

**Table 1** Summary of parameters, matrices, and analytical methods used for this study. Additional parameters were collected but not used in this study

| Matrix | Parameter | Abbreviation | Analytical method | Reference |
|---|---|---|---|---|
| Surface water and porewater | Dissolved organic carbon | DOC | SM 5310B | Clesceri et al. (1998) |
| | Total mercury | THg | EPA 1631E | US EPA (2002a) |
| Soil | Total phosphorus | TP | EPA 365.2 | US EPA (1971) |
| | Total nitrogen | TN | EPA 440.0 | US EPA (1997) |
| | Total carbon | TC | SM 9060A | Clesceri et al. (1998) |
| | Total organic carbon | TOC | SM 9060A | Clesceri et al. (1998) |
| | Cellulose | – | AOAC 973.18 | Möller (2009) |
| | Lignin | – | AOAC 973.18 | Möller (2009) |
| | Total mercury | THg | EPA 1631 Appendix | US EPA (2002b) |
| Fish tissue | Total mercury | | EPA 1631 Appendix | US EPA (2002b) |
| Periphyton | Total mercury | | EPA 1631 Appendix | US EPA (2002b) |
| | Total organic carbon | DOC | SM 9060A | Clesceri et al. (1998) |
| | Total carbon | TC | SM 9060A | Clesceri et al. (1998) |

*ASTM* American Society for Testing and Materials, *EPA* Environmental Protection Agency, *SM* Standard Methods, *AOAC* Association of Official Agricultural Chemists

data 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 method detection limit (MDL) were assigned a value equal to the MDL, unless otherwise noted.

## 2.3 Data Analysis

All surface water, porewater, periphyton, and soil constituent concentrations were converted from mass (mg kg$^{-1}$ or mg L$^{-1}$) to molar concentrations (mmol kg$^{-1}$ or mM). Molar ratios were calculated for soil total Hg (THg) and total C (THg/TC); THg and total organic C (THg/TOC); TC and total N (TC/TN); TC and total P (TC/TP); and TN and TP (TN/TP). Periphyton molar ratios between THg and TC (THg/TC) as well as THg and TOC (THg/TOC) were computed as were surface water and porewater THg and DOC (THg/DOC) molar ratios. Lignocellulose index (LCI) was calculated as the ratio between lignin and cellulose concentrations as a proxy to organic matter decomposition in soil, as consistent with Melillo et al. (1989). Soil THg data were assessed against the 174 ng Hg g$^{-1}$ Hg sediment quality guideline (SQG) threshold effect level (TEL) for freshwater sediments, as suggested by Mac-Donald et al. (2000). The TEL is operationally defined as the concentration below which adverse biological effects

rarely occur. Fish tissue concentrations were assessed against the USEPA criterion of 77 ng Hg g$^{-1}$ for the protection of piscivorous wildlife (USEPA 1997).

The strength of periphyton's Hg stoichiometric homeostasis was characterized using the homeostasis coefficient $H$ (Eq. 1):

$$\frac{1}{H} = \frac{\log(y) - \log(c)}{\log(x)} \tag{1}$$

where $x$ is the resource THg/OC, $y$ is the periphyton THg/OC, and $c$ is a constant (Sterner and Elser 2002). To determine the constant $c$ for our system, log-transformed periphyton and surface water THg/OC molar ratios were regressed using a Theil-Sen single median linear model (mblm R-library). The $c$ constant is estimated from the intercept of the log-log regression between resource (i.e., surface water THg/OC) and consumer (i.e., periphyton THg/OC). Values of $1/H$ range between 0 and 1 with values approaching 1, indicating a non-homeostatic relationship; meanwhile, values approaching 0 signify consumer-resource homeostasis (Sterner and Elser 2002). Degree of homeostasis was defined as follows: (1) $0 < 1/H < 0.25$, homeostatic; (2) $0.25 < 1/H < 0.50$, weakly homeostatic; (3) $0.50 < 1/H < 0.75$, weakly non-homeostatic; and (4) $0.75 < 1/H \leq 1.00$, non-homeostatic consistent with previous studies (Makino et al. 2003; Persson et al. 2010; Feijoó et al. 2014).

Soil THg concentration, TC/TN, TC/TP, TN/TP, THg/TC, and THg/OC molar ratios, periphyton THg concentration, THg/TC and THg/OC molar ratios, and porewater and surface water THg/OC molar ratios were compared between hotspot and non-hotspot stations using the Kruskal-Wallis Rank Sum Test. Spearman rank correlation analysis was used to assess the relationship between soil THg concentrations and soil TC, nutrient (TN and TP) concentrations and associated nutrient ratios (TC/TN, TC/TP, and TN/TP); soil THg concentrations with soil TC/TP and TN/TP molar ratios of hotspot and non-hotspot stations separately; LCI and soil THg/TC molar ratios; and mosquitofish THg compared with separate soil molar nutrient ratios (TC/TN, TC/TP, and TN/TP). Additionally, soil molar nutrient ratios were compared to mosquitofish THg concentrations using multiple regression analysis. Periphyton and surface water THg concentrations and THg/OC ratios were compared using Spearman rank correlation analysis. Periphyton-surface water THg/OC homeostatic coefficient ($H_{THg/OC}$) was compared between hotspot and non-hotspot stations using the Kruskal-Wallis Rank Sum Test. Spearman rank correlation analysis was used to compare $1/H_{THg/OC}$ and periphyton THg concentration to mosquitofish THg. All statistical operations were performed with R© (Ver 3.1.2, R Foundation for Statistical Computing, Vienna Austria), unless otherwise stated all statistical operations were performed using the

Base R library. The critical level of significance was set at $\alpha = 0.05$.

# 3 Results

A total of seven sampling events occurred between the four monitoring stations from 2010 to 2013. As expected, elevated mosquitofish and soil THg concentrations were observed at monitoring stations associated with Hg hotspots. Meanwhile, periphyton, porewater, and surface water THg concentrations remained relatively constant between stations. Soil TP concentrations were higher at non-hotspot stations relative to hotspot stations and the inverse occurred for soil TN concentrations. Total and organic C remained relatively constant between stations for periphyton and soil, but porewater and surface water were more variable (Table 2). As a result, stoichiometric relationships between THg, TC, and OC differed between ecosystem compartments.

## 3.1 Soil Stoichiometry

All soil nutrient ratios differed between hotspot and non-hotspot stations with hotspot stations experiencing significantly higher soil nutrient ratios than non-hotspot stations. Soil TC/TN ratios ranged from 10.8 to 14.6 with hotspot stations experiencing significantly higher

**Table 2** Summary statistics for total mercury, carbon and organic carbon (dissolved or total) for each ecosystem compartment, soil total nitrogen, and soil total phosphorus between hotspot and non-hotspot categories. All solid matrices are expressed as wet weight concentration

| Parameter | Matrix | Units | Mean ± standard error (N) | |
|---|---|---|---|---|
| | | | Hotspot | Non-hotspot |
| Total mercury | Mosquitofish | ng Hg g$^{-1}$ | 154.9 ± 19.1 (12) | 17.7 ± 3.1 (12) |
| | Periphyton | ng Hg g$^{-1}$ | 2.9 ± 0.4 (10) | 2.9 ± 0.9 (7) |
| | Soil | ng Hg g$^{-1}$ | 237.6 ± 28.3 (8) | 117.2 ± 11.8 (7) |
| | Porewater | ng Hg L$^{-1}$ | 0.6 ± 0.1 (36) | 0.6 ± 0.1 (33) |
| | Surface water | ng Hg L$^{-1}$ | 2.1 ± 0.3 (14) | 1.9 ± 0.5 (13) |
| Total carbon | Periphyton | g TC kg$^{-1}$ | 314 ± 19.7 (10) | 333.8 ± 27.9 (6) |
| | Soil | g TC kg$^{-1}$ | 477.5 ± 4.3 (8) | 440.9 ± 15.5 (7) |
| Total organic carbon | Periphyton | g OC kg$^{-1}$ | 271.1 ± 25.6 (10) | 285.7 ± 38.3 (6) |
| | Soil | g OC kg$^{-1}$ | 473.4 ± 5.6 (8) | 437.1 ± 16.4 (7) |
| Dissolved organic carbon | Porewater | mg DOC L$^{-1}$ | 33.7 ± 1.9 (42) | 81.3 ± 8.3 (39) |
| | Surface water | mg DOC L$^{-1}$ | 26 ± 3.4 (14) | 36 ± 3.4 (13) |
| Total nitrogen | Soil | g TN kg$^{-1}$ | 33.3 ± 0.9 (8) | 28.7 ± 1.0 (7) |
| Total phosphorus | | mg TP kg$^{-1}$ | 379.5 ± 33.6 (8) | 699.3 ± 150 (7) |

TC/TN ratios with a 15% difference between hotspots and non-hotspots (Fig. 2; $\chi^2 = 5.9$, $df = 1$. $\rho < 0.05$). Soil TC/TP ratios ranged from 108 to 887 with hotspot stations experiencing 63% significantly higher TC/TP ratios with than non-hotspot stations (Fig. 2; $\chi^2 = 10.5$, $df = 1$. $\rho < 0.01$). Soil TN/TP ratios ranged from 8.3 to 60.6 with hotspot stations experiencing significantly 66% higher TN/TP ratios (Fig. 2; $\chi^2 = 10.5$, $df = 1$. $\rho < 0.01$).

Soil THg concentrations ranged from 95 to 325 ng g$^{-1}$ and as expected hotspot stations experienced 67% higher concentrations than non-hotspot stations (Table 2; $\chi^2 = 7.1$, $df = 1$. $\rho < 0.01$). A total of seven soil samples (23%) were observed above the 174 ng Hg g$^{-1}$ Hg SQG TEL. Soil THg concentrations were significantly correlated with soil TN concentrations ($r = 0.66$, $S = 190$, $\rho < 0.01$), but not correlated with soil TC ($r = 0.12$, $S = 492$, $\rho = 0.67$) or TP ($r = -0.27$, $S = 712$, $\rho = 0.32$) concentrations. Separate distinct populations are apparent relative to soil THg and soil TP while TC
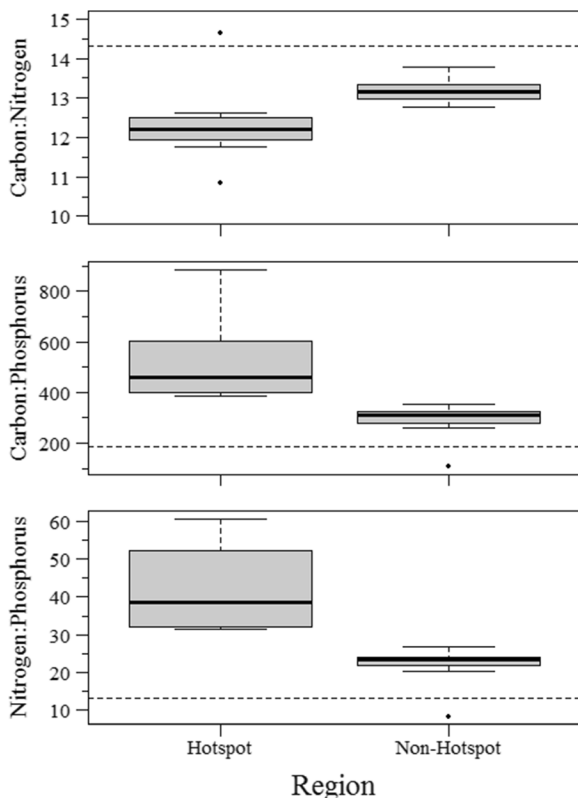
and TN relationships with THg are well mixed among sites (Fig. 3). Soil THg concentrations were positively correlated with soil TP concentrations for hotspot stations ($r = 0.88$, $S = 10$, $\rho < 0.01$), and no significant correlation was observed between soil THg and soil TP concentration at non-hotspot stations ($r = 0.53$, $S = 26$, $\rho = 0.24$). Soil THg concentration was negatively correlated with soil TC/TN ratio ($r = -0.74$, $S = 972$, $\rho < 0.01$) (Fig. 3). When hotspot and non-hotspot station data were aggregated, soil THg concentrations were not significantly correlated with soil TC/TP ($r = 0.34$, $S = 368$, $\rho = 0.21$) or soil TN/TP ratios ($r = 0.36$, $S = 358$, $\rho = 0.19$). Separate populations are apparent in Fig. 4 between soil THg, TC/TP, and TN/TP molar ratios. Soil THg concentrations were negatively correlated with TC/TP ($r = -0.88$, $S = 158$, $\rho < 0.01$) and TN/TP ($r = -0.88$, $S = 158$, $\rho < 0.01$) molar ratios for hotspot stations. Soil THg concentrations were not correlated with TC/TP ($r = -0.64$, $S = 92$, $\rho = 0.14$) and TN/TP ($r = -0.50$, $S = 84$, $\rho = 0.27$) molar ratios for non-hotspot stations.

Soil THg/TC molar ratios were significantly different between stations ($\chi^2 = 5.90$, $df = 1$, $\rho < 0.05$) with hotspots experiencing greater THg/TC across a larger
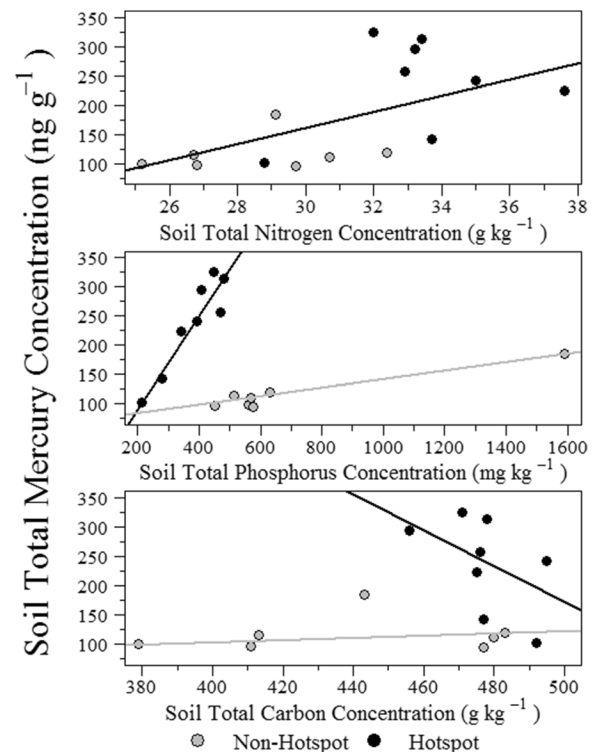


**Fig. 2** Soil molar nutrient ratios between hotspot ($N = 8$) and non-hotspot ($N = 7$) stations within the Everglades ecosystem. *Dashed lines* on each plot indicate stoichiometric relations consistent with the established "Redfield" like ratio of 186:13:1 for C/N/P determined by Cleveland and Liptzin (2007) for soil



**Fig. 3** Soil total mercury concentrations by soil nutrient concentrations with stations designations identified. *Lines* through data represent correlation trends identified by Theil-Sen estimates
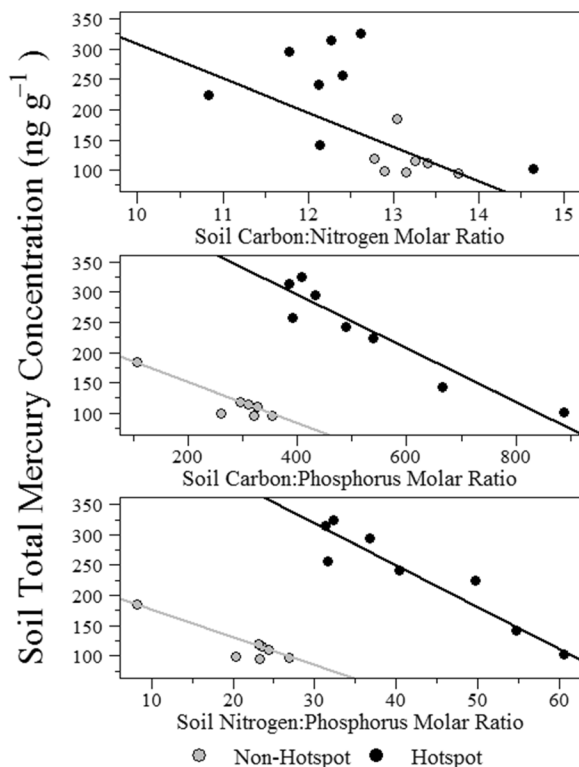
**Fig. 4** Soil total mercury concentrations by soil nutrient molar ratio with stations designations identified. *Lines* through data represent correlation trends identified by Theil-Sen estimates

range of values. This relationship was similar between soil THg/OC molar ratios with a significant difference between stations ($\chi^2 = 5.90$, $df = 1$, $\rho < 0.05$). Lignocellulose index was significantly correlated with soil THg/TC molar ratio ($r = -0.74$, $S = 974$, $\rho < 0.01$), soil THg concentration ($r = -0.62$, $S = 908$, $\rho < 0.05$), and soil TC concentration ($r = 0.53$, $S = 261$, $\rho < 0.05$) across all stations. Meanwhile, periphyton THg/TC and THg/OC molar ratios were not significantly different between stations ($\chi^2 = 0.05$, $df = 1$, $\rho = 0.83$ and $\chi^2 = 0.29$, $df = 1$, $\rho = 0.59$, respectively). Porewater and surface water THg/OC molar ratios were not significantly different between stations ($\chi^2 = 2.18$, $df = 1$, $\rho = 0.14$ and $\chi^2 = 3.40$, $df = 1$, $\rho = 0.07$, respectively).

### 3.2 Periphyton Homeostasis

Periphyton THg concentrations ranged from 1.06 to 6.81 ng Hg g$^{-1}$ with no apparent difference between hotspot and non-hotspot stations (Table 2; $\chi^2 = 0.86$, $df = 1$, $\rho = 0.35$). Surface water THg concentrations

ranged from 0.16 to 5.93 ng Hg L$^{-1}$ with no difference between hotspot and non-hotspot stations (Table 2; $\chi^2 = 0.46$, $df = 1$, $\rho = 0.50$). Periphyton and surface water THg were positively correlated ($r = 0.53$, $S = 319.7$, $\rho < 0.05$), periphyton and surface water THg/OC were positively correlated ($r = 0.70$, $S = 206$, $\rho < 0.01$), and a statistically significant linear model ($V$ value $= 5732$, $\rho < 0.001$) between periphyton and surface water THg/OC molar ratios resulted in an intercept ($\log(c)$ value for Eq. 1) of 15.7. Periphyton $1/H_{THg/OC}$ varied from 0.11 to 0.16 exhibiting no difference between hotspot and non-hotspot stations ($\chi^2 = 0.58$, $df = 1$, $\rho = 0.45$). Based on the range of $1/H_{THg/OC}$ and an overall mean ($\pm$SE) of $0.14 \pm 0.004$, periphyton can be classified as homeostatic with respect to surface water THg concentrations.

### 3.3 Mosquitofish THg

Mosquitofish THg concentrations ranged from 8.45 to 312.00 ng Hg g$^{-1}$ with 46% of the samples (11/24) exceeding the USEPA criterion for the protection of piscivorous wildlife (USEPA 1997). All of the samples that exceeded the protection criteria were observed at hotspot stations. Mosquitofish THg concentrations were positively correlated with soil TC/TP ($r = 0.82$, $S = 102$, $\rho < 0.001$) and soil N/P ($r = 0.82$, $S = 100$, $\rho < 0.001$) and negatively correlated with soil C/N ($r = -0.70$, $S = 950$, $\rho < 0.01$) across hotspot and non-hotspot stations (Fig. 5). The associated data and resulting model using soil TC/TN, TC/TP, and TN/TP molar ratios to predict mosquitofish THg concentration in a multiple regression model framework conforms with the assumptions of linear models (H$_0$: Data fit assumption of linear models; Global Statistic: 5.53, $\rho = 0.24$). The multiple regression model of soil TC/TN, TC/TP, and TN/TP and mosquitofish THg concentrations resulted in statistically significant model with a high degree of certainty (Table 3; $R^2 = 0.89$, $F(3,11) = 30.99$, $\rho < 0.001$). Both soil TC/TN and TC/TP were statistically significant model terms; however, soil TN/TP was not a statistical significant parameter in the model (Table 3). Mosquitofish THg concentrations was positively correlated with periphyton and surface water $1/H_{THg/OC}$ ($r = 0.55$, $S = 306$, $\rho < 0.05$) irrespective of station designation. Additionally, periphyton THg and mosquitofish THg concentrations were positively correlated ($r = 0.48$, $S = 421.52$, $\rho < 0.05$).
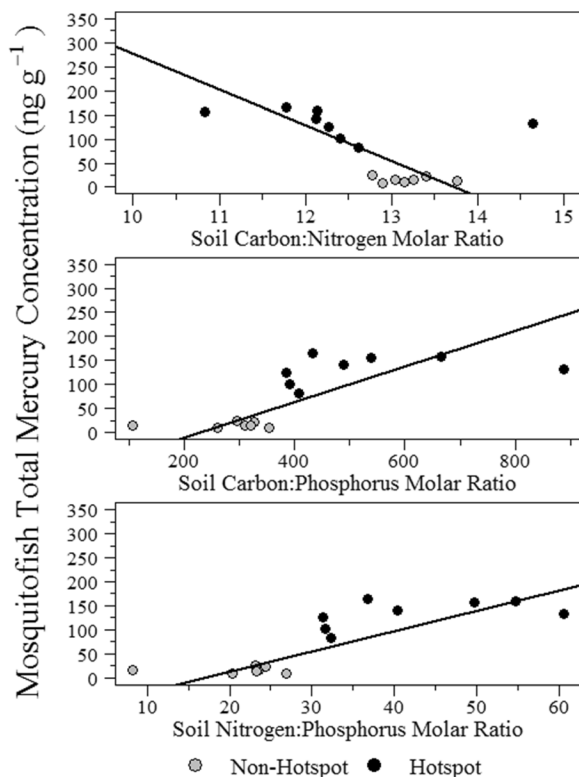
**Fig. 5** Mosquitofish total mercury concentrations by soil nutrient molar ratio with stations designations identified. *Lines* through data represent correlation trends identified by Theil-Sen estimates

## 4 Discussion

### 4.1 Confirmation of Hotspot Designation

No formal, data-driven criterion has been established to determine and designate Hg accumulation hotspots within natural ecosystems. However, some studies have utilized wildlife tissue threshold/criteria (Evers et al. 2007; Hutcheson et al. 2008) to define biological Hg

**Table 3** Mosquitofish total mercury and soil nutrient ratio multiple regression model results using data collected between 2010 and 2013 within the Everglades Protection Area and Stormwater Treatment Area-1W from stations identified as suspected mercury accumulation hotspots and non-hotspot stations

| Model term | Estimate | Std. error | $t$ value | $\rho$ value |
|---|---|---|---|---|
| Intercept | 1326.28 | 426.43 | 3.11 | <0.01 |
| Soil C/N molar ratio | −104.24 | 32.41 | −3.21 | <0.01 |
| Soil C/P molar ratio | 1.56 | 0.66 | 2.37 | <0.05 |
| Soil N/P molar ratio | −17.26 | 8.74 | −1.98 | 0.07 |

RMSE = 20.08, $R^2$ = 0.89, $F(3,11)$ = 30.99, $\rho < 0.001$

hotspots. Evers et al. (2007) suggests that biological Hg hotspots can be defined by concentrations exceeding established human or wildlife health criteria. There are important considerations in identifying and delineating biological Hg hotspots especially when the sources of Hg contamination are not easily differentiated across ecosystems. Ecosystems have unique characteristics that influence Hg transport, translocation, conversion, and accumulation in biota (Lange et al. 1993; Bodaly et al. 1993; Driscoll et al. 2007). In the Northeastern United States and Southeastern Canada, biological hotspots were identified using Hg data from several major taxa spread assessed against USEPA human health criteria advisory level for fish tissue. Secondary indicators based on statistically determined adverse-effect levels for several species within the study were also used to identify Hg accumulation hotspots (Evers et al. 2007). Biological hotspots identified by Evers et al. (2007) correlated to areas of elevated Hg deposition from far and near-field emissions. Similarly, Hutcheson et al. (2008) utilized a fish tissue dataset, depositional modeling projections, historical mercury accumulation records from lake sediments, and emission data to evaluate occurrences and drivers of biological Hg hotspots.

The current study evaluated suspected hotspots against both a soil quality guideline/threshold and a biological threshold. Soil quality guidelines allow for evaluation of ambient site conditions over a period of time. This study limited soil samples to the top 0–10 cm which accounts for Hg burial rates >92% of the annual deposition mass (Liu et al. 2008b) and represents recent Hg enrichment from atmospheric sources (Arfstrom et al. 2000). The application of the TEL SQG was deemed more applicable than other limits or concentrations (i.e., background) since a TEL is linked to the biology of indicator species. Assessing fish tissue against protection criteria represents an ecological hazard to higher (or equal) trophic status wildlife. Generally, soils collected from hotspot stations had the greatest number of data points observed above the TEL threshold although a couple of samples collected from hotspot stations were observed below the TEL which could be the result of high spatial variability in soil parameters (Osborne and Cohen, *unpublished data*). Therefore, a second level of evaluation in the form of fish tissue concentrations was used to further confirm hotspot status. Fish tissue samples collected during this study all were above the USEPA criterion at hotspot stations. Historic ambient monitoring data (Julian et al. 2015a) corroborate the hotspot designations confirmed in this study.

### 4.2 Wetland Soil Mercury Stoichiometric Relationships

Soil THg concentrations were consistent with other studies throughout the Everglades region (Stober et al. 2001; Scheidt and Kalla 2007; Liu et al. 2008b; Osborne et al. 2011) with hotspot stations having higher THg concentrations than non-hotspots. Soil THg concentrations are an integrated measure of surface water Hg transport and translocation, Hg atmospheric bulk deposition, throughfall and litterfall within a given ecosystem. Surface water concentrations of THg were relatively low and consistent with concentrations reported by others within the Everglades ecosystem (Stober et al. 2001; Scheidt and Kalla 2007; Julian et al. 2015a) (Table 2). Due to relatively low surface water Hg concentrations, THg surface water loads are relatively low (ca. $0.51 \pm 0.07$ µg Hg m$^{-2}$ year$^{-1}$) when compared to loads from wet deposition (Julian et al. 2015a). Atmospheric deposition is the predominate source of Hg to the Everglades ecosystem with greater than 95% of the Hg load to the Everglades being attributed to atmospheric wet deposition (Florida Department of Environmental Protection 2013). Annual fluxes of Hg throughfall (26 µg Hg m$^{-2}$ year$^{-1}$) within the Everglades ecosystem is similar to wet (23–24 µg Hg m$^{-2}$ year$^{-1}$) and bulk (22–23 µg Hg m$^{-2}$ year$^{-1}$) atmospheric deposition of THg (Guentzel et al. 1998). A recent study determined Hg flux throughfall from cattail and sawgrass within the EPA to be 0.13 and 0.33 µg Hg m$^{-2}$ year$^{-1}$, respectively (F. Dierberg, *unpublished data*). Over a 20-year period, annual fluxes of wet Hg deposition have ranged from 10 to 21 µg Hg m$^{-2}$ year$^{-1}$ (Julian et al. 2015a) which correspond to those observed elsewhere (Guentzel et al. 1995, 1998, 2001). Litterfall Hg flux rates from the Everglades ecosystem have been estimated to range between 1 and 40 µg Hg m$^{-2}$ year$^{-1}$ (Guentzel et al. 1998). Additionally, using cattail and sawgrass THg tissue data (F. Dierberg, *unpublished data*) combined with estimated litterfall (Appendix 1 Table 1), Hg litterfall flux rates for cattail and sawgrass can range between 7 and 109 µg Hg m$^{-2}$ year$^{-1}$, depending on species, site conditions, and state of litter (i.e., live versus sensence). As a result of Hg source dynamics within the Everglades ecosystem, Hg accumulation in soils is a combination of new (direct bulk deposition onto soil) and old Hg (throughfall). Ecological characteristics such as vegetation type, site-specific productivity (Appendix 1, Table 1), and litter decomposition rates (Appendix 1, Table 2) could drive the large disparity of

soil THg concentrations between hotspot and non-hotspot stations. Generally, vegetative productivity can vary dramatically between hotspot and non-hotspot stations in that biomass/litterfall can range from 3405 to 4865 kg m$^{-2}$ year$^{-1}$ in non-hotspot stations (i.e., cattail) and 208 to 740 kg m$^{-2}$ year$^{-1}$ in hotspot locations (Appendix 1, Table 1). Juxtaposed ecosystem features such as rookery tree islands and the associated wading bird feeding excretion and wash-out could be an additional source of THg (Zhu et al. 2014), as proximity to these features could also influence soil THg concentrations further driving the differences between stations.

Lignocellulose index has been used as a proxy for litter decomposition in soils with higher values resulting in more recalcitrant organic matter. Lignin is relatively resistant to microbial decomposition since degradation requires an additional de-lignifying step before hydrolysis (Melillo et al. 1989). Lignocellulose index can also signify the relative age of soils due to the differences between lignin and cellulose along the decay continuum (DeBusk and Reddy 1998). In this study, higher LCI values corresponded with lower THg/TC ratios. Upon further examination of this relationship, LCI was positively correlated with soil C concentrations due to the decomposition of litter and the net accumulation of C. Organic matter and C accumulation varies across the landscape within the Everglades ecosystem (Appendix 1, Table 3). More specifically, C accumulates at a rate of $215.5 \pm 37$ g C m$^{-2}$ year$^{-1}$ in high nutrient areas and $123.7 \pm 21$ g C m$^{-2}$ year$^{-1}$ (Reddy et al. 1993), with 15 to 24% of the accumulated C being buried when accounting for net annual primary production (Craft and Richardson 1993). Meanwhile, soil THg concentrations are negatively correlated with LCI indicating that older soils have less THg than younger soils due to volatilization or evasion, translocation from soil to surface water, burial or methylation biotic uptake, and transport of Hg (St. Louis et al. 1996; Hintelmann et al. 2002; Liu et al. 2008a).

Litterfall can play a significant role in Hg flux dynamics to the soil. Flux of Hg from litterfall to soil is a significant pathway in forested ecosystems, especially deciduous forests (Demers et al. 2007). The role of litterfall and its decomposition have a duel role by altering soil organic matter and contributing THg to the soil. Litterfall initially increases both C and N in soil as biomass decomposes which can alter organic matter characteristics. This control of organic matter characteristics can cause a cascade of biogeochemical reactions

including influencing the THg binding capacity of organic matter (Ravichandran 2004; Aiken et al. 2011a; Demers et al. 2013). Furthermore, litter N is known to be conserved during the decomposition process ultimately enriching the soil below, but C is lost due to microbial degradation, assimilation, and respiration typically as carbon dioxide. Therefore, as litterfall progresses through the decay continuum, soil N concentrations increase, soil TC/TN ratios decrease (Melillo et al. 1989), and thereby THg is transported to the soil and accumulates (Åkerblom et al. 2007; Obrist et al. 2009). Decomposition of litter also provides organic substrate to facilitate Hg methylating microbes and promotes an anoxic environment by consuming dissolved oxygen during decomposition. Much like relationships observed in forested ecosystem, litterfall in wetlands plays a dual role. During this study, soil THg was positively correlated with soil N and negatively correlated with soil C/N ratios, which suggests that a mechanism of initial THg enrichment through the decomposition of leaf litter and biotic mechanisms was involved in the breakdown of litter. As litter and soil age, THg is translocated. Soil N and C concentrations are qualitatively higher at hotspot stations relative to non-hotspot stations (Table 2) which could provide some clarity for explaining differences in Hg dynamics between hotspot and non-hotspot stations within the Everglades ecosystem.

Soil C/P and N/P molar ratios provide an indication of trophic status and limiting nutrients (Sinclair et al. 2000; Cleveland and Liptzin 2007). During this study, hotspot stations were observed in oligotrophic portions of the marsh that experience low surface water nutrient concentrations (Julian et al. 2015b) which has resulted in relatively low soil TP concentration (this study) relative to non-hotspot stations (Table 2). Oligotrophic areas of the marsh are typically characterized by extensive sawgrass sloughs. Non-hotspot stations occurring in eutrophic or mesotrophic portions of the marsh with moderate to high surface water concentrations had relatively elevated soil TP concentrations (Julian et al. 2015b) (Table 2). Cattail stands typically occupy eutrophic portions of the marsh and serve as an indicator of nutrient enrichment (Newman et al. 1998). As a result, soil C/P and N/P ratios are elevated and more variable in hotspot locations relative to non-hotspots (Fig. 2), which could indicate that differences in trophic structure, the source of litterfall (i.e., cattail versus sawgrass), or decomposition dynamics contribute to the formation of Hg hotspots.

### 4.3 Periphyton Mercury: Carbon Homeostasis

Periphyton THg concentrations observed in this study were consistent with other studies throughout the Everglades region (Stober et al. 2001; Scheidt and Kalla 2007; Liu et al. 2008b), with very little overall difference between hotspot and non-hotspot stations (Table 2). Cleckner et al. (1998) observed large spatial differences in periphyton THg within the Everglades ecosystem along trophic gradients from several stations including sites resampled as a part of this study (i.e., WCA2F1, WCA2U3, and WCA315). Cleckner et al. (1998) noted that at some stations a simple partitioning of Hg between periphyton and the water column was not apparent with large differences in concentrations between these two compartments. In this study, surface water and periphyton THg concentrations were significantly correlated with no consistency among stations. This partitioning of THg between periphyton and surface water is of importance since periphyton is the base of the Everglades foodweb (Davis and Ogden 1994) and marks an entry point of Hg into the trophic cascade and suggests that trophic transfer is a key for the formation hotspot.

Several studies have demonstrated that periphyton biomass and tissue THg have an asymptotic relationship at which point periphyton biomass become "saturated" with Hg (Pickhardt et al. 2002; Bell and Scudder 2007). In vitro and in situ studies did not find this consistent relationship (Stober et al. 2001; Moye et al. 2002; Scheidt and Kalla 2007). The use of OC in this study relative to THg concentrations with respect to periphyton is twofold, as periphyton OC was used to indicate periphyton biomass and account for the role that OC plays in complexing with THg (Skyllberg 2008; Skyllberg and Drott 2010; Aiken et al. 2011b). The homeostatic stoichiometric relationship between surface water and periphyton THg/OC presented in this study may provide additional clarity to the partitioning of Hg between periphyton and surface water observed by Cleckner et al. (1998) in that OC aids in regulating THg between the two partitions.

The concept of stoichiometric homeostasis has been applied to a variety of trophic levels from algae, vascular plants, invertebrates, and vertebrates to investigate nutrient dynamics (Vitousek 1984; Sterner and Lampert 1998; Vanni 2002; Yu et al. 2011; Feijoó et al. 2014). Based on the $1/H_{THg/OC}$ values reported in this study, periphyton was considered homeostatic relative to surface water. Periphyton maintains a constant

stoichiometric relationship with surface water relative to THg. The stoichiometric THg/OC relationship presented in this study also suggests that OC (proxy for organic matter) may, in part, regulate the availability of THg for uptake which is consistent Aiken et al. (2011b). Once THg enters the periphyton matrix, it either remains unreactive due to complexation with the abundance organic matter, ions, or macro-biomolecules present or THg can then enter into several mercury methylation pathways depending on the dominate Hg methylation microbes present (sulfate-reducing bacteria, iron-reducing bacteria, and methanogens) (Cleckner et al. 1999; Hagerthey et al. 2011; Hamelin et al. 2011). However, the fate or effect of Hg in the periphyton matrix is not clear and more study is needed.

### 4.4 Mosquitofish Hg Relationship

Mosquitofish THg concentrations observed in this study were consistent with other studies throughout the Everglades region (Stober et al. 2001; Scheidt and Kalla 2007; Liu et al. 2008b), with large observed differences in concentrations between hotspot and non-hotspot stations. Several hypotheses regarding the differences in mosquitofish Hg concentrations between hotspot and non-hotspot stations have been proposed including differences in trophic and habitat dynamics between stations and bio-dilution of Hg due to higher growth rates and overall size due to excessive nutrients (Bates et al. 2002; Karimi et al. 2007, 2010; Abbey-Lee et al. 2013; Julian et al. 2014, 2015a, 2016). It is likely that a combination of hypotheses would be appropriate as soil nutrient ratios were significantly correlated and well explained by mosquitofish THg concentrations (Fig. 5 and Table 3) better than water column metrics (Liu et al. 2009; Julian 2013, 2014). While soil is a long-term integrator of ecosystem inputs, soil nutrient ratios can indicate both a relative trophic level indicator (Grevilliot et al. 1998; Sinclair et al. 2000) and the degree of nutrient limitation (Cleveland and Liptzin 2007) by influencing bottom-up trophic interactions influencing site-specific trophic cascades. For example, stations with higher soil TP concentrations would have a lower soil C/P and N/P molar ratios in P limited areas.

Mosquitofish are known to be meso-consumers with a highly variable and diverse diet (Loftus 2000; Blanco et al. 2004). Mosquitofish diets are influenced by food availability, niche partitioning, and seasonal influences of marsh processes resulting in spatial variation in food-

chain length resulting in differences of biomagnification (Williams and Trexler 2006; Abbey-Lee et al. 2013). Therefore, two distinct pathways of Hg uptake are possible through direct uptake of THg or MeHg from the water column or dietary bioaccumulation. Pickhardt et al. (2006) demonstrated that the majority of Hg accumulated in mosquitofish was derived from dietary bioaccumulation, although a small fraction was derived from water column uptake. As a part of its diet, mosquitofish will feed on periphyton when present. In this study, it was determined that the degree of homeostasis of Hg between water column and periphyton as indicated by $1/H_{\text{THg/OC}}$ significantly influences mosquitofish THg tissue concentration suggesting that periphyton is an important vector of dietary sources of Hg to mosquitofish. Factors that influence the availability of Hg and the potential mechanism(s) of uptake/exchange of Hg with respect to periphyton need to be investigated further.

## 5 Conclusions

Based on this study, several factors can explain the development and persistence of Hg hotspots within wetland ecosystems by evaluating several different pathways of the formation of Hg accumulation hotspots. The use of soil and biological criteria/guidelines confirmed the status of long standing suspected Hg accumulation hotspots originally designated through the use of only fish tissue ecological and human health protection criterion. Mercury enters the Everglades ecosystem largely from uniform atmospheric deposition; however, areas of elevated Hg in several ecological compartments (soil and fish) persist. Initial hotspot formation could be linked to deposition and degradation of litterfall which provides an initial pulse of THg and is driven largely by vegetation dynamics with different vegetative communities (i.e., cattail versus sawgrass) potentially contributing variable fluxes of THg to the soil surface from litterfall. As litter and soil age, soil THg concentrations decrease presumably due to evasion, translocation, or transformation processes leaving "old" recalcitrant THg behind.

Other biotic controls of hotspot formation or persistence could be linked to the interaction of periphyton and surface water THg. Periphyton is an important component to the trophic structure of the Everglades marsh ecosystem and is a viable entry point of Hg into the trophic cascade. Therefore, periphyton THg

accumulation and trophic transfer of Hg to higher organisms could explain factors involved in hotspot formation. Food-chain length and periphyton are important vectors of dietary sources of Hg to mosquitofish, but further study is needed to further investigate the role of periphyton and other drivers of Hg trophic transfer.

A significant amount of effort has been placed in studying Hg dynamics within the Everglades ecosystem, but the picture is still not complete. Mercury accumulation, MeHg formation, and bioaccumulation are a complex issue with several confounding factors. Further study is needed to elucidate hotspot formation, persistence, and trophic transfer of Hg with an emphasis on food-chain length, trophic continuum, and the interaction of ecological components that contribute to Hg accumulation in the soil.

**Compliance with Ethical Standards**

**Conflict of Interest**    The authors declare that they have no conflict of interest.

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