Trophic transfer of methyl mercury in the northern Florida Everglades

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Key words: Everglades, Hg transfer, methyl mercury, periphyton

Abstract. There are spatial differences in methyl mercury (MeHg) concentrations in biota in Water Conservation Areas 2 and 3 in the Everglades, with higher concentrations generally found in the southern areas. Fish and hemipterans had the most MeHg on a wet weight basis, with levels exceeding 30 ng g⁻¹. The magnitude of MeHg accumulation in biota varies seasonally and does not always appear to be associated with changes in water column concentration. This is exemplified by periphyton, the base of the foodweb in the Everglades, at a high nutrient sampling site. Although limited in scope, MeHg concentrations presented for biota provide insight into beginning to understand the dynamic nature of Hg transfer in the Everglades foodweb on a spatial and temporal basis.

Introduction

Fish consumption advisories have been issued for Southern Florida, including the Everglades, due to high mercury (Hg) levels in sport fish such as largemouth bass (*Micropterus salmoides*) (Ware et al. 1990). In addition, deleterious health effects in wading birds, alligators and the Florida panther have been associated with high Hg levels (Spalding et al. 1994; Roelke et al. 1991; Sundlof et al. 1994; Sepulveda et al. 1995). Since these animals can derive a significant portion of their diet from aquatic organisms, it is important to determine how Hg is bioaccumulating through the trophic structure of this region.

The propensity or ability of Hg to increase concentration several orders of magnitude from surface waters to fish is well documented (Driscoll et al. 1994; Watras et al. 1994; Hudson et al. 1994), although the exact processes governing bioconcentration and bioaccumulation are not yet fully understood. It is known, however, that phytoplankton and zooplankton play an inter-

mediary role in the bioconcentration and bioaccumulation of Hg (Back et al. 1995; Mason et al. 1996).

The trophic structure of the Everglades, and wetlands in general, is much different from lacustrine and marine systems. In the Everglades, periphyton serves as the base of the food web rather than phytoplankton (Browder et al. 1994). Invertebrates associated with this periphyton, in addition to zooplankton, provide a link between primary producers and larger fish such as sunfish (*Lepomis* spp.) and largemouth bass (*Micropterous salmoides*). These links potentially include freshwater shrimp (*Palaemonetes paludosa*), amphipods (*Hyalella* spp.), mayfly nymphs, mosquitofish (*Gambusia* sp.), least killifish (*Heterandia formosa*) and bluefin killifish (*Lucania goodei*) (Browder et al. 1994).

A limited number of studies have measured Hg concentrations in insects and invertebrates other than zooplankton (Jackson 1988; Parkman & Meili 1993; Tremblay et al. 1996; Suchanek et al. 1995; Bodaly et al. 1996; Hall et al. 1996; Beverly et al. 1996; Rask et al. 1994). Of these, only a subset have measured methyl mercury (MeHg) (Parkman & Meili 1993; Tremblay et al. 1996; Bodaly et al. 1996; Hall et al. 1996). Since this is the form of Hg that is most toxic and bioaccumulates most readily, it is important to determine MeHg levels to begin to understand how Hg is transferred through the food web. Measurements of MeHg concentrations for organisms in the FL Everglades other than fish, which is assumed to be 95%+ of total Hg (Hg_T), are virtually non-existent. Thus, little is known about Hg transfer through the trophic structure in the Everglades, and wetlands in general (Zillioux et al. 1993).

A component of the U.S. Geological Survey's Aquatic Cycling of Mercury in the Everglades (ACME) project (Krabbenhoft 1996; Hurley, this issue) is focused on determining the bioconcentration and bioaccumulation of MeHg and Hg_T in the lower levels of the food web in the northern Florida Everglades. The specific objectives are (1) to measure methyl and total Hg in biota from primary producers to small fish and (2) to determine spatial and temporal changes in these Hg levels in the food web of the Everglades. This paper represents the preliminary results from this investigation and focuses mainly on the first year of data collection.

Methods

Sampling sites

The primary study areas for this investigation are Water Conservation Areas (WCA) 2 and 3 located between the Everglades Nutrient Removal Area and

Everglades National Park (Figure 1). Characteristics of the specific study sites are described in more detail by Hurley et al. (this issue). Samples were collected in July and December 1995 from fixed South Florida Water Management District sites F1, F4 and U3. More sites such as 2BN and 3A-15 were added in December as the sampling effort progressed to more southern locations. Due to the limited number of fish that were collected in 1995, additional data from fish sampling in December 1996 at these same locations are also presented, when significantly more fish were collected.

The northern part of WCA 2 is eutrophic and very different from the rest of the Everglades with respect to nutrients, dissolved anions and cations, and vegetation (McCormick et al. 1996). The dominant vegetation at F1 is cattails (*Typha domingensis*) which have replaced the native sawgrass (*Cladium jamaicense*) found at U3 and the rest of the Everglades. Also, the algae comprising the periphyton shift from eutrophic types in the north to oligotrophic species in the south (McCormick et al. 1996)

Field collection

All sampling was completed using clean techniques (Patterson & Settle 1976; Fitzgerald & Watras 1989). For water, filtered samples were collected in Teflon (any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government) bottles using a peristaltic pump with acid-cleaned C-flex pumphead tubing and Teflon sampling line (Hurley et al. this issue) with an in-line 0.45 μ m Calyx capsule filter (pre-cleaned by filling with 50% HNO₃ for two days, rinsing with Milli-Q water, filling with 50% HCl for two days, rinsing, and filling with Milli-Q water until use). Water samples were frozen until analysis. Periphyton, defined as a free-floating mat or film, was collected with gloved hands and placed into widemouth Teflon bottles. An effort was made to remove detritus and/or any visible invertebrates. Splits of the periphyton were saved for pigment analysis and identification.

For invertebrate and fish sampling, organisms were collected using a non-metallic Nitex dip net with a mesh size of 153 μ m. Many different substrata were sampled at each site including the stems and undersides of vegetation as well as larger particulate matter in the water column. The material collected in the net was put into 1-L Teflon jars in the field and diluted with water from the site.

Field processing

Material from the 1-L jars was placed into Teflon petri dishes under a HEPA-filtered clean hood. Organisms were visually separated using Teflon-coated

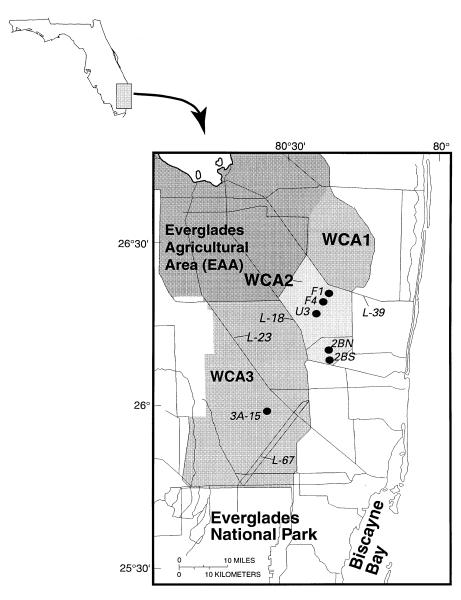


Figure 1. Location of study area in South Florida with sampling sites in WCA 2A, WCA 2B and WCA 3A.

forceps and placed into smaller Teflon petri dishes within 48 hours of collection. Once all the material from a site was examined, individual organisms or groups of similar organisms from the same taxa were placed into Teflon vials and frozen until analysis. For the invertebrates, one to two organisms

were collected from each site due to their typically low density in the Everglades, with the exception of amphipods where at least 10 organisms were collected. The Everglades is a very oligotrophic ecosystem and does not support a large amount of biomass, particularly at the primary consumer level. Also, water conditions are variable within a year and between years which makes it difficult for many organisms to be collected from the same sites over time.

Analysis

For analysis, whole organisms were prepared by weighing wet to the nearest 0.01 mg. No grinding was completed as total homogenization of the organisms occurred during acid digestion (Hg_T analysis) or distillation (MeHg analysis). Since there were only one to three organisms per site for each sampling time, analysis was completed on individuals rather than composites for all biota but the amphipods which were analyzed in groups of 5 to 10. Thus, either a MeHg or Hg_T measurement was completed for individual organisms.

Total Hg. Pre-weighed (to the nearest 0.01 mg) organisms were placed in Teflon bombs to which 7 ml of a mixture (5:2, v/v) of trace metal grade HNO₃ and H₂SO₄ were added. Fish and shrimp were analyzed for Hg_T because preliminary experiments showed that the levels of MeHg and Hg_T were approximately equal in these organisms. Bombs were sealed tightly and microwaved for a period long enough to dissolve biotic tissue. This was typically two rounds of heating at 20% power in a 900 watt microwave oven for two minutes each. Bombs were allowed to cool to room temperature between heating cycles. After organisms were dissolved, 23 ml of Milli-Q (Millipore) water were added to each bomb. Blanks consisted of the acid mixture microwaved for the same amount of time to which 23 ml of Milli-Q were added.

For analysis, an aliquot of digestate was placed into a bubbler with 0.5 ml SnCl₂ and analyzed using the dual amalgamation, cold vapor atomic fluorescence spectroscopy (CVAFS) technique described by Bloom & Crecelius (1983). Typically, 5 to 10 ml of the mixture were analyzed for each bomb in duplicate. The mean percent difference of the measurements was less than 10%.

In order to determine whether the microwave method was sufficient to extract Hg_T from biotic samples, a NIST reference material (8406), Tennessee River sediments, was analyzed using the methodology described above. While this is not the same matrix found in biological samples, it is probable that this sediment is a more difficult matrix from which to extract Hg_T . Also, the Hg_T concentration of these sediments is close to the levels of Hg_T that were

measured during this investigation. The results of the analysis of the sediment were within 10% of the nominal value of 60 ng $\rm g^{-1}$.

Methyl Hg. Methyl mercury in water and biota was analyzed by the distillation/ethylation method of Horvat et al. (1993). A more complete description of the quality assurance procedures as well as the precautions taken to circumvent matrix interference effects in water for the ACME project can be found in Hurley et al. (this issue) and Olson et al. (in press). Briefly, 100 ml of water were distilled for each sample and distillation blanks were completed for each round.

For biota analysis, pre-weighed (nearest 0.01 mg) organisms were placed whole into Teflon jars with 0.2 ml KCl, 0.5 ml $\rm H_2SO_4$ and approximately 50 ml of Milli-Q water, and distilled using the method of Horvat et al. (1993) and Liang et al. (1994). Blanks, consisting of reagents and Milli-Q water, were subtracted from the sample distillations. Distillates were ethylated with 100 μ l of sodium tetraethylborate (NaTEB) and analyzed using gas chromatography separation and CVAFS detection. Bubbler spikes typically consisting of 50 pg of MeHg were completed to ensure that there was enough NaTEB present to ethylate MeHg in samples. A reference material, dogfish muscle (DORM-2), was analyzed using this technique. The analyzed concentration from our laboratory was within the confidence limit of the certified value, 4.47 ± 0.32 ng g $^{-1}$.

Pigments. In order to determine the major types of algae comprising the periphyton mats, chlorophyll a, bacteriochlorophylls a and d, and accessory pigments were determined for the periphyton samples. A known weight of periphyton was extracted with 90% acetone, and the pigments were quantified using a high performance liquid chromatographic technique (Van Heukelum et al. 1994; Hurley & Watras 1991).

Results and discussion

Organisms collected

The most common and numerous biota sampled include periphyton, amphipods (*Hyalella* spp.), hemipterans such as gator fleas (*Pelocoris* sp.) and belastomatids (*Belastoma* sp.), odonates, freshwater shrimp (*Palaemonetes paludosa*) and small fish (*Gambusia* sp., *Heterandia formosa, Lucania goodei*). The Everglades does not support a diverse assemblage of organisms due to changing water conditions (Lodge 1994). A similar variety of biota was collected from sites for the various sampling periods based on a presence

or absence during different sampling periods. Thus, there do not appear to be large changes in biotic communities sampled between sites and over time. While a large number of specific organisms were difficult to collect, these analyses represent some of the first MeHg data on important trophic levels in the Everglades food chain. Initial data on food chain Hg bioaccumulation, in turn, has led to more detailed studies during this ongoing investigation.

Biota concentrations along north-south transect

The concentrations of Hg in filtered water, periphyton and fish from ACME sampling sites in December 1995 are plotted in Figure 2a. Data are plotted from this month since this was the first sampling period when samples were collected along a large spatial gradient including WCA 2B and WCA 3A. In general, there is an increase in Hg concentration in all three components as one moves from north to south in the study area. A similar pattern of increasing Hg levels has been observed by other investigators in this region (Stober et al. 1995; Hurley et al., this issue; Gilmour et al., this issue). There are exceptions to this trend, however. The MeHg level in periphyton at F1 is higher than the more southern site, F4, and is similar in magnitude to the WCA 2B sites. The fish Hg concentration at 2BS is lower than at U3 and 2BN. Nonetheless, Hg levels in water, periphyton and fish were highest at the most southern site, 3A-15. This particular location has been identified as having high fish Hg concentrations by other investigators (Stober et al. 1996), but the exact mechanisms have not been identified. Since there were few fish collected in December 1995, additional fish data are presented for December 1996 when more fish were collected (Figure 2b). Once again, F1 fish had the lowest Hg concentration of the sites while 3A-15 fish were highest in Hg levels.

Looking at only the periphyton from December 1995, there is an increase in percent of Hg_T as MeHg as one moves from north to south in the study area (Table 1). This increase in percent of Hg_Tas MeHg is also observed in sediments (Gilmour et al. this issue), although the magnitude of the percent of Hg_T as MeHg in periphyton is higher than the sediments from the same sites. Sites in WCA 2B do not follow a consistent increase in percent of Hg_T as MeHg for periphyton. However, this area is hydrologically separate from WCA 2A, as the main source of water to 2B is precipitation rather than sheet flow from WCA 2A. Inconsistencies in surface water Hg dynamics along this north to south sampling transect, and in particular WCA 2B, have also been noted (Hurley et al. this issue).

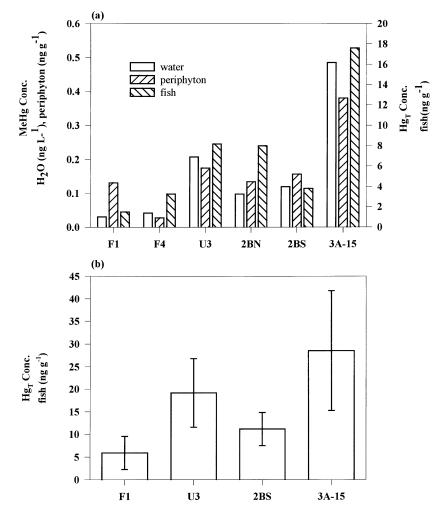


Figure 2. (a) Mean concentrations of MeHg in filtered water and biota along north–south sampling transect in December 1995 ($n \le 3$ for all organisms except amphipods). Water is presented as ng L⁻¹ while biota are ng g⁻¹ on a wet weight basis. (b) Mean concentrations of Hg_T in fish (ng g⁻¹ wet weight) along selected sites of north–south sampling transect in December 1996 (n = 10). Error bars are one standard deviation.

Comparison of Hg in biota from F1 and U3

Since there are limited biota data available for all sampling sites over time, we focus on two contrasting locations to compare Hg dynamics of periphyton and water seasonally as well as Hg accumulation in organisms representing different trophic levels of the Everglades.

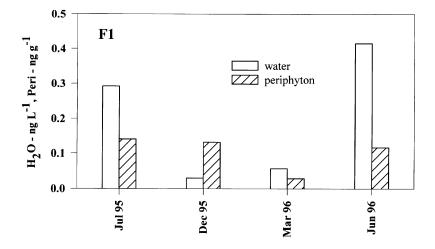
Table 1. Concentrations of methyl and total Hg (ng g⁻¹ wet weight) in Everglades periphyton along north–south sampling transect in December 1995.

Site	MeHg (ng g ⁻¹)	HgT (n g ⁻¹)	% of Hg T as MeHg
F1	0.13	4.02	3.3
F4	0.03	2.09	1.2
U3	0.17	0.77	22.6
2BN	0.14	1.38	9.8
2BS	0.16	1.85	8.4
3A	0.44	1.60	27.5

Seasonal Hg dynamics. The relationship between MeHg in the water column and periphyton for sites F1 and U3 is seasonally dynamic (Figure 3). At U3, the highest levels of MeHg in water and periphyton were seen in March 1996, while at F1, highest MeHg concentrations were observed in the summer months, July 1995 and June 1996. There have been very few recent studies of MeHg levels in periphyton (Simmons 1994), although in earlier work periphyton was shown to be an indicator for Hg levels in fish, but not necessarily water (Stokes et al. 1983). There appear to be differences in the relationship between water and periphyton MeHg levels between sites F1 and U3, as there does not appear to be a simple partitioning of MeHg from the water to the periphyton at F1. This has implications for entrance of MeHg into the food web since periphyton is the base (Browder 1994). A continuing focus of the ACME project will be to determine what site-specific processes might be occurring to influence these differences.

Trophic accumulation. Levels of Hg in biota from F1 and U3 in July 1995 and March 1996 are presented in Figure 4. There were no hemipterans or shrimp collected at U3 and F1, respectively, in July 1995. Generally, there are few hemipterans present at a site since they occupy a high position in the trophic structure of this ecosystem. In July, water and periphyton MeHg concentrations were similar at the two sites (Figure 4), but there were vast differences in MeHg levels in primary consumers (amphipods, shrimp, fish) and predators (hemipterans). Organisms at site U3 had much higher Hg concentrations than F1. In fact, the fish Hg levels increased by a factor of about 200 from the periphyton (wet weight basis) at U3. This is a valid comparison since the small fish in the Everglades consume periphyton as well as some zooplankton (Browder 1994).

At U3 in July, fish had the highest concentration of Hg of any organism, while hemipterans at F1 were highest. Predatory insects such as hemipterans



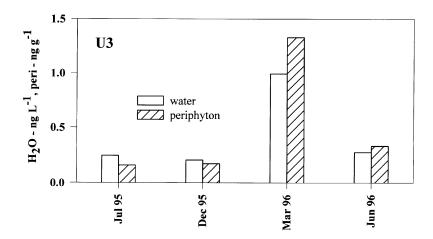


Figure 3. Seasonal changes in MeHg levels in filtered water and periphyton at sites F1 and U3. Water is presented as $ng L^{-1}$ while periphyton is $ng g^{-1}$ on a wet weight basis.

have been found to have high levels of MeHg relative to other invertebrates by researchers in the Experimental Lakes Area (Bodaly et al. 1996; Hall et al. 1996). It is likely that hemipterans are also at a higher trophic position than the small fish collected during this study as reflected by their relative Hg concentrations. It has been reported that hemipterans eat small fish in the Everglades (Lodge 1994).

In March 1996 (Figure 4), U3 organisms had higher Hg levels than those from F1 for all trophic levels examined, which is in contrast to July when the

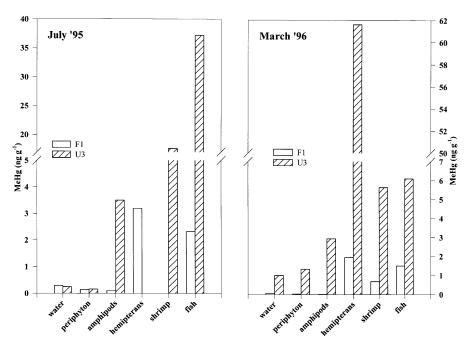


Figure 4. Mean levels of MeHg in organisms from different trophic levels at sites F1 and U3 for December 1995 and March 1996 ($n \le 3$ for all organisms except amphipods). Water is presented as ng L⁻¹ while periphyton is ng g⁻¹ on a wet weight basis.

periphyton and water MeHg levels at F1 and U3 were similar. The fish concentrations at U3 were only about 5 times higher than periphyton. This may be partly due to the fact that the periphyton MeHg levels were approximately 4 times higher in March compared to December. Also, small numbers of fish (n < 3) were analyzed for these sites so there may be an inherent variability of fish Hg levels within sites not determined from this investigation. Another possible explanation may be that different generations of fish are being collected from the sites over time since small fish of the Everglades have short life spans (Loftus & Eklund 1994), typically less than six months. Finally, a shift in diet may be contributing to differences in fish Hg levels over time. As the hydrologic conditions change, small fish diets change to take advantage of the food available. In the winter months, Gambusia sp. will shift to periphyton for food while in the summer they eat more zooplankton (Browder 1994). Finally, there may be a time lag associated with incorporating MeHg into the food web. Since December periphyton and water levels were relatively low at U3 (Figure 3), it is possible that the fish collected in March were eating food lower in Hg for the preceding months. It is not known how long it takes for Hg to bioaccumulate in Everglades organisms.

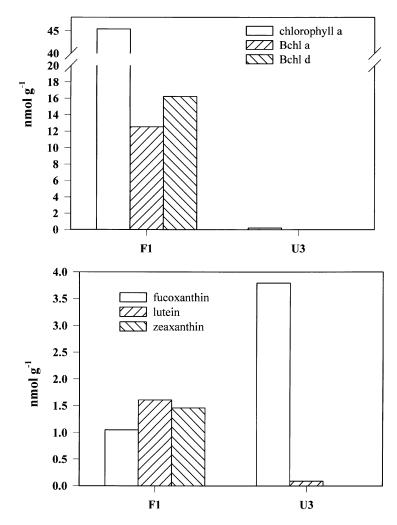


Figure 5. Chlorophylla and accessory pigments (nmol g^{-1} wet weight) of periphyton collected at sites F1 and U3 in December 1995.

It is possible that the types of periphyton available at a particular site are related to the accumulation of Hg in the upper trophic levels. The base of the food web, periphyton, is very different between the two sites, with higher chlorophyll a levels found at the more eutrophic F1 (Figure 5). Also, accessory pigments reveal the presence of green (lutein), blue-green (xeathanthin) and golden brown (fucoxanthin) algae at F1 while the predominant type at U3 is golden brown (Figure 5). In addition, the presence of bacteriochlorophylls a and d at F1 indicates that there are sulfide oxidizing bacteria and, thus, anoxic conditions at times. It is known that golden brown algae, and more specifically

diatoms, are a preferred food source for grazing invertebrates relative to the harder-to-digest blue-greens and greens. Thus, at U3, periphyton may be assimilated into the trophic structure more efficiently and more often leading to higher Hg levels in consumers and predators.

Conclusions

This paper presents some of the first measurements of MeHg in organisms in the lower levels of the food web of the Florida Everglades. There are discernible trends in biota Hg concentrations between sampling sites in December 1995 and 1996 with an increase in Hg concentrations when moving from north to south in the study area. However, over time, levels of MeHg and Hg_T in biota can change dramatically at the same site. These changes are not always related to changes in water and periphyton concentrations of MeHg. Possible explanations include: a time lag associated with uptake into the food web, the short life span of small fish, a shift in diets and the quality of food available for consumers. The data presented here are preliminary but provide an initial look at how Hg is transferred and biomagnified in organisms that are a major component of the food web of the Everglades.

Acknowledgements

Funding for the Aquatic Cycling of Mercury in the Everglades is provided by the U.S. Geological Survey, South Florida Ecosystem Program. The authors are grateful for the field and laboratory support efforts of Tim Heelan, Sue King and Jennifer Knepel. Larry Fink, Pete Rawlik and Kim Jacobs of South Florida Water Management District provided logistical assistance. The authors thank two anonymous reviewers for critically reviewing this manuscript.

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