Mercury Concentrations in Fish from Treatment Wetlands in the Northern Everglades

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Abstract

In comparison to values reported elsewhere, mercury concentrations in mosquitofish (*Gambusia affinis)* from the Everglades Nutrient Removal Project, a constructed wetland, are relatively low (<40 ng/g, wet weight). However, mercury concentrations in mosquitofish from Cell 4, an area dominated by periphyton, are significantly higher (<16 ng/g wet weight, on average) than those from Cell 3, an area dominated by macrophytes (<5 ng/g wet weigh, on average). Gut content studies suggest that mosquitofish from Cell 4 consume more animal material in comparison to Cell 3 mosquitofish (31% by volume and 22% by volume, respectively). Additionally, the ratios of animal species consumed are different with the Cell 3 mosquitofish diet being dominated by ostracods, chironomids and rotifers (57%) while the Cell 4 mosquitofish diet wax dominated by dipterans, copepods and leeches (95%). Despite these differences, upper trophic level species such

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as bass and sunfish do not show the same mercury concentration patterns. Thus the differences at lower trophic levels do not appear to cascade up the food chain, and thus may not be ecologically significant.

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Executive Summary

Quarterly from June 1996 until September 2000, staff of the South Florida Water Management District collected mosquitofish (*Gambusia affinis)* from Cells 3 and 4 of Stormwater Treatment Area 1 West (STA-1W) and analyzed them for mercury concentrations. In comparison to values reported elsewhere, mercury concentrations in STA-1W mosquitofish are relatively low. However, mercury concentrations in mosquitofish from Cell 3, an area dominated by macrophytes were on average less than half the concentrations from Cell 4, an area dominated by submerged aquatic vegetation (SAV) and periphyton. Studies carried out in the Everglades suggest that periphyton may be an important source of mercury to Everglades fish and invertebrates. This study suggests that the ratio of SAV/periphyton habitat to macrophyte habitat may affect mercury concentrations in biota. Additionally, examination of the gut contents of mosquitofish revealed differences in feeding behaviors between the two cells.

Given the differences in mercury concentrations in mosquitofish between cells, it was theorized that a similar pattern would emerge among large fish. Analysis of sunfish and largemouth bass do not show large fish to be higher in mercury concentrations in Cell 4 compared to Cell 3. The lack of a difference in larger fish may be explained by the fact that mosquitofish are not a major portion of the sunfish or largemouth bass diet and thus do not have a significant impact on the mercury concentrations of these predators.

While it appears that periphyton habitat effects mercury concentrations in mosquitofish through methylation and food web structure, these differences do not appear to cascade up to predatory species. Consequently it is suggested that sportfish may be deriving their mercury content from a different food web possibly one that is detrital in origin. Regardless, these data do support the concept that periphyton based wetlands may increase mercury concentrations in some lower trophic level species. Therefore, it would be prudent to monitor mercury concentrations routinely in constructed wetlands dominated by periphyton.

1. Introduction

Phosphorus rich stormwater runoff has been identified as a factor in anthropogenic eutrophication in the Everglades (SFWMD 1999). One source of phosphorus rich stormwater runoff to the Everglades is the Everglades Agricultural Area (EAA). The EAA is a 291,000 hectare area south of Lake Okeechobee dominated by farming for sugar cane, winter vegetables, and rice (SFWMD, 1992). In an effort to reduce phosphorus loads to the Everglades, the South Florida Water Management District (District) is in the process of building a series of constructed wetlands called Stormwater Treatment Areas (STAs) to lower phosphorus concentrations in surface waters through macrophyte and algal growth, and peat accumulation. One design concept proposes using a STA dominated by submerged aquatic vegetation and associated periphyton (SAV-STA).

In a prototype STA operated by the District, the Everglades Nutrient Removal Project (ENR Project), Cell 4 is a 147 ha marsh that has been manipulated to promote submerged aquatic vegetation with associated periphyton as the dominant community (see Chimney and Goforth, this volume). Consequently, Cell 4 can be viewed as a prototype SAV-STA and therefore used to compare to other types of STA communities particularly ENR Project Cell 3, which receives similar water quality but is a mixed marsh wetland dominated by emergent vegetation.

However, the creation of SAV-STA may create additional environmental problems. Current Everglades research suggests that extensive periphyton communities, are one habitat in which anaerobic sulfate–reducing bacteria readily transform mercury into methylmercury (Gilmour et al., 1992, Gilmour et al., 1998; Cleckner et al., 1999, Hurley et al., 1999). Elevated mercury concentrations exceeding 0.5 ng/g in sportfish including largemouth bass (*Micropterus salmoides*)

and sunfish (*Lepomis* spp.) (Ware et al., 1990) have warranted the issuing of consumption advisories throughout the Everglades. In addition to the human health risk, other species which are linked to the aquatic food web, may also be at risk including wading birds, panthers (*Felis concolor coryi*), otters (*Lutra canadensis*) and mink (*Mustela vison evergladensis*) many of which are threatened or endangered (Lodge, 1994). Since many of these species are likely to take advantage of newly created habitats, it has been hypothesized that constructed wetlands dominated by SAV and periphyton communities could potentially expose endangered species to unacceptable levels of mercury (Cleckner et al., 1998).

This study documents mercury concentrations in fish species common to both the STAs and the Everglades. The primary focus of this study was the eastern mosquitofish (*Gambusia affinis*) an ubiquitous omnivore that has been used by other Everglades mercury studies as a sentinel species (Rumbold et al., 2001). Additional species collected included sailfin mollies (*Poecilia latipinna*) and sunfish which are important in the diets of wading birds (Rumbold, 2000) and largemouth bass, an important sportfish. Using these species differences in mercury concentrations between cells and possible causal relationships are discussed.

2. Materials and Methods

Mosquitofish, a small ubiquitous omnivore native to South Florida, were collected on a quarterly basis beginning in June 1996 at established sites in Cell 3 (ENR302 and ENR303) and Cell 4 (ENR401 and ENR402) (Chimney and Goforth, this volume, Figure 3). Two sites in each cell were sampled until June 1999 when sampling was decreased to one site per cell (ENR302 and ENR401). Sampling at one site per cell continued through September 2000. Mosquitofish were collected using dipnets and placed on ice in freezer bags with *in situ* water. For each site,

individual fish were sorted by weight into three classes, with the focus of this study being on the medium size class (0.07 g - 0.28 g) which served to exclude both large gravid females and small fry fish. Medium size class fish were homogenized in a stainless steel blender and/or a polytron. Homogenate was subsampled to create split samples for quality assurance purposes. The remaining homogenate was frozen and archived, while samples were shipped on ice, overnight to the analytical laboratory. Total Hg (THg) analysis was carried out for all samples by two laboratories using variations on the cold vapor atomic fluorescence USEPA standard method 1671 (REF).

In May 2000, a sufficient amount (greater than 2 grams) of sailfin mollies were incidentally collected at the Cell 4 site to allow for processing and mercury analysis of this species. As with mosquitofish, analyses of sailfin mollies were carried out on homogenates of multiple fish, but in this case the animals were not divided into size categories.

In October 1999, May 2000 and September 2000, sunfish and largemouth bass were collected in both Cell 3 and Cell 4. These fish were collected from a number of sites throughout the two cells using both electro-shocking and hook and line. Animals were filleted and fillets were shipped frozen to the laboratory for analysis.

3. Results and Discussion

Concentrations of mercury in mosquitofish at both sites ranged from less than 5 ng Hg/g wet weight to 36 ng Hg/g wet weight (Figure 1). Mean concentrations of mercury mosquitofish from Cell 4 were, on average three times higher than in mosquitofish from Cell 3. These differences were statistically significant ($t = -6.4$; $\alpha = 0.05$; $\beta = 0.90$). Sailfin mollies collected from Cell 4 averaged 11 ng Hg/g wet weight. Sunfish collected from Cell 3 and Cell 4 both averaged 21 ng

Hg/g wet weight in October 1999 but averaged 19 ng Hg/g wet weight and 11 ng Hg/g wet weight respectively in May 2000 (Table 1). Largemouth bass collected from Cell 3 in October 1999 and May 2000 averaged 53 ng Hg/g wet weight and 24 ng Hg/g wet weight respectively. Largemouth bass collected in Cell 4 in October 1999 were all less than the detection limit of 21 ng Hg/g wet weight.

For the most part, the mean THg concentrations in mosquitofish in Cell 3 (~5 ng Hg/g) and Cell 4 (~16 ng THg/g) are comparable to the lowest values reported for mosquitofish in the Everglades (Cleckner et al., 1998). However, the differences between cells suggest differences in net methylmercury (MeHg) production or differences in food web structure.

Preliminary studies of Hg methylation rates in periphyton mats of Cell 3 and Cell 4 (C. Gilmour, unpublished data) indicated higher gross methylation rates in periphyton mats from Cell 3 when compared to Cell 4 (1.08 ng/g/d compared to 0.25 ng/g/d, respectively). Thus it appears that gross methylation rate alone cannot explain the differences between the cells. However, sparse SAV and periphyton mats occupied only 51 ha (12.5%) of Cell 3, compared to 139 ha (94.5%) of dense SAV and periphyton in Cell 4. Given both the higher density and habitat coverage of periphyton in Cell 4, it is likely that a higher net spatial methylation occurs in Cell 4 in comparison to Cell 3. Monitoring data has found that the average surface water MeHg concentration in Cell 4 increased from 0.053 ng/L at the inflow to 0.071 ng/L at the first monitoring site. In contrast, Cell 3 showed no such change (SFWMD, 1999). This would indicate that at least the upper portion of Cell 4 was methylating Hg in sufficient quantities to change the concentration in the water column while methylation in Cell 3 was insufficient to affect a change. This suggests significant differences in the net methylation rates for each cell.

Another possible source of differences between cells is differences in diet. Hurley et al. (1999) found differences in the diets of mosquitofish collected in Cell 3 and Cell 4 (Table 3). Mosquitofish in Cell 3 consumed an average of 22% (by weight) animal material while Cell 4 mosquitofish consumed an average of 31% animal material. Thus Cell 3 mosquitofish function more as grazers and detritivores than Cell 4 fish. Cell 4 mosquitofish also consumed different ratios of animal taxa than those in Cell 3, with the Cell 3 diet being dominated by ostracods (29%), chironimids and rotifers (both 14%). In contrast the animal portion of the Cell 4 mosquitofish diet was dominated by dipterans (52%), copepods (29%), and leeches (14%).

The ratios of taxa consumed may play an important role in relative exposure in mosquitofish. Hurley et al. (1999) found copepods in Cell 4 to have average MeHg concentrations of 0.09 ng/g dry weight, nearly four times that of copepods in Cell 3 (0.024 ng/g dry weight). Copepods make up 9% of the Cell 4 mosquitofish diet, but only 1% of the Cell 3 mosquitofish diet. Since the diet analysis was done on a percent-weight basis, the concentration of mercury in the copepods can be multiplied by the percent copepods to determine relative mercury exposure. For each gram of food consumed, Cell 4 mosquitofish consume 0.09 g of copepods with MeHg concentrations of 0.09 ng/g or 0.0078 ng MeHg / g food. In contrast for each gram of food consumed, Cell 3 mosquitofish consume 0.01 g of copepods with a MeHg concentration of 0.024 ng/g or 0.0002 ng MeHg/g food. Consequently, the exposure to mercury via copepods is more than 45 times higher in Cell 4 than in Cell 3.

In comparison, ostracods were the dominant animal in the Cell 3 mosquitofish diet (7%) but only a small part of the Cell 4 mosquitofish diet (0.3%). Ostracods from Cell 3 and Cell 4 had comparable MeHg concentrations (0.16 and 0.17 ng/g, dry, respectively). Exposure analysis

carried out as shown above found that MeHg exposure from ostracods in Cell 3 mosquitofish was 22 times higher than exposure in Cell 4.

However, the differences in mosquitofish diets and mercury exposure between cells may not be ecologically significant. Given the differences in mosquitofish Hg concentrations between Cell 3 and Cell 4, it had been hypothesized that a similar pattern would appear in bass and sunfish. However, the majority of largemouth bass and sunfish collected in Cells 3 and 4 had tissue Hg concentrations under 25 ng Hg/g wet weight. Largemouth bass collected in October 1999 from Cell 3 had an average Hg concentration of 53 ng Hg/g with values ranging up to more than 150 ng Hg/g. In comparison, largemouth bass collected in May 2000 from Cell 4 only ranged up to 50 ng Hg/g. While this data appears to have a trend opposite that of the mosquitofish data, this may be a result of both small sample size and small fish size in Cell 4 fish (Table 1).

The lack of significant differences or reversal of patterns of Hg concentrations in sportfish between Cell 3 and Cell 4 would suggest that the differences in mosquitofish Hg concentrations are not having an affect on concentrations of either sunfish or largemouth bass. This could be because mosquitofish are not a major component of the sportfish diet (Lange et al., 1999).

5. Conclusions

In comparison to other sites in the Everglades (Table 4), both Cell 4 and Cell 3 have mosquitofish populations with low Hg concentrations. However, it appears that Hg concentrations in mosquitofish in the submerged aquatic vegetation community of Cell 4 are elevated in comparison to mercury concentrations in mosquitofish from the emergent macrophyte dominated communities of Cell 3. These differences are most likely the result of a combination of factors including net methylation rate, dominant habitats, and differences in diet. Despite differences in

mercury concentrations in mosquitofish between cells, the same pattern does not appear to repeat at higher trophic levels, indicating that mercury concentrations in ENR Project largemouth bass and sunfish may not be linked to mosquitofish or similar prey species.

Overall, these data suggest that the SAV- STA did elevate Hg concentrations in several lower trophic level species in comparison to wetlands dominated by emergent macrophytes but not in higher trophic level organisms. Since higher trophic levels must be linked to some portion of the lower trophic structure, it is likely that elevated Hg methylation rates and bioaccumulation are not affecting some portions of the lower trophic levels. This may be indicative of two distinct pathways in the food web of Cell 4. One food web could transport relatively high amounts of Hg through zooplankton to mosquitofish and another could transport lower amounts to sportfish. The possibility that mosquitofish feed on an algal-based food web and that sportfish feed on a detrital based food web should be investigated.

Acknowledgements

We would like to thank Ted Lange of the Florida Fish and Wildlife Conservation Commission for help in sample collection. This manuscript was improved based on comments from Dr. Darren Rumbold, Dr. Garth Redfield and Larry Fink.

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Figure Legends

Figure 1. Mean and ranges of mercury concentrations in mosquitofish from Cell 3 (ENR302 and ENR303) and Cell 4(ENR401 and ENR402) in the ENR Project quarterly from 6/96 to 9/00.

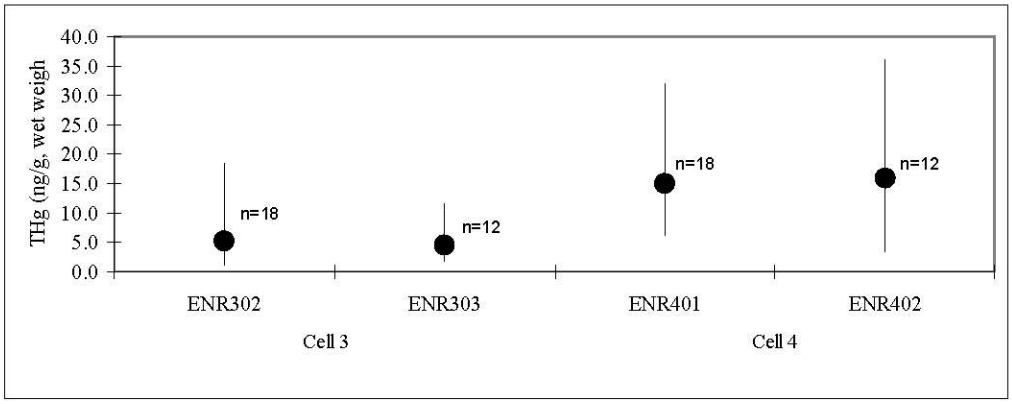
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
THg (ng/g, wet weigh
n=18
n=12
n=18
n=12
ENR302
ENR303
ENR401
ENR402
Cell 3
Cell 4

Table 1. Summary of analytical results for sunfish collected from Cell 3 and Cell 4 of the Everglades Nutrient Removal Project.

Collection Date	Oct-99		May-00	
Site	Cell 3	Cell 4	Cell 3	Cell 4
N	9	22	10	10
Weight Range (g, wet weight)	50-205	117-230	60-180	60-75
THg Range (ng/g, wet weight)	<21	<21-27	6-57	5-22
THg Mean (ng/g, wet weight)	21	21	19	11

Table 2. Summary of analytical results for largemouth bass collected from Cell 3 and Cell 4 of the Everglades Nutrient Removal Project.

Collection Date	Oct-99		May-00	
Site	Cell 3	Cell 4	Cell 3	Cell 4
N	20	4	3	0
Weight Range (g, wet weight)	104-1092	47-111	246-324	
THg Range (ng/g, wet weight)	<21-160	<21	12-49	
THg Mean (ng/g, wet weight)	53	<21	24	

Table 3. Gut content analysis by percent weight, of mosquitofish from Cell 3 and Cell 4 of the ENR Project.

Prey Item	Cell 3		Cell 4	
	% Weight	MeHg (ng/g dry weight)	% Weight	MeHg (ng/g dry weight)
Macrophyte	3%		32%	
Fil. Algae	44%		31%	
Diatoms	32%		5%	
Rotifer	3%		1%	
Copepod	1%	0.02	9%	0.09
Ostracod	7%	0.16	0%	0.17
Dipteran (adult)	1%		16%	
Other	10%		5%	

Table 4. Mean mercury concentrations (1998 and 1999) in mosquitofish and sportfish in the Everglades (After Rumbold et al., 2001).

Site	Mosquitofish	Sunfish	Largemouth Bass
WCA2A-F1	40	88	337
WCA2A-U3	169	131	474
WCA2A-3A15	197	373	994
ENP-P33	164	547	1097