Appendix 7-15: Mercury Concentrations in Mosquitofish from Treatment Wetlands in the Northern Everglades

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INTRODUCTION

Fish consumption advisories have been issued throughout the Everglades due to elevated mercury concentrations exceeding 0.5 ng/g in sportfish including largemouth bass (*Micropterus salmoides*) and sunfish (*Lepomis* spp.) (Ware et al., 1990). In addition to the human health risk, other species which are linked to the aquatic food web, may be at risk including wading birds, panthers, otters and mink. Since many of these species are threatened or endangered, it is important to understand the ecological processes influencing the transformation of mercury into methylmercury and the accumulation of methylmercury in the Everglades foodweb.

The transformation of mercury into methylmercury appears to be carried out by anaerobic sulfate –reducing bacteria (Gilmour et al., 1992). In the Everglades and associated ecosystems, there are three areas that appear to be capable of supporting these microbes: the sediment water interface, the extensive periphyton mats, and the root zones of floating macrophytes such as water hyacinth and water lettuce (Gilmour et al., 1998; Cleckner et al., 1999, Hurley et al., 1999). Consequently, it has been hypothesized that areas dominated by periphyton communities could potentially have higher rates of mercury methylation than other wetland communities (Cleckner et al., 1998).

In addition to elevated mercury concentrations, excess phosphorus entering the Everglades from Everglades Agricultural Area runoff has been acknowledged as a factor in anthropogenic eutrophication in the Everglades (SFWMD, 1999). In response, the South Florida Water Management District (SFWMD) has and continues to build constructed wetlands designated Stormwater Treatment Areas (STAs) to lower phosphorus concentrations in surface waters through macrophyte and algal growth, and peat accumulation. One subcategory of the STAs, Periphyton-Based Stormwater Treatment Areas (PSTAs), will use periphyton (epiphytic and epibenthic algae and metaphyton) as the dominant community for phosphorus removal. However, since periphyton communities provide habitat for mercury methylating bacteria (Cleckner et al., 1999), the possible increases in mercury methylation and bioaccumulation must be examined.

In the prototype STA, the Everglades Nutrient Removal Project, Cell 4 is a 147 ha marsh that is maintained such that submerged vegetation with an associated periphyton complex is the dominant community. Consequently, Cell 4 can be viewed as a prototype PSTA and therefore used to compare to other types of STA communities. This study identifies differences in mercury concentrations in mosquitofish (Gambusia affinis) collected from Cell 4 and a more traditional constructed wetland, Cell 3, and provides possible causal relationships.

MATERIALS AND METHODS

LOCATION

The Everglades Nutrient Removal (ENR) Project is located in western central Palm Beach County of Florida, in the northern Everglades, at the northwestern tip of Water Conservation Area 1, and is comprised of 1538 ha of former agricultural fields. Agricultural stormwater runoff is pumped into the ENR Project into the 55 ha Buffer Cell. The Buffer Cell provides dampening of storm surges and serves to convey water to two parallel and independent treatment trains. The eastern treatment train is comprised of the 525 ha Cell 1, dominated by submersed vegetation (approximately 44%) and Cattail (approximately 35%), and the 407 ha Cell 3, dominated by Cattail (approximately 42%). The western treatment train is comprised of the 414 ha Cell 2, dominated by Cattail (approximately 65%) and submersed vegetation (approximately 25%), and the 147 ha Cell 4, dominated by a periphyton and submerged aquatic vegetation complex (approximately 94.5%).

QUARTERLY COLLECTIONS

Mosquitofish were collected on a quarterly basis at established sites in Cell 3 and Cell 4. Initial collections were at two sites in each cell sites but this was later lowered to a single site per cell. Mosquitofish were collected using dipnets and placed on ice in freezer bags with ambient water. For each site, individual fish were sorted by weight into three classes, with the focus being on the medium size class (0.07- 0.28 g). Medium size class fish were then composited and homogenized using a blender and/or a polytron. Some homogenates were then subsampled to create split samples for quality assurance purposes. Remaining homogenate was frozen and archived, while samples were shipped on ice, overnight for analysis. Total mercury analysis was carried out for all samples by three laboratories all using variations on a CVAFS method that have since been accepted as equivalent to USEPA standard method 1671.

Laboratory quality assurance for accuracy was achieved using standard reference materials. Homogeneity of the samples was checked by comparing the results of the split samples. All results were within the accuracy and precision quality assurance criteria, which were both set at 25%.

RESULTS

Analytical results for the routine monitoring of mercury in mosquitofish are shown in **Figure A7-15-1**. Concentrations of mercury in mosquitofish at both sites ranged from less than 5 ng/g, wet to 70 ng/g, wet. Concentrations of mercury in homogenates of fish from Cell 3 were on average three times higher than in homogenates of fish from Cell 4. Statistical analysis of data using a Student's t test showed that results from Cell 4 were significantly different than results from Cell 3 (a = 0.05, B = 0.90).

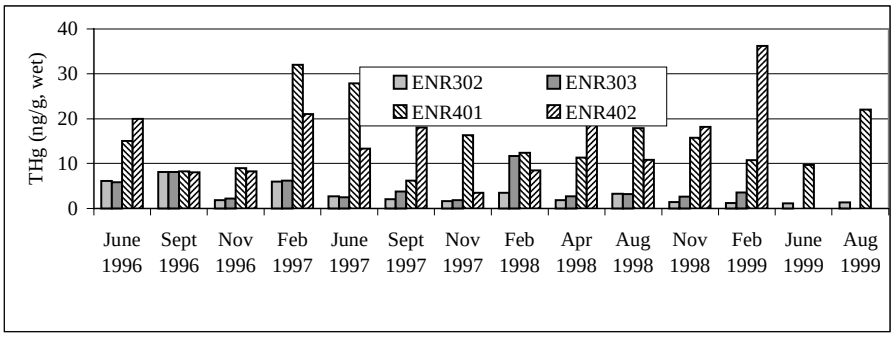

Figure A7-15-1. Mercury concentrations in mosquitofish from sites in the ENR Project

DISCUSSION

The differences in mercury concentrations in mosquitofish between the two cells are striking and significant. For the most part, the concentrations of mercury in mosquitofish in Cell 3 (approx. 6 ng/g, wet) and Cell 4 (approx. 18 ng/g, wet) are comparable to the lowest values reported for mosquitofish in the Everglades (Cleckner et al., 1998).

There are several factors that may be responsible for the differences in the mosquitofish mercury concentrations including 1) differential mercury loadings; 2) differences in water quality factors that regulate mercury methylation and bioaccumulation; 3) differences in net methylmercury production; 4) differences in food web structure. Finally it is possible that all or some of these factors could be operating in combination.

Differential mercury loadings to the cells is an unlikely candidate for driving the differences. Extensive monitoring by the SFWMD (Miles and Fink, 1998) suggests that loadings to the two cells from both atmospheric deposition and surface water inflows are proportional to their size. Similarly, surface water quality entering the cells are very similar (SFWMD, 1998) and thus it is unlikely that this is a factor engendering differences.

Preliminary and limited studies of mercury methylation rates in periphyton mats of Cell 3 and Cell 4 carried out by Gilmour (unpublished data) suggests higher gross methylation rates in mats from Cell 3 when compared to Cell 4. Thus, at first glance it appears that this cannot explain the differences between the cells. However, periphyton habitat is only 12.5% or 51 ha of cell 3, compared to 94.5% or 139 ha in Cell 4. Thus, on a direct comparison of area, periphyton habitat in Cell 4 is nearly three times that of Cell 3, and consequently, the potential area for methylation of mercury is proportionally larger as well. This may be sufficient to offset the higher methylation rates in Cell 3 and create higher net spatial methylation in Cell 4.

There does seem to be some evidence for the theory that the net methylation rate in Cell 4 was higher than that in Cell 3. Monitoring found that the average MeHg concentrations of inflows to Cell 3 and Cell 4 were very similar, being 0.057 ng/L and 0.053 ng/L, respectively (SFWMD 1999). Monitoring at the first downstream site in Cell 3 found an average MeHg concentration of 0.055 a slight decrease. In contrast, monitoring of the first downstream site in Cell 4 reports a significant increase in MeHg concentrations to 0.071 ng/L (Student's t-test, a=0.95). This would seem to indicate that at least the upper portion of Cell 4 was methylating mercury in sufficient quantities to change the concentration in the water column. This may also be linked to the short-circuiting of water in Cell 4 which has created some areas of relative low flow in the northern portions of the cell (DB Labs, 2000).

Another possible source for between-cell differences is food web structure. Hurley et al. (1999) reported gut contents of mosquitofish collected in Cell 3 (n=50) and Cell 4 (n=10) and noted differences between the two cells (**Figure A7-15-2**). Mosquitofish in Cell 3 consumed an average of 22% 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.

In addition to consuming more animal material, Cell 4 mosquitofish consume different ratios of animal taxa (**Figure A7-15-3**). The animal portion of the Cell 3 mosquitofish diet was 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 have an important part to play in relative exposure in mosquitofish. Analysis by Hurley et al. (1999) found copepods in Cell 4 to have average methylmercury concentrations of 0.09 ng/g, dry, nearly four times that of Cell 3 (0.024 ng/g, dry). Copepods make up 28% of the Cell 4 mosquitofish diet, but only 4% of the Cell 3 mosquitofish diet. Consequently, the exposure to mercury via copepods is 28 times higher in Cell 4 than in Cell 3.

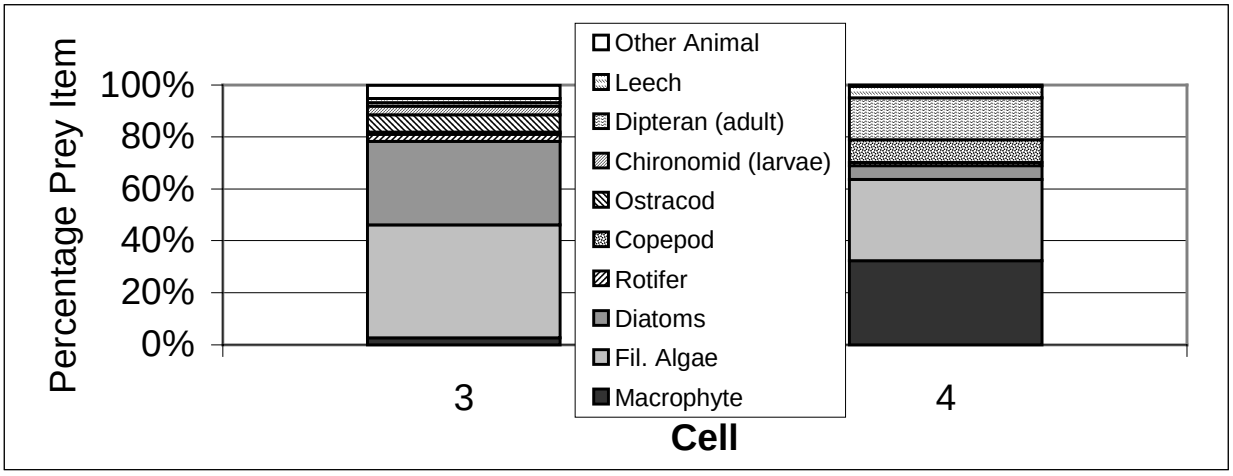

Figure A7-15-2. Diets of mosquitofish at sites in the ENR Project.

In comparison, ostracods were the dominant animal in the Cell 3 mosquitofish diet (29%) but only a small part of the Cell 4 mosquitofish diet (0.3%). Ostracods from Cell 3 and Cell 4 had comparable methylmercury concentrations (0.16 and 0.17 ng/g, dry, respectively). Analysis found that methylmercury exposure from Ostracods in Cell 3 mosquitofish was 77 times higher than exposure in Cell 4. However, if exposure from both Copepods and Ostracods are combined, methylmercury exposure in Cell 4 is still 4.6 times higher than exposure in Cell 3.

The analysis of mercury concentrations in Copepods and Ostracods and the diets, suggests that Copepods are an important exposure pathway in Cell 4. This may be related to the placement of Copepods in the aquatic food web. The only Copepod identified by Rader (1994) in the northern Everglades was *Argulus* sp., which is a common fish parasite. Argulus has also been identified in the gut contents of bluegills (*Lepomis macrochirus*) in Everglades National Park (Loftus et al., 1998). These parasites are commonly consumed when smaller fishes "clean" infested fishes (Sulak, 1975). While it is not believed that all Everglades copepods are Argulus, the possibility exists that a percentage of these organisms may be parasites. Thus the diet of mosquitofish in Cell 4 may contain a significant percentage of parasites, which could be cycling mercury from higher trophic levels such as bass or sunfish.

However, while the differences in mosquitofish may be real, they may not be ecologically significant. In October of 1999, the Florida Fish and Wildlife Conservation Commission carried out annual sportfish sampling of STA-1W including Cells 3 and 4. From this collection largemouth bass, and several species of sunfish became available for determination of mercury tissue concentrations. The majority of sportfish collected in

Cells 3 and 4 were at or near the detection limit, with only bass in Cell 3 being substantially higher with an average of 53 ng/g but values ranging up to more than 150 ng/g.

Given the differences in mosquitofish mercury concentrations between Cell 3 and Cell 4, it had been hypothesized that a similar pattern would appear in bass and sunfish, with the Cell 4 populations having higher mercury concentrations than the Cell 3 populations. In contrast, the mercury concentrations appear to be very similar in sunfish.

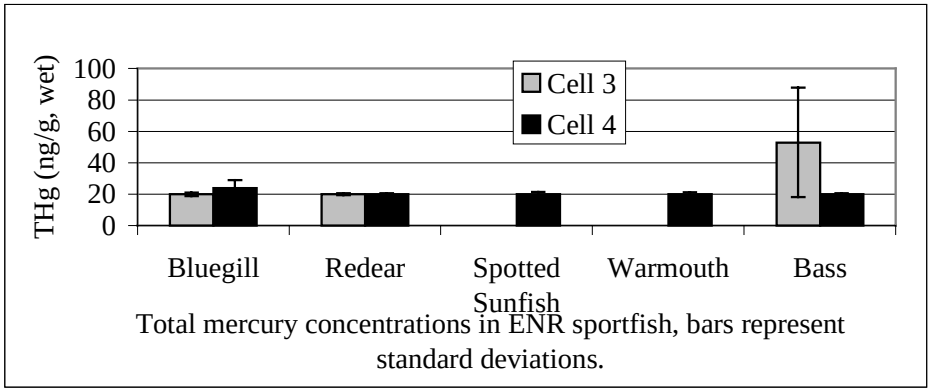

Figure A7-15-3. Mercury concentrations in Cell 3 and Cell 4 sportfish.

Additionally, the apparent elevation of Cell 3 bass over Cell 4 bass may be a result of a small sample size in Cell 4 (n=3) and the small size of the Cell 4 fish (47-111 g) compared to Cell 3 (61-1092 g). This lack of difference between mercury concentrations in sportfish between Cell 3 and Cell 4 would suggest that the differences in mosquitofish mercury concentrations are not having an affect on concentrations of either sunfish or bass. This could be because mosquitofish are not a major component of the sportfish diet (Lange, 1999), or perhaps the spatial variability between the cells is less important than other factors such as variations in diet or prey size. Regardless, it appears that the differences in mercury concentrations in mosquitofish between Cell 3 and Cell 4 may not be of ecological significance. Further sampling to validate the sportfish results on a quarterly basis is needed.

CONCLUSIONS

In comparison to other sites in the Everglades both Cell 4 and Cell 3 have mosquitofish populations with low mercury concentrations. However, it appears that mercury 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 gross methylation rate, habitat coverage, hydrology, and differences in diet. Despite these differences in mercury concentrations in mosquitofish, the same pattern does not appear to repeat at higher

trophic levels, indicating that the differences may not be ecologically significant. Further sampling is required to verify the sportfish results.

Overall, these data suggest that periphyton based STAs do elevate mercury concentrations in lower trophic levels in comparison to wetlands dominated by emergent macrophytes. Given the sensitivity of mercury methylation to water quality variables, if periphyton-based STAs are developed, mercury concentrations in biota should be routinely monitored to assure that concentrations in biota are acceptable.

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