Mercury in Fish from a Sulfate-Amended Wetland Mesocosm

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total number of words in text, references, tables, and figure legends = 4,245

MERCURY BODY BURDENS IN *Gambusia holbrooki* AND *Erimyzon sucetta* IN A WETLAND MESOCOSM AMENDED WITH SULFATE

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ABSTRACT

This study used an experimental model of a constructed wetland to evaluate the risk of mercury methylation when the soil is amended with sulfate. The model was planted with *Schoenoplectus californicus*, and the sediments were varied during construction to provide a control and two levels of sulfate treatment. This allowed characterization of sulfate's effect on mercury bioaccumulation in periphyton and two species of fish – eastern mosquitofish (*Gambusia holbrooki*) and lake chubsucker (*Erimyzon sucetta*). After one year in the experimental model, mean dry-weight normalized total mercury concentrations in mosquitofish from the non-sulfate treated controls (374 ± 77 ng/g) and the reference location (233 ± 27 ng/g) were significantly lower than those from the low and high sulfate treatments (520 ± 73 and 613 ± 80 ng/g, respectively). For lake chubsucker, mean total mercury concentration in fish from the high sulfate treatment (276 ± 63 ng/g) was significantly elevated over that observed in the control (109 ± 47 ng/g), the low sulfate treatment (122 ± 42 ng/g), and the reference population (41 ± 2 ng/g). Methylmercury in periphyton ranged from 6.6 ng/g (dry weight) in the control to 9.8 ng/g in the high sulfate treatment, while total mercury concentrations ranged from 1148 ng/g in the control to 1297 ng/g in the low sulfate treatment. Fish methylmercury bioaccumulation factors from sediment ranged from 52 to 390 and from 495 to 3059 for water. Based on these results, it can be concluded that sulfate treatments add a factor of risk due to elevated production of methylmercury in sediment and porewater which biomagnified into small fish, and may potentially increase through the food web.

Keywords: mercury, methylmercury, *Gambusia holbrooki*, *Erimyzon sucetta*, sulfate

INTRODUCTION

Mercury is a ubiquitous environmental contaminant spread globally from natural and anthropogenic sources through a complex geochemical cycle. Elevated mercury concentrations in aquatic systems have caused human health concerns world-wide due to the consumption of contaminated fish. The most toxic form of mercury is methylmercury, the mercury species that most easily bioaccumulates and biomagnifies through the aquatic food web. Methylmercury has been detected in all species of fish and fish-consuming mammals, including humans [1]. From the standpoint of human health risk, the accumulation of methylmercury in edible fish tissue has resulted in fishing restrictions, numerous health advisories, and much public apprehension [2,3]. There are over 1000 fish consumption advisories currently in effect across the United States due to mercury contamination [4].

Once in the aquatic environment, inorganic mercury (Hg^{2+}) may be transformed into methylmercury, primarily through the activity of anaerobic sulfate-reducing bacteria in sediment [5-8]. As inorganic mercury is taken up by sulfate reducers, methylation occurs through a side reaction within the bacteria's normal metabolic pathway [9-11]. As methylmercury is lipophilic, this means of methylmercury production is a key mechanism affecting the quantity of mercury accumulated in fish [7,8,12].

Natural wetlands are often conducive to mercury methylation and can be contributors of methylmercury to downstream environments [13,14]. Methylmercury research,

therefore, often focuses on natural wetland systems such as the Everglades [15-18], boreal wetlands and peat bogs [19-22], or natural depression wetlands in the southeastern United States [23]. Currently, however, little information exists regarding the behavior of mercury in man-made wetland treatment systems or on the use of wetland-based systems to remove inorganic mercury from regulated wastewater discharges.

Wetland treatment systems depend upon several processes for the immobilization of cationic metals from the wastestream [24], including the formation of solid metal-sulfide precipitates. Treatment systems amended with sulfate-rich compounds (e.g., gypsum) for the enhanced production of sulfide potentially favor microbial selection for sulfate-reducing bacteria. While these bacteria produce the sulfide needed to immobilize metals (including mercury) from the wastestream, they are also responsible for simultaneous mercury methylation [8]. Under these conditions, an increase in methylmercury production can occur if inorganic mercury is bioavailable. If inorganic mercury is present in discharge water, efforts must be made to ensure that constructed wetland systems augmented with sulfur-derived compounds are not increasing a source of more harmful mercury species.

To study methylmercury bioaccumulation in gypsum-amended sediments, we used a pilot-scale experimental model of an actual constructed wetland built to reduce copper, mercury, and metal-related toxicity in a wastestream at the Department of Energy's Savannah River Site (SRS) on the Upper Coastal Plain of south-central South Carolina (Aiken and Barnwell counties). Use of an experimental model allowed us to vary soil

preparation and provide a controlled environment for both replication and comparison between soil treatments. Like the full-scale wetland, the experimental model was planted with a single species of vegetation, *Schoenoplectus californicus*, and the model received effluent directly from the wastestream. The soil of the model, however, was varied to provide a control and two sulfate treatments. This allowed characterization of sulfate's effect on mercury methylation and bioaccumulation by fish and periphyton.

We selected two fish species for determination of mercury bioaccumulation – lake chubsucker (*Erimyzon sucetta*) and eastern mosquitofish (*Gambusia holbrooki*). These species are known to inhabit SRS wetlands [25], and they represent different trophic niches within these wetlands [26]. This study also compared how sulfate treatments affected mercury uptake and methylation within periphyton communities which provide food to these fishes.

MATERIALS AND METHODS

The experimental field model consisted of a flow-through system of twelve 1268-liter rectangular fiberglass tanks (Aquaculture Systems Technologies, New Orleans, LA), placed adjacent to the wastestream. Soil indigenous to the Savannah River flood plain was placed in each tank to a depth of 46 cm then amended with organic material (wood mulch and chopped plant material) so that the organic material made up 6% of the soil volume. Soils were composed of 85% sand and 15% silts/clays. Horticultural lime (Hoffman, Lancaster, NY) and ammonium nitrate fertilizer (Royter Clark, Norfolk, VA)

were added at rates of 95 g/tank and 75 g/tank, respectively. Aquatic plants, giant bulrush (*Schoenoplectus californicus*), were obtained from a commercial vendor (Horticultural Systems, Inc., Parrish, FL) and planted on 15-cm centers in the saturated soil.

The experimental design included a control and two levels of sulfate treatment – each with four replicates. Pelletized agricultural gypsum ($CaSO_4 \cdot 2H_2O$; Southdown, Easton, PA) was added to the soil of the treatment tanks prior to planting and flooding; soil in the control tanks contained no added gypsum. Two sulfate concentrations were selected: a low sulfate addition (3 kg/tank), and a high sulfate addition (12 kg/tank). Treatment and control replicates were spaced within the experimental system to ensure statistical independence.

Water directly from the wastestream was continuously pumped into a 265-liter elevated holding tank (US Plastics Corp., Lima, OH) before being gravity fed into each of the twelve tanks. Discharge was through a slotted pipe set 30 cm above the sediment surface. Flow rate through the system was 0.15 L/minute for an estimated 48-hour hydraulic retention time for wastewater passing through the system.

Surface water sampling for each tank was conducted at the outflow device using a syringe submerged 5 cm below the surface. Interstitial water (porewater) was collected using *in situ* samplers commonly referred to as sippers. Sippers are constructed of hollow stakes having a porous Teflon collar located at the appropriate sampling depth (0-

3, 3-6, 6-9, and 9-12 cm). Mercury and methylmercury samples were filtered in the field through 0.45 µm Acrodisc® syringe filters. (Gelman Laboratory, Ann Arbor, MI) and collected in fluoropolymer bottles which had been acid cleaned and double sealed in an ultra clean laboratory.

The system was allowed to stabilize for eight weeks prior to the introduction of fish. In late August 2001, twenty juvenile mosquitofish collected from an uncontaminated impoundment were introduced into each of the tanks after a four-day acclimation period. In early December 2001, ten juvenile lake chubsuckers from a laboratory population described by Hopkins *et al.* [27] were acclimated and introduced into each tank.

Both fish species were harvested in September 2002 using standard minnow traps. After collection, fish were held in the laboratory for 24 hours to clear gut contents. Standard measurements were determined, and the fish were frozen for analysis. The number of chubsuckers recovered from the tanks was limited to one or two per tank, while mosquitofish were collected in abundance. To reduce variability and generational differences, analysis was performed on larger (>0.8 g) female mosquitofish, and five individuals per tank were homogenized into one sample. Whole fish were analyzed. A subsample of each species was weighed, dried for 48 hours at 100°C, then weighed again to determine the percent moisture for the dry weight normalization calculations.

Both species of fish were also harvested from reference sites with no known source of contamination, either present day or historical. Lake chubsuckers were harvested from a

depression wetland, commonly referred to as a "Carolina Bay" within the boundary of SRS. This bay has been identified as Bay # 142 and has been described by Schalles *et al.* [28]. Mosquitofish were collected from Fire Pond, a small impoundment often used as a reference site for studies of heavy metal contamination [29].

Periphyton was allowed to colonized for six weeks on acid-washed plastic plates inserted into the tanks for this purpose. Colonies were scraped from the plates and collected in polypropylene sample tubes. Samples from replicated tanks were composited to ensure adequate volume, centrifuged to remove excess water, and frozen until analysis. A subsample from each tube was dried and weighed for percent moisture determination.

Whole sediment cores were taken the end of this experiment for measurement of total and methylmercury. Sediment cores were collected using a plastic core barrel sampler (15 cm length and 2 cm diameter) with a Teflon plunger. Sediment cores were sliced into 2 depths (0-6 and 6-12 cm) then frozen in polypropylene sample tubes until analysis. A subsample of each core was weighed, dried at 100°C for 48 hours, then weighed again to determine the percent moisture in the sample.

Analysis of total and methylmercury

Low-level total mercury in sediment, periphyton, and fish tissue was measured via Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) using the cold-vapor reduction, isotope dilution method described by Smith [30]. Tissue samples (2.5 g) were acidified with nitric acid, spiked with 0.5 mL of an enriched ^{201}Hg isotope solution, then

microwave digested. Aliquots of the digested samples were then reacted with a combined solution of potassium hydroxide (0.1%) and sodium borohydride (5%) for the reduction and volatilization of all mercury species which were carried into the instrument in an argon stream. Total mercury concentration in the sample was determined based on the ratio of $^{202}Hg/^{201}Hg$. This analysis was carried out using a VG Plasma Quad 3 ICP-MS (Thermo VG Scientific, West Sussex, England). Data were normalized for dry weight by dividing the measured mercury (or methylmercury) concentration by the percent dry weight of the sample.

Low-level methylmercury analyses of water, sediment, and tissue were preformed using a technique modified from US EPA Method 1630 [31]. Organics were removed from the samples through subboiling distillation of acidified samples [32]. Mercury in the distillate was then ethylated with sodium tetraethylborate and purged with argon onto a trap packed with Tenax [33]. The Tenax was flash heated in a stream of argon to release the mercury which was speciated chromatographically [34], combusted to Hg^0, and measured using cold vapor atomic fluorescence spectrometry (CVAFS) [35]. The CVAFS mercury analyzer was a Tekron Model 2500 (Tekron Inc, Ontario, Canada) and the integrator was a Hewlett-Packard Model HP3394A (Hewlett Packard Co., Boise, ID). The detection limit was 0.02 ng/L as Hg.

Statistical analysis

Data distribution and variance were evaluated through Kolmogorov-Smirnov Tests for Normality [36] and Levene's Test for Equal Variance [37]. A One-way Analysis of

Variance combined with a Least Significant Difference procedure [38] compared mercury concentrations in fish tissue within each species. Statistical analyses were performed with SPSS Base 10.0 statistical software [39].

RESULTS

Mean dry-weight normalized total mercury concentration measured in mosquitofish (*Gambusia holbrooki*) from the control tanks was 374 ng/g, and the mean of the field-collected reference samples was 233 ng/g. Both of these values were significantly lower than those from the low and high sulfate treatment which were 520 and 613 ng/g, respectively (Table 1). Mean total mercury concentrations measured in lake chubsuckers (*Erimyzon sucetta*) from the experimental tanks were 109 ng/g in the control, 122 ng/g in the low sulfate treatment, and 276 ng/g in the high sulfate treatment. Mean total mercury in the reference population was 41 ng/g (Table 1). For this species, only those from the high sulfate treatment were significantly elevated; all others, including the reference, were statistically equivalent. Previous work [40] has shown that most of the mercury body burdens in fish are accumulated as methylmercury, and we assumed that was the case in this study. Inorganic mercury is absorbed much less efficiently across the gut and gills and is eliminated much more rapidly [41,42].

Periphyton is generally abundant in wetland systems and serves as a plant-based food source at the base of the wetland food web [43]. Dry-weight normalized methylmercury concentrations in periphyton ranged from 6.6 in the control to 9.8 ng/g in the high sulfate

treatment. There were no statistical differences among treatments for methylmercury; however, the general increase in methylmercury concentrations from the control through the high sulfate treatment mirrors the increase in fish mercury body burdens (Figure 1). Mean total mercury concentrations measured in periphyton from the experimental model were unexpectedly high at 1148 ng/g in the control, 1297 ng/g in the low sulfate treatment, and 1477 ng/g in the high sulfate treatment. There were no statistical differences between total mercury concentrations in the control or the treatments. These data are in agreement with other studies which have shown that mercury concentrations in periphyton are largely inorganic and that consumers of periphyton preferentially bioaccumulate methylmercury [15,44].

Sediment methylmercury concentrations ranged from 1.4 to 2.3 $ng/g_{(dry\ wt.)}$ with the highest concentration observed in the low sulfate treatment (Figure 2). Overall mean porewater methylmercury concentrations in the sulfate-treated tanks were three-fold higher than the control (1.7 vs. 0.5 ng/L; Figure 3A). However, these elevated porewater methylmercury concentrations in sulfate treatments were not detected in surface waters (Figure 3B). Despite the fact that the highest methylmercury concentrations were confined to the benthos, results indicate that methylmercury has been efficiently transferred from water and/or sediment into the food web. Sediment methylmercury bioaccumulation factors ranged from 52 to 390, and methylmercury bioaccumulation factors in water ranged from 495 to 3059 (Table 2). Other studies have noted that the magnitude of mercury accumulation in biota does not always appear to be associated with changes in water column concentration [15], because dietary exposure has been shown to

be the dominant route of methylmercury uptake in fish [45]. In fact, the propensity of mercury to increase concentration several orders of magnitude from water to fish is well documented [46-48]. As methylmercury fluxes from sediment and porewater, it accumulates in phytoplankton such as algae and diatoms [49,50]. It is then efficiently concentrated by herbivorous zooplankton species [51] and subsequently accumulated by fish preying upon these organisms [45,52,53].

The differences in mercury burdens between the two fish species reflect the somewhat longer exposure period for the mosquitofish combined with the fact that these two species occupy different trophic niches [26]. Lake chubsuckers primarily feed upon algae, benthic detritus, and associated benthic organisms, while mosquitofish are typically carnivorous water column feeders [26]. Although mosquitofish may shift to periphyton in the winter months [43], plant material has been shown to make up less than 25% of the overall mosquitofish diet [54] with zooplankton prey species making up the balance [55]. In contrast, plant material comprises up to one-half of the diet of lake chubsuckers [54]. Higher overall mercury body burdens in mosquitofish probably reflect this increased dependence upon prey items.

Data presented here indicate that mercury accumulation by small fish species in a constructed wetland model was enhanced by sulfate addition, and this was presumably due to increased mercury methylation by sulfate-reducing bacteria and ensuing transfer into the aquatic food web. Mercury concentrations in both species of fish from the non-sulfate treated control were somewhat elevated over those from reference populations

(Table 1) but the differences were not statistically significant. Based on these results, it can be concluded that sulfate amendments add a factor of risk due to elevated production of methylmercury in sediment and porewater which can biomagnify into small fish, and may potentially spread or increase through the food web.

Acknowledgement – The authors would like to thank Debbie Wells, James Bowers, and Robert Ray for analytical and technical assistance. Mercury samples were analyzed at the Skidaway Institute of Oceanography, Savannah, GA. This work was funded by the U.S. Department of Energy and Westinghouse Savannah River Company through a research program administered by the Oak Ridge Institute for Science and Education.

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Table 1. Mean total mercury concentrations measured in fish tissue from the experimental model. Values in parentheses represent one standard deviation from the mean.

Treatment	Dry-weight Normalized Mercury Concentration in Mosquitofish (*Gambusia holbrooki*) (ng/g)	Dry-weight Normalized Total Mercury Concentration in Lake Chubsuckers (*Erimyzon sucetta)* (ng/g)
Control	374 (±77)	109 (±47)
Low Sulfate	520[a] (±73)	122 (±42)
High Sulfate	613[a] (±80)	276[b] (±63)
Reference	233 (±27)	41 (±2)

[a]Mercury concentrations in mosquitofish were statistically higher in the low and high sulfate treatments when compared to fish from the control and the reference samples.

[b]Mercury concentrations in lake chubsuckers were significantly higher in fish from the high sulfate treatment when compared to the control, the low sulfate treatment, and the reference samples.

Table 2. Bioaccumulation factors for methylmercury in each species for each treatment. These factors are based upon mercury concentration in tissue compared to the methylmercury concentration in the top 6 cm of sediment or the overall mean methylmercury concentration in surface water.

Species	Treatment	Sediment	Water
		Methylmercury BAF	Methylmercury BAF
Gambusia	Control	275	1700
	Low	223	3059
	High	390	2358
Erimyzon	Control	80	495
	Low	52	718
	High	176	1062

FIGURE CAPTIONS

Figure 1. Mean methylmercury concentrations in periphyton and total mercury concentrations measured in mosquitofish (*Gambusia holbrooki*) and lake chubsuckers (*Erimyzon sucetta*) from the experimental wetland model.

Figure 2. Mean methylmercury concentrations in sediment from the experimental model. Asterisk (*) indicates that the mean value was significantly higher than all others.

Figure 3. Overall methylmercury concentrations in porewater (A) and surface water (B) from the experimental model. Porewater methylmercury concentrations were significantly lower ($p<0.05$) in the control than in either of the treatments. There were no significant differences in surface water methylmercury concentrations.

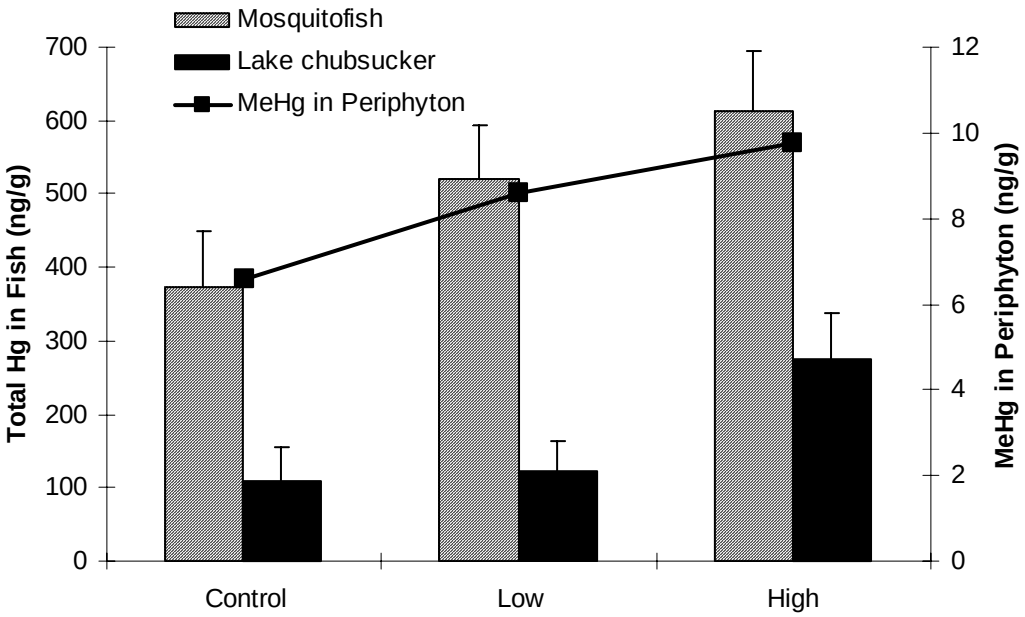
Mosquitofish
Lake chubsucker
MeHg in Periphyton
Total Hg in Fish (ng/g)
MeHg in Periphyton (ng/g)
0
100
200
300
400
500
600
700
0
2
4
6
8
10
12
Control
Low
High

Figure 1

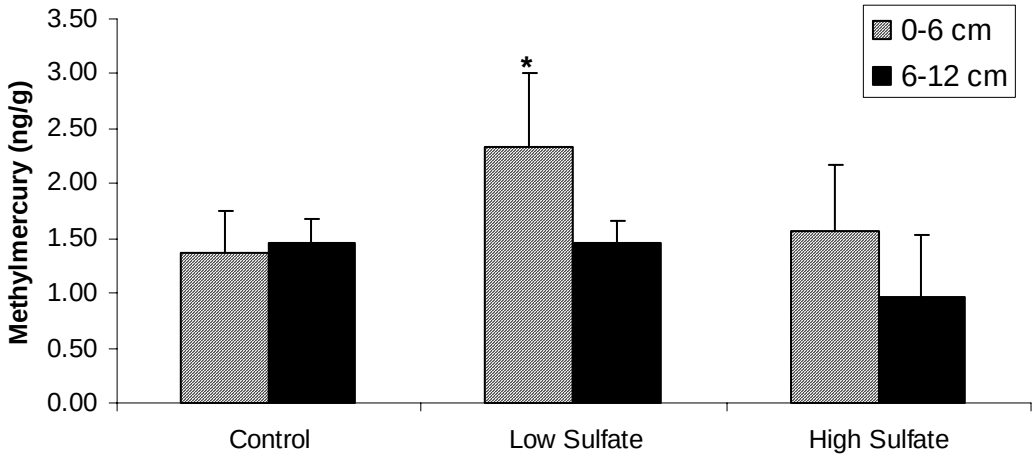
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Methylmercury (ng/g)
*
0-6 cm
6-12 cm
Control
Low Sulfate
High Sulfate

Figure 2

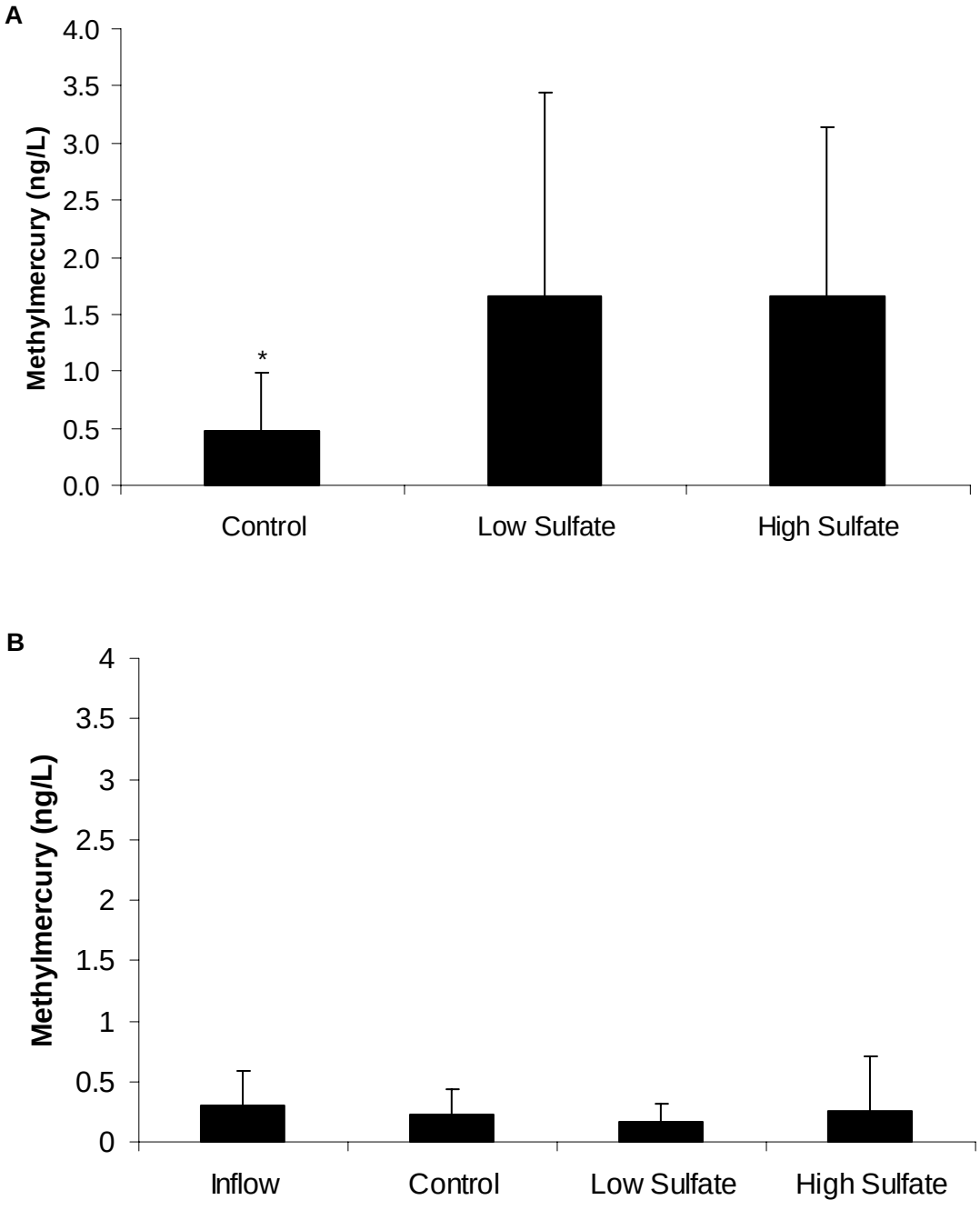
A
Methylmercury (ng/L)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
Low Sulfate
High Sulfate
B
Methylmercury (ng/L)
4
3.5
3
2.5
2
1.5
1
0.5
0
Inflow
Control
Low Sulfate
High Sulfate

Figure 3