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MERCURY ACCUMULATION IN PERIPHYTON OF EIGHT RIVER ECOSYSTEMS[1]

Amanda H. Bell and Barbara C. Scudder[2]

ABSTRACT: In 2003, the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program and U.S. Environmental Protection Agency studied total mercury (THg) and methylmercury (MeHg) concentrations in periphyton at eight rivers in the United States in coordination with a larger USGS study on mercury cycling in rivers. Periphyton samples were collected using trace element clean techniques and NAWQA sampling protocols in spring and fall from targeted habitats (streambed surface-sediment, cobble, or woody snags) at each river site. A positive correlation was observed between concentrations of THg and MeHg in periphyton (r^2 = 0.88, in log-log space). Mean MeHg and THg concentrations in surface-sediment periphyton were significantly higher (1,333 ng/m^2 for MeHg and 53,980 ng/m^2 for THg) than cobble (64 ng/m^2 for MeHg and 1,192 ng/m^2 for THg) or woody snag (71 ng/m^2 for MeHg and 1,089 ng/m^2 for THg) periphyton. Concentrations of THg in surface-sediment periphyton had a strong positive correlation with concentrations of THg in sediment (dry weight). The ratio of MeHg:THg in surface-sediment periphyton increased with the ratio of MeHg:THg in sediment. These data suggest periphyton may play a key role in mercury bioaccumulation in river ecosystems.

(KEY TERMS: algae; sediment; rivers/streams; periphyton; mercury/methylmercury; bioaccumulation.)

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INTRODUCTION

The U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program began a study in 2003 to examine mercury in precipitation, surface water, streambed sediment, sediment porewater, predator fish, forage fish, and benthic macroinvertebrates. Methylation potential in streambed sediment and dissolved organic carbon in surface water was also sampled (Brigham *et al.*, 2003). Our study focused on periphyton (attached benthic algae) and was a cooperative effort between the USGS and U.S. Environmental Protection Agency (USEPA) to address an important aspect of bioaccumulation, and complement biological aspects of the larger study. This article focuses on mercury levels in periphyton and the physicochemical environment from which periphyton were sampled.

Mercury (Hg) is a priority pollutant for many federal and state agencies, as well as private programs, because of human and ecological receptors, such as fish and wildlife that feed on fish. The concern is especially focused on methylmercury (MeHg) because

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[2]Respectively, U.S. Geological Survey, Wisconsin Water Science Center, 8505 Research Way, Middleton, Wisconsin 53562 (E-Mail/Bell: ahbell@usgs.gov).

of its high toxicity and its propensity for high bioaccumulation in aquatic food webs (USEPA, 2005). Hg is the leading chemical cause of fish-consumption advisories in the United States, with 13,068,990 lake acres and 766,872 river miles under advisories reported in 2003 (USEPA, 2004). Most Hg released to the environment in the United States is from anthropogenic sources such as mining runoff and atmospheric deposition from coal-fired combustion, chlor-alkali manufacturing, and waste incineration (USEPA, 1997).

Previous studies have shown that MeHg biomagnifies as it moves up aquatic food chains to top predators (Bloom, 1992; Krabbenhoft, 1996; Cleckner *et al.*, 1998, 1999; Krabbenhoft *et al.*, 1998; Morel *et al.*, 1998; Neumann and Ward, 1999; USEPA, 2001). Consumers from invertebrates to top level predator (piscivorous) fish accumulate Hg primarily though their diets (Watras and Bloom, 1992; Mason *et al.*, 1996). Most of these studies have been conducted in lakes, reservoirs, and wetlands and have focused on top predator or game fish, the water column, and bed sediment. Few detailed studies of Hg bioaccumulation in aquatic food webs have been conducted in streams and rivers, despite their importance for recreational and subsistence fishing.

Based on data from lakes, Hg accumulation in phytoplankton or suspended algae, the base of the aquatic food web in those systems, is the single largest step in bioaccumulation but does not occur at a constant rate. Previous studies have found MeHg bio-concentration factors (bioaccumulation factors hereafter) in the 10^4 to 10^6 range between surface water and phytoplankton (Watras and Bloom, 1992; Watras *et al.*, 1998; Miles *et al.*, 2001). Phytoplankton blooms in a mesocosm study resulted in reduced bioaccumulation in algal-rich eutrophic lake-type systems due to decreases in the concentration of Hg per algal cell (Pickhardt *et al.*, 2002). Hill and Larsen (2005) also found growth dilution of Hg concentration and uptake in microalgal biofilms in a flow-through laboratory set-up.

In small and medium-sized rivers, periphyton are primary producers and the base of the food web, as opposed to phytoplankton in most larger rivers and lakes (Lowe and LaLiberte, 1996). The term periphyton, in this study, refers to the matrix of attached benthic algae and other heterotrophic bacteria or microbes that are affixed to the submerged substrata in freshwater systems. This matrix also includes some allocthonous sources of carbon, such as detritus, that the periphyton are growing on. The autotrophic and heterotrophic nature of the matrix enhances bioavailability of carbon and other essential nutrients, but also enhances bioavailability of non-essential elements, such as Hg (Hill and Larsen, 2005). Although periphyton are an important aspect in bioaccumulation and trophic transfer of MeHg to organisms higher on the food chain, there has been little research to date on bioaccumulation of MeHg in natural periphyton communities in rivers. The emphasis of this study was on the natural periphyton communities in river ecosystems.

It has yet to be established by the scientific community whether periphyton passively assimilate Hg, actively assimilate Hg, or adsorb Hg to the surface of their cells. One hypothesis, according to Morel *et al.* (1998) and Moye *et al.* (2002), is that hydrophobic inorganic mercury [Hg(II)] and MeHg diffuse through cellular membranes at approximately the same rate in diatom cells. The Hg(II) binds to the cellular membrane which is then egested by the consumer of the diatom, while MeHg becomes associated within the soluble fraction of the cell which the consumer then assimilates as its food source (Mason *et al.*, 1995, 1996). Another hypothesis is that the periphyton are actively assimilating Hg because of a ligand attached to the MeHg, including organic carbon, chloride, sulfide, hydroxide and others (Sunda and Huntsman, 1998; Watras *et al.*, 1998). The third hypothesis is that the periphyton are assimilating some inorganic form of Hg, through active or passive means, and methylating that Hg within the periphyton matrix (Cleckner *et al.*, 1999; Mauro *et al.*, 2002). Finally, Miles *et al.* (2001) found that rinsing the sample with EDTA to remove any extra-cellular-bound MeHg did not change the Hg concentration of the sample, hypothesizing that MeHg is not simply adsorbed to the cellular membrane.

STUDY DESIGN

The objectives of this periphyton study were to investigate THg and MeHg bioaccumulation in periphyton in a diverse set of rivers, explore relations of periphytic THg and MeHg with other geochemical measures, and gain insight concerning the role of periphyton in transfer of MeHg to higher trophic levels. This study used three approaches: (1) measure THg and MeHg concentrations in periphyton, (2) determine chlorophyll *a* concentrations and ash-free dry mass as measures of algal biomass, and (3) identify gross periphyton taxonomic composition to division level.

Periphyton were collected at eight study sites from watersheds of different hydrological, biogeochemical, and land use characteristics (Table 1). These eight study rivers are in three USGS NAWQA Basins: Willamette Basin in Oregon (Oregon), Western Lake

TABLE 1. Table Describing Surface Water and River Morphology Characteristics of the Eight Study Sites.

USGS Station ID	Station Name	River Code	Basin Area (square miles)	Major Landuse/ Land cover (%)	Percent Wetland (%)	Major Streambed Substrate	Major Inputs	Filtered MeHg Concentration (ng/L)	Dissolved Organic Carbon (mg/L)	pH
14206435	Beaverton Creek at SW 216th Ave., near Orenco, Oregon	BT	36.9	Urban (78)	0.2	Gravel/cobble and fines	Runoff during winter, ground water during low flow	0.04 (<0.04-0.23)	4.5 (3.2-7.3)	7.3 (7.0-7.5)
14161500	Lookout Creek near Blue River, Oregon	LO	24.1	Forest (97)	0	Gravel/cobble with areas of low organic sand on bedrock	Runoff during winter, ground water during low flow	<0.04 (<0.04-0.15)	1 (0.6-2.0)	7.2 (6.8-7.7)
04066500	Pike River at Amberg, Wisconsin	PR	255	Forest (76)	18	Cobble and low organic sand	Ground water	0.1 (<0.04-0.26)	7.9 (2.2-18.7)	7.7 (6.9-8.2)
04075365	Evergreen River below Evergreen Fallsnear Langlade, Wisconsin	EG	64.5	Forest (76)	9.1	Cobble and high organic sand and fines	Ground water	0.05 (<0.04-0.12)	5.1 (1.7-15.6)	7.8(7.2-8.4)
04087204	Oak Creek at South Milwaukee, Wisconsin	OC	25	Urban (68)	1.3	Cobble with areas of low organic sand	Runoff, Wastewater, ground water during low flow	0.06 (<0.04-0.25)	6.7 (3.5-13.1)	7.5 (7.1-7.9)
02322500	Santa Fe River near Fort White, Florida	SF	1020	Forest(47)	18	Low organic sand on limestone hard pan	Black water lake outflow, ground water during low flow	0.2 (<0.04-0.93)	11.3 (1.6-43)	7.2 (5.7-7.9)
02231000	St. Marys River near MacClenny, Florida	SM	700	Forest (48)	38	Low organic sand	Ombrotrophic wetlands	0.4 (<0.04-1.03)	39.3 (8.8-77)	4.8 (2.9-7.5)
02234998	Little Wekiva River near Longwood, Florida	LW	44.5	Urban (73)	5.3	Low organic sand with areas of high organic sand	Ground water and stormwater runoff	0.08 (<0.04-0.44)	4.9 (1.7-10.3)	7.2 (6.9-7.5)

Note: Surface water filtered MeHg, dissolved organic carbon and pH values are means with range in parentheses. Land characteristics data is based on enhanced 1993 Multi-Resolution Land Characteristic data (Nakagaki and Wolock, 2005; USGS, 1990; Vogelmann *et al.*, 2001).

Michigan Drainages in Wisconsin (Wisconsin), and Georgia-Florida Coastal Drainages (Florida) (Figure 1). In each basin, we examined one river in an urban watershed and one reference river in a rural watershed with minimal or no cultivated agriculture and low wetland density. Additionally, in the Florida and Wisconsin basins, we sampled one reference river in a high-wetland-density watershed with minimal or no cultivated agriculture. Land cover percentages for sampling site drainage basins were determined in a Geographic Information Systems environment. The 30-m resolution raster of the 1992 National Land Cover Dataset (Vogelmann *et al.*, 2001) was enhanced to include selected land categories from the USGS Land Use and Land Cover dataset (Anderson *et al.*, 1976; Nakagaki and Wolock, 2005). This raster was then further enhanced to include 1990 and 2000 population density data by block group (U.S. Bureau of the Census, 1991, 2001; Kerie J. Hitt, USGS, written communication, 2006). The resulting layer was clipped to sampling site drainage basins (delineated using USGS 1:24,000 topographic maps); land cover data for each basin were summarized to level 1 classes, with the exception of urban/recreational grasses class, which was considered developed.

Periphyton sampling was closely coordinated in space and time with the larger study's spring and fall Hg sampling of surface water, higher biota (invertebrates and small fish), streambed sediment, and sediment porewater at each site. Streambed sediment and porewater were collected five times over the course of 1 year, with the last sampling effort being an intensive spatial sediment sampling to determine streambed substrate throughout the river reach sampled. Surface water was collected 12-18 times a year for 2 years for analysis of THg, MeHg, pH, dissolved organic carbon, and other ancillary measures. Water and sediment samples were collected according to Olson and DeWild (1999). Other biota, including three species of invertebrates, two types of forage fish, and one species of predator fish were collected at each river during the spring and fall sampling events when the periphyton were collected; however, those data are not included in our report.

Periphyton samples were collected using protocols developed for the USGS NAWQA program modified for clean techniques to minimize the potential for sample contamination due to sampling procedures (Bell and Scudder, 2004; Moulton *et al.*, 2002; Porter *et al.*, 1993). Two composite periphyton samples were collected from substrates where periphyton growth dominates in rivers. Those substrates were: (1) cobble or woody snags and (2) streambed surface-sediment (surface-sediment periphyton hereafter) collected from each of the eight sites during spring and fall of 2003.

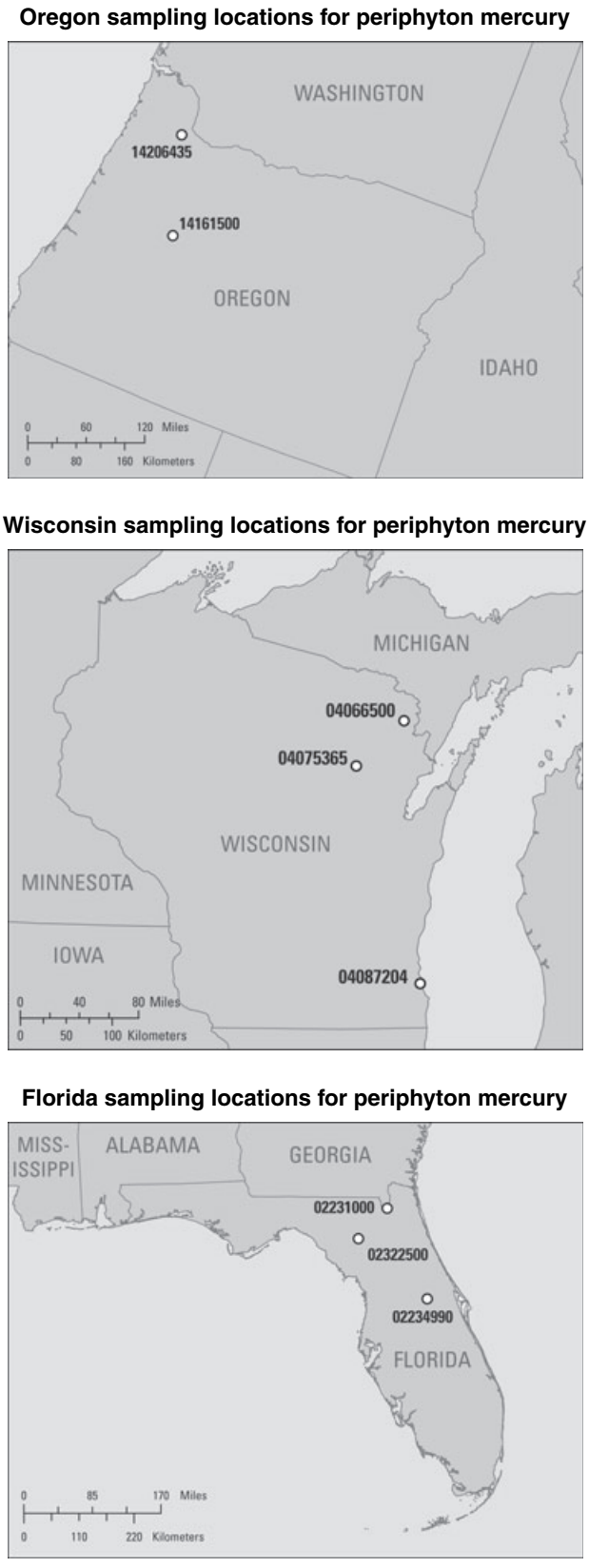

FIGURE 1. Locations of the Eight Studied Rivers (Bell and Scudder, 2004).

Cobble or woody snags were collected so that the substrate was consistent throughout each NAWQA basin. In Oregon and Wisconsin streams, cobbles were the preferred substrate; in Florida streams, woody snags dominated the otherwise sandy streambeds. Five cobble or woody snag sub-samples were collected from five locations within each river reach and composited into a single sample per river. Cobble and woody snag periphyton samples were collected by brushing and scraping periphyton from a measurable area into a Teflon bottle. Each collection area was measured using the foil template method after periphyton removal; areas for composited samples ranged from 570 to 5,720 cm^2 for cobble and 1,430 to 2,270 cm^2 for woody snags (Porter *et al.*, 1993).

Five surface-sediment periphyton samples at three locations within each river reach were collected and composited per river from fine sediment such as silt/clay or sand, as appropriate, for a total area of 294.5 cm^2 per composite sample. The surface-sediment sample was collected using an inverted Teflon Petri dish and sheet to capture the upper 0.5 cm of periphyton and sediment in a shallow quiescent depositional zone with visible algal growth. The surface-sediment periphyton sampling technique does not discern between periphyton, bacteria and other microbes, detritus, and sediment that were captured during collection; however, each of the surface-sediment samples collected for this study were decanted to remove sand and larger sediment particles. Additional details of the sampling methodology used in this study can be found in Bell and Scudder (2004).

Separate sub-samples were removed from each composite periphyton sample and analyzed for THg, MeHg, chlorophyll *a*, ash-free dry biomass, and taxonomy. Concentrations of THg and MeHg in the sediment, surface water, porewater, and periphyton samples were determined at the USGS Mercury Research Laboratory in Middleton, Wisconsin, using Hg analysis methods described in USEPA Method 1631 (USEPA, 2002) for THg and in DeWild *et al.* (2002, 2004) for MeHg. The USGS National Water-Quality Laboratory in Denver, Colorado, determined periphyton chlorophyll *a* and ash-free dry biomass (Arar and Collins, 1997). Periphyton taxonomy was determined according to Prescott (1962, 1970) and Wehr and Sheath (2003).

The ash-free dry biomass (biomass hereafter) and chlorophyll *a* data were used to calculate the Hg concentrations in periphyton on a dry weight basis and estimate the relative biovolume of live algal cells in the periphyton sample. The biomass of samples was analyzed to determine the mass of biological organic material in the sample; whereas, chlorophyll *a* in periphyton samples was used to estimate the standing crop of algae in the sample (Steinman and Lamberti, 1996).

Quality control procedures for the collection and processing of periphyton samples included collection of approximately 17% replicate samples. Replicate values for all periphyton analytical parameters were found to be within 5% of targeted values. For analysis of THg and MeHg, a certified biological reference material of mussel tissue (IAEA-2976) was used. This reference material was chosen because at the time of analysis, there was no certified reference material (CRM) for plant tissue for THg and MeHg. Additionally, the mussel tissue CRM was used because the CRM for sediments does not contain the organic matter content that the periphyton samples do, and the THg and MeHg concentrations in the mussel tissue CRM were similar to those in the periphyton samples.

RESULTS AND DISCUSSION

Data were examined for potential correlations between THg and MeHg in periphyton and ash-free dry biomass, chlorophyll *a*, and taxonomy. Correlations were also examined between THg and MeHg in periphyton samples and (a) surface water (dissolved and particulate THg and MeHg), (b) sediment (THg and MeHg,), and (c) filtered sediment porewater (THg and MeHg). To determine whether any apparent differences among groupings of data were statistically significant, the nonparametric Kruskal-Wallis rank analysis of variance test was used, followed by a Tukey multiple-comparison procedure (SAS Institute Inc., 1989). Unless otherwise stated, all significant correlations (r) discussed are for values of $p < 0.05$.

Mercury in Periphyton

Periphyton samples were collected and compared based on the area sampled. An areal burden was determined by multiplying the concentration of Hg in the periphyton sample by the volume of sample collected and filtered, divided by the known area of sampled substrate. MeHg and THg (ng/m^2) areal burden in periphyton showed a strong positive correlation when all samples were used (Figure 2, $r^2 = 0.88$, $p < 0.001$, $n = 32$, in log-log space). No difference in concentration of MeHg or THg was found between seasons. Both MeHg and THg areal burden in periphyton had a strong positive correlation to biomass (Figure 3a and b, $r^2 = 0.44$, $p < 0.001$ and $r^2 = 0.51$, $p < 0.0001$, respectively).

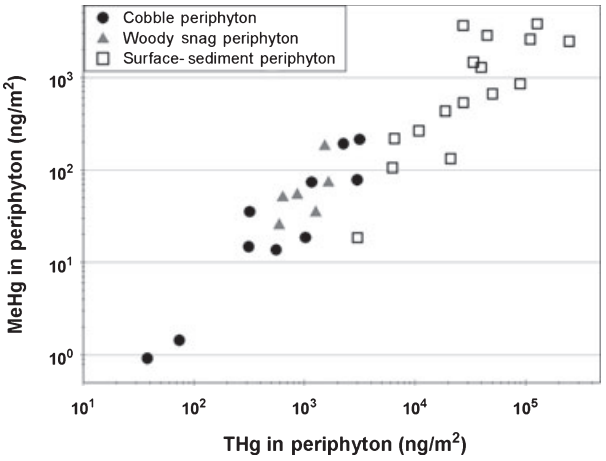

FIGURE 2. Areal Burden of Methylmercury (nanogram per square meter) *vs.* Total Mercury (nanogram per square meter) in Periphyton on Dry Weight Basis. Cobble periphyton, n = 10; woody snag, n = 6; surface-sediment, n = 16.

Generally, samples with higher biomass had higher MeHg and THg areal burdens, but it appeared that a leveling off of Hg areal burden occurred as biomass increased after roughly 100 g/m^2 of biomass. These findings were consistent with those from Pickhardt *et al.* (2002) who stated that as algal cell abundance increases, Hg burden per cell decreases. However, Moye *et al.* (2002) found that, for the alga *Cosmarium*, older cultures experiencing stationary growth had greater MeHg uptake rates than cultures that were experiencing exponential growth. The apparent equilibrium response of our samples may be because of a combination of those two situations. One might predict that Hg concentrations would decrease with increasing biomass; however, the periphyton matrix could have been incorporating MeHg at a higher rate because the periphyton cells were older.

Biomass and chlorophyll *a* are typical analyses performed to determine periphyton growth and live or standing crop of algal cells. All of the sediment areas sampled displayed active algal growth as indicated by green to yellow-brown surface film, presence of small bubbles on the surface of the sediment suggesting active photosynthesis, and measured chlorophyll *a* concentration. Cobble/woody snag periphyton samples showed an increase of biomass from spring to fall sampling events (Figure 3c). There were many possible reasons why biomass for cobble and woody snag periphyton samples increased throughout the

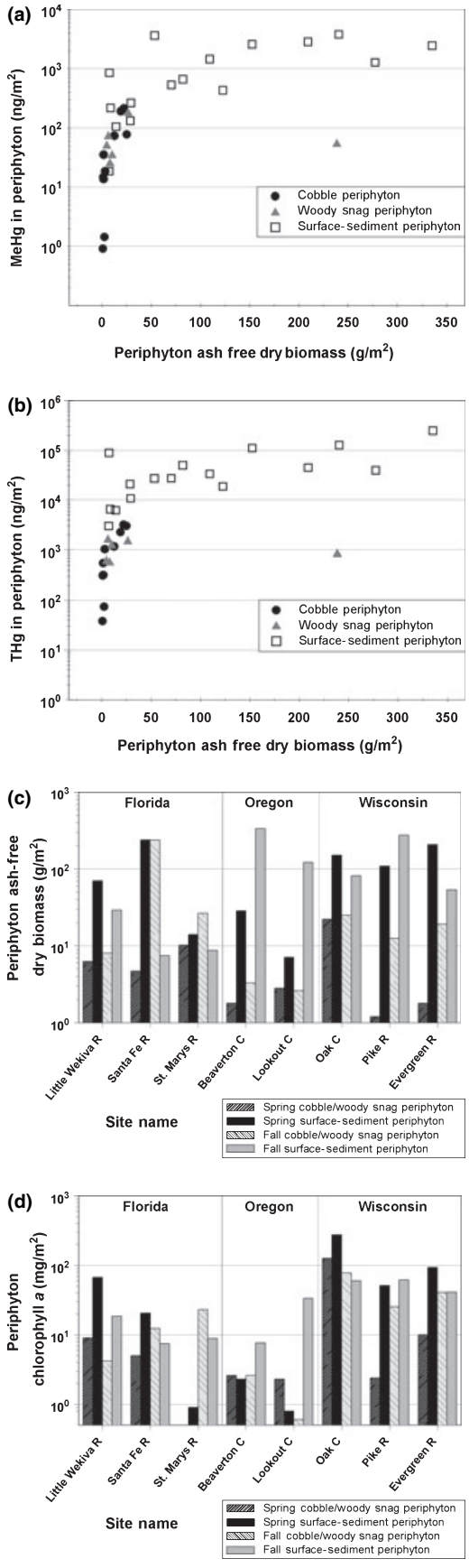

FIGURE 3. Substrate-Based Regression of Dry Weight Methylmercury (a) and Total Mercury (b) Areal Burden in Periphyton (nanogram per square meter), *vs.* Biomass (gram per square meter), and Seasonal comparisons of Biomass (c) (gram per square meter) and Chlorophyll *a* (d) (milligram per square meter). Cobble periphyton, n = 10; woody snag n = 6; surface-sediment, n = 16.

growing season, including normal succession in the periphyton community and increased algal abundance as the year progressed because of nutrient enrichment, increased daylight, increased water temperatures, and organic matter deposition. There was no pattern for chlorophyll *a* concentration in any periphyton samples as the year progressed, suggesting that the standing crop of live algal cells did not undergo a consistent change across substrates or study basins (Figure 3d).

Although there was no significant difference, on a concentration basis, between THg and MeHg in periphyton substrate types (Figure 4a), the areal burden of THg and MeHg in surface-sediment periphyton was higher than in cobble or woody snag periphyton (Figure 2). With the exception of one outlier, the percentage of THg as MeHg in surface-sediment periphyton was the lowest of all three substrates (Figure 4b). Tukey's Studentized Range test showed that the percentage of THg as MeHg for surface-sediment periphyton was significantly lower than woody snag periphyton ($p < 0.01$), but not significantly lower than cobble periphyton. There was also no significant difference between the cobble periphyton and the woody snag periphyton for percentage of THg as MeHg.

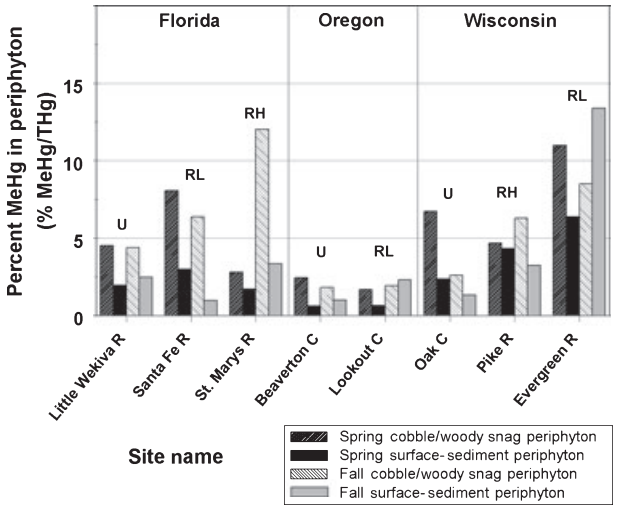

FIGURE 4. Percentage of Total Mercury as Methylmercury in Periphyton. U = urban river, $n = 3$; RL = reference river with low wetland percentage, $n = 3$; RH = reference river with high wetland percentage, $n = 2$; Cobble periphyton, $n = 10$; woody snag periphyton $n = 6$; surface-sediment periphyton, $n = 16$.

In general, surface-sediment periphyton samples had greater biomass than cobble or woody snag periphyton samples. Surface-sediment periphyton samples were collected in depositional areas with little or no flow whereas cobble and woody snags were collected in areas with relatively faster flow. Therefore, the higher Hg areal burdens in surface-sediment periphyton may have been due to the nature of the area sampled; greater amounts of Hg-containing sediment may have been deposited on top of established periphyton mats providing more Hg for accumulation. It was also possible that, despite decanting, fine sediment remaining in the surface-sediment periphyton samples contributed to higher Hg concentrations. Higher mercury (THg and MeHg) areal burdens in surface-sediment periphyton samples compared to cobble/woody snag samples may also be because of the algal species found in those periphyton communities. In all of the surface-sediment periphyton samples, diatoms (division Chrysophyta) were the dominant cells found (>40% of total periphyton cells), followed by blue-green algae (division Cyanophyta) (>20%), and green algae (division Chlorophyta) (>10%). In contrast, the cobble and woody snag periphyton samples contained more blue-green and green algal cells than diatom cells (Bell and Scudder, 2004).

There was considerable variation in the percentage of THg as MeHg in periphyton (Figure 5). This variation may be the result of several factors including the variety of periphyton taxa in the samples collected. Each division, genus, or even species of periphyton accumulates Hg at different rates similar to the way different fish species accumulate Hg at different rates (Hacon *et al.*, 1997; Neumann and Ward, 1999; Miles *et al.*, 2001; Moye *et al.*, 2002). Moye *et al.* (2002) found that different algal divisions (diatoms, greens, blue-greens) accumulated Hg at different rates, and accumulation rates were significantly different at the species level within the green algae division. Natural periphyton populations may contain several different algal divisions and tens to hundreds of different algal species in each area sampled, as was the case with this study. This conglomeration of periphyton could lead to a large range in the amount of THg and the percentage of MeHg within the sites sampled. Geochemical microenvironments in which the periphyton reside also could have a strong influence on Hg methylation and uptake of MeHg (Planas *et al.*, 2004).

No significant difference was observed between landscape types for areal burdens of Hg in periphyton (Figure 6a). With the exception of the Evergreen River in Wisconsin, there was no significant difference in the percentage of THg as MeHg between landscape types (Figure 6b). However, the ranges of percentages varied greatly among the different landscape types. This may be because of highly varying rates of Hg methylation by bacteria in these rivers, and presence, absence, or proximity of more actively methylating environments, such as wetlands in the watershed (Hurley *et al.*, 1995; Marvin-DiPasquale and Agee, 2003).

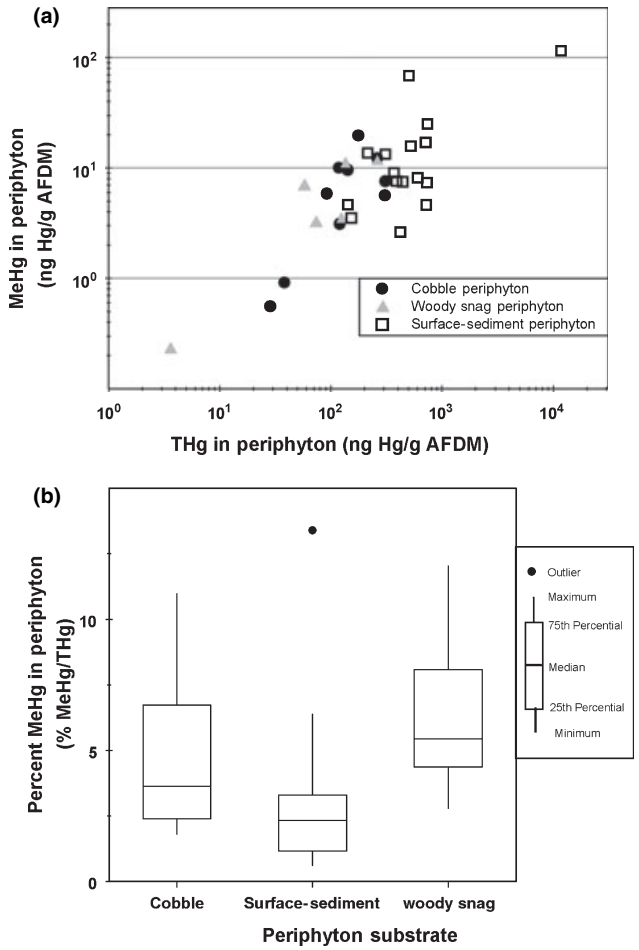

FIGURE 5. Substrate-Based Comparisons of Methylmercury (MeHg) Concentration *vs.* Total Mercury (THg) Concentration (nanogram per gram) and Percent THg as MeHg in Periphyton. Periphyton concentrations are expressed per gram of periphyton ash-free dry mass. Cobble periphyton, $n = 10$; woody snag periphyton, $n = 6$; surface-sediment periphyton, $n = 16$.

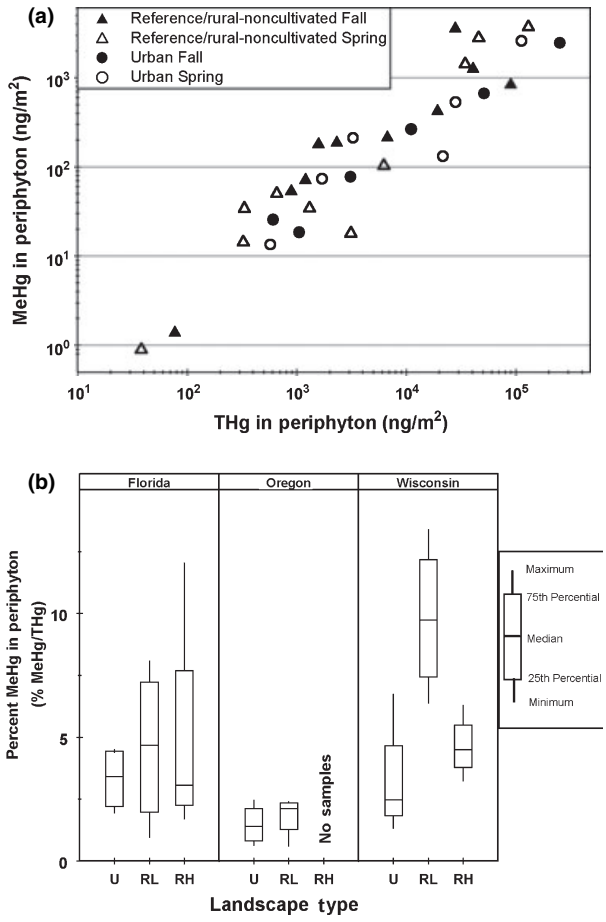

FIGURE 6. Landscape-Based Comparisons of Percentage of Total Mercury (THg) as Methylmercury (MeHg) in Periphyton and Percent THg as MeHg in Periphyton. U = urban river, $n = 3$; RL = reference river with low wetland percentage, $n = 3$; RH = reference river with high wetland percentage, $n = 2$.

Relation of Periphyton Mercury to Other Geochemical Characteristics

The relation between Hg concentrations in periphyton on different substrates and Hg concentrations in filtered surface water created wedge-shaped distributions (Figure 7a and b). THg and MeHg concentrations in cobble periphyton were not significantly related to filtered surface water MeHg or THg concentrations. Woody snag periphyton MeHg concentrations increased with higher concentrations of THg and MeHg in filtered surface water, while surface-sediment periphyton MeHg concentrations decreased with increasing concentrations of THg and MeHg in filtered surface water. The wedge-shaped distributions showed considerable variability in periphyton THg and MeHg at low filtered surface water THg and MeHg concentrations. In contrast, at higher filtered surface water THg and MeHg concentrations, periphyton Hg concentration became less variable; suggesting that Hg concentrations in surface water were not a limiting factor for Hg assimilation in periphyton. These distributions indicate that processes in addition to sorption from the surface water were likely important in contributing to Hg concentrations in periphyton, depending on the specific environment sampled. Our results were limited by sparse data at higher filtered surface water Hg concentrations. Surface-sediment periphyton concentrations of THg or MeHg were not related to porewater Hg concentrations. The THg concentrations in surface-sediment periphyton were positively correlated ($p < 0.0001$, $r^2 = 0.50$) to THg in sediment when the high values of both surface-sediment periphyton and streambed sediment from the Santa Fe River, Florida were not included.

A positive relation was found between MeHg in periphyton and MeHg in the particulate fraction of surface water ($r^2 = 0.52$) and between THg in

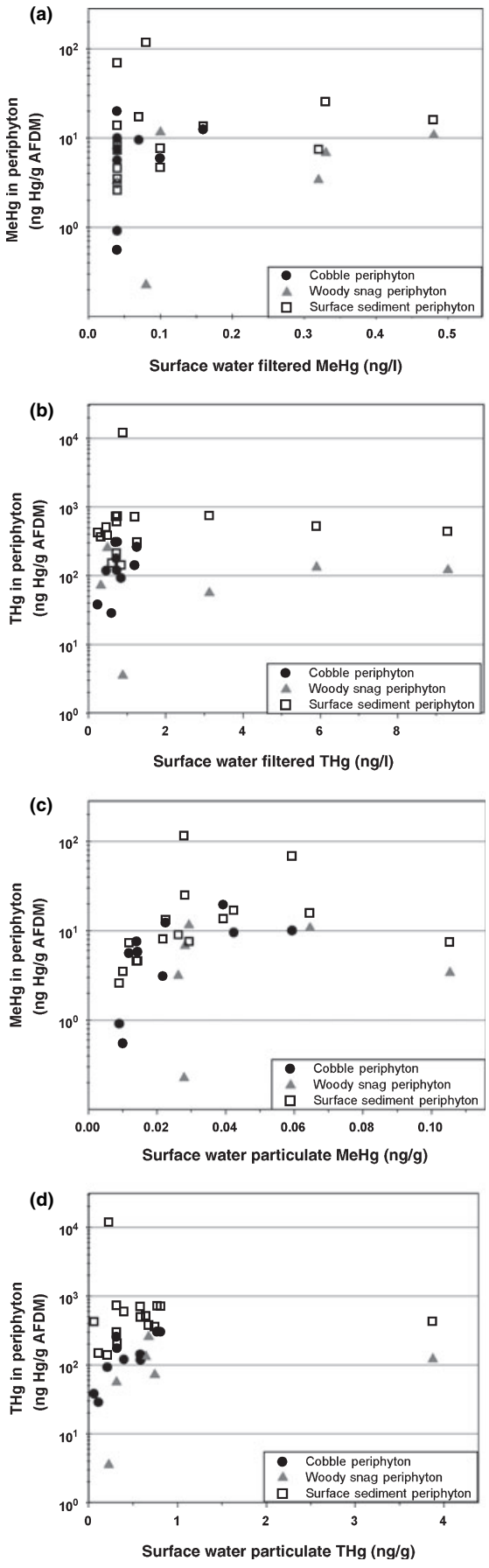

FIGURE 7. Methylmercury (MeHg) Concentrations and Total Mercury (THg) Concentrations in Periphyton (nanogram per gram) *vs.* Filtered and Particulate Surface MeHg and THg. All periphyton concentrations are expressed per gram of periphyton ash-free biomass.

TABLE 2. Table Showing Mean $\log_{10}$-Transformed Bioaccumulation Factors (logBAF) for Periphyton [$_{\log}$BAF=$\log_{10}$ (Cb/Cm), Where Cb and Cm Are the Mercury Concentrations in Biota and the Medium of Interest].

	THg	MeHg
Surface water, filtered (n = 32)	5.36 (0.68)	4.92 (0.58)
Surface water, particulate (n = 32)	5.73 (0.62)	5.42 (0.48)
Streambed, sediment (n = 16)	4.44 (0.67)	4.29 (0.60)
Porewater, filtered (n = 16)	5.02 (0.65)	4.40 (0.54)

Note: Standard deviations are in parentheses.

periphyton and THg in the particulate fraction of surface water (r^2 = 0.57, Figure 7c and d). These correlations indicate that Hg in periphyton was more closely related to Hg in the particulate fraction than in the dissolved fraction of surface water. Suspended particulates in the surface water can settle out of the water column and be incorporated into the periphyton matrix, which could explain the relation of periphyton Hg concentrations to Hg concentrations in the particulate fraction of surface water.

The $\log_{10}$-tranformed bioaccumulation factor [logBAF= $\log_{10}$ (*Cb/Cm*), where *Cb* and *Cm* are the Hg concentrations in biota and the medium of interest, respectively] (log BAF hereafter) is the ratio between the dry weight Hg concentrations of the organism and the Hg concentration of the medium that organism is found in or on (Table 2) (Watras and Bloom, 1992). Lower logBAFs indicate less difference in Hg concentrations between biota and the medium of interest. The range of our logBAFs (3.46-6.23) for MeHg in biota and filtered surface water was comparable to published phytoplankton and surface water logBAFs from Watras *et al.* (1998) and Miles *et al.* (2001), with values from previous studies ranging from 3.5 to 6.5 for MeHg. Median periphyton logBAFs from this study were slightly lower than literature values, possibly because of the differences in the organisms and ecosystems sampled. The logBAFs can vary greatly depending on the organism, the river that organism was found in, the medium the organism lives on, and the time of year the samples were collected (Watras and Bloom, 1992; Watras *et al.*, 1998; Miles *et al.*, 2001). The Watras *et al.* (1998) and Miles *et al.* (2001) studies were conducted in lakes and laboratories where conditions were more stable; whereas, in our study, water surrounding the periphyton in the river systems was constantly moving and Hg concentrations were temporally and hydrologically dynamic.

The logBAFs for streambed sediment and porewater were based solely on the surface-sediment periphyton because cobble and woody snag periphyton do not directly interact with the sediment or porewater. Although not statistically significant, the surface-sediment periphyton to streambed sediment logBAFs reflect smaller increases in periphyton Hg concentrations over sediment concentrations than from surface water or porewater. This was likely due to surface-sediment periphyton closely interacting with streambed sediment. The logBAFs are simply a tool to compare concentrations across trophic levels, and it is unclear whether the source of Hg to the surface-sediment periphyton is more from streambed sediment, surface water, or porewater constituents.

Periphyton Transfer of Methylmercury to Higher Trophic Levels

The high areal burdens of THg and MeHg in surface-sediment periphyton samples suggest that periphyton in the sampled streams could be a significant pool of MeHg at the base of aquatic food webs and a key pathway for transfer of THg and MeHg to higher trophic levels. Previous studies have shown that most (95-99%) THg in fish is MeHg and that fish accumulate the majority of Hg from their diet (Huckabee *et al.*, 1979; Grieb *et al.*, 1990; Bloom, 1992). Preliminary data from the Wisconsin rivers showed that some older blacknose dace had surprisingly high THg concentrations for small forage fish. Some of the blacknose dace THg concentrations were similar to, and even exceeded THg concentrations of older brown trout (*Salmo trutta*) collected at the same river (Scudder *et al.*, 2004) and the THg concentration for one blacknose dace (*Rhinichthys atratulus*) was at the USEPA criterion (0.3 μg/g wet weight) (USEPA, 2001). Blacknose dace are known to consume diatoms and other surface-sediment periphyton in amounts up to 25% of their diet (Breder and Crawford, 1922; Becker, 1983). If blacknose dace ate diatoms found in the surface-sediment periphyton, which has been shown in this study to have the highest Hg and biomass concentrations, they would have been consuming more Hg than if they were grazing on cobble periphyton or woody snag periphyton.

CONCLUSIONS

Results of this study indicate that the importance of Hg contribution from periphyton depends greatly on ecosystem-specific factors, such as periphyton biomass in the habitat, periphyton as a food source, background Hg concentrations in the physiochemical environment, and sources of Hg to the ecosystem. High bioaccumulation factors between aqueous and periphyton MeHg show a substantial bioconcentration of Hg in the periphyton matrix. In the studied rivers, areal burdens of both THg and MeHg in periphyton have a strong positive correlation to periphyton biomass, and surface-sediment periphyton had the highest median values for both areal burden and biomass but the lowest ratio of MeHg:THg. We found no differences in Hg areal burdens between the urban or reference landscape types. Differences in watershed land cover, wetland density, wetland proximity, and Hg loading and availability to the rivers may be more important controlling factors than land use. Concentrations of MeHg in periphyton communities were highly variable at low Hg concentrations in filtered surface water, suggesting the possibility that periphyton accumulate Hg via multiple pathways or mechanisms; however, at higher Hg concentrations in filtered surface water, periphytic Hg had low variability within a stream, regardless of habitat or substrate, suggesting Hg sorption from the aqueous environment is important.

This study provides several lines of evidence that suggest that periphyton communities in rivers play a key role in Hg accumulation in riverine food webs. Periphyton were a vital source of organic carbon and nutrients to higher trophic levels in the rivers studied, and high THg and MeHg concentrations in the periphyton matrix can be passed onto the consumers when they graze on periphyton communities, especially periphyton communities with a higher number of diatoms. Surface-sediment periphyton were found to have the highest areal burden of MeHg and contained a greater number of diatoms relative to cobble or woody snag periphyton communities. Diatoms, in turn, can be a large portion of the diet of some consumers such as many benthic invertebrates and blacknose dace. Based on the findings of this study, periphyton in riverine systems may play a significant role in trophic transfer of Hg between the water column and higher trophic levels.

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