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Mercury methylation in periphyton of the Florida Everglades

Abstract—Trophic accumulation of mercury (Hg) in aquatic ecosystems is of global concern due to health effects associated with eating fish with elevated Hg levels. The methylated form of Hg bioaccumulates so it is important to understand how inorganic Hg is transformed to methylmercury in the environment. Here, a new site for Hg methylation, the periphyton communities that are prevalent in the Florida Everglades, is described. It is hypothesized that periphyton communities that support an active microbial sulfur cycle support Hg methylation. This new methylation site has implications for trophic transfer of methylmercury since periphyton can be the base of the food web in aquatic ecosystems.

Mercury accumulation in food webs is a global problem (Clarkson 1990; U.S. Environmental Protection Agency 1997) that is exacerbated in wetlands (St. Louis et al. 1994), including the Florida Everglades (Ware et al. 1990; Cleckner et al. 1998). Increased rates of atmospheric mercury (Hg) deposition relative to historical times worldwide (Mason et al. 1994) may contribute to increased production of methylmercury (MeHg) in aquatic ecosystems. Mercury is methylated in situ through the action of microorganisms (Winfrey and Rudd 1990; Gilmour and Henry 1991), and it is the methylated form that bioaccumulates in food webs (Bloom 1992). Because anthropogenically derived Hg must undergo methylation before it bioaccumulates in food webs, MeHg production is a key process in the complex biogeochemical cycle of Hg.

Wetlands in particular support elevated Hg methylation rates and MeHg concentrations (St. Louis et al. 1994; Hurley et al. 1995; Krabbenhoft et al. 1995; Gilmour et al. 1998). Specifically, aquatic sediments and wetland soils have been identified as key locations for MeHg production (Korthals and Winfrey 1987; Ramlal et al. 1993; Gilmour et al. 1998; Krabbenhoft et al. 1998*a*), with sulfate-reducing bacteria (SRB) being the important mediators (Compeau and Bartha 1985; Gilmour et al. 1992). Here, a novel site for MeHg production, the periphyton communities that are an abundant component of biomass in many wetlands, including the Everglades, are discussed. The data presented support the following hypothesis: periphyton communities that support an active microbial sulfur cycle, including dissimilatory sulfate reduction, also support Hg methylation. This finding is particularly important because, more so than sediments, periphyton is a direct food source to higher organisms such as invertebrates and small fish (Browder et al. 1994; Hill et al. 1996).

We have been examining factors that affect MeHg bioaccumulation in the Florida Everglades as part of the Aquatic Cycling of Mercury in the Everglades (ACME) study. Our initial studies on Hg and MeHg distribution in water (Hurley et al. 1998), MeHg distribution and production in sediment (Gilmour et al. 1998), diel cycling of Hg (Krabbenhoft et al. 1998*b*), and trophic transfer of MeHg (Cleckner et al. 1998) in the northern Everglades provide detailed site descriptions and background information for the locations discussed here.

Briefly, the main study area is Water Conservation Areas (WCA) 2A and 3A—two large, diked marshes in the northern portion of the remnant Everglades (Hurley et al. 1998). About 60% of the inflow water to WCA 2A originates from the Everglades Agricultural Area and is discharged through a distribution canal on the northern edge of the marsh. As a result, a strong nutrient gradient exists across WCA 2A, with high nutrient levels in the north and lower levels in the south. Sites F1 and U3 were chosen as end members of this gradient in WCA 2A (Fig. 1). WCA 3A is somewhat less affected by agricultural runoff, with precipitation accounting for about 60% of the input water. Our site numbers within WCA 3A begin with a 3A designation and run from 3A33 in the north to 3ATT in the south, with 3A15, our main study site, in central WCA 3A. Cattail (*Typha* spp.) is the dominant vegetation in the eutrophic area of WCA 2A (site F1), while sawgrass (*Cladium jamaicense*), spike rush (*Eleocharis* spp.), and bladderwort (*Utricularia* spp.) are codominant at the other sites.

Everglades periphyton communities, consisting of living algae, bacteria, detrital particulate organic matter, and, in some cases, particulate calcium carbonate, are complex structures that range from filamentous green mats (main algal types are *Spirogyra* spp. and *Mougeotia* spp.) in eutrophic areas to calcareous mats (main algal types are diatoms and blue-green algae) in less impacted areas (Browder et al. 1994; McCormick et al. 1996). The term ''periphyton'' in this paper refers to both ''true periphyton,'' immobile organisms attached to substrates, and ''pseudoperiphyton,'' organisms that are ''free-living, mobile, or creeping within the true periphyton'' (Vymazal et al. 1994). Calcareous periphyton mats are usually attached to submerged macrophytes, particularly *Utricularia* spp. and *Eleocharis* spp., and/or to sediments. However, many mats break away from their substrates and become free-floating because of trapped oxygen formed during photosynthesis (Browder et al. 1994).

Periphyton samples and filtered surface-water samples were collected using clean techniques from ACME study sites (Olson et al. 1997; Cleckner et al. 1998; Hurley et al. 1998). To maintain the integrity of periphyton and associated redox microstructure, there was no effort to separate algae from macrophytes or to remove invertebrates from the periphyton.

Water and periphyton samples were analyzed for MeHg using distillation, ethylation and cold vapor atomic fluorescence spectroscopy (Horvat et al. 1993; Olson et al. 1997). All periphyton samples were analyzed in triplicate, with an average relative standard deviation (RSD) of 19%, while approximately 15% of the water samples were analyzed in duplicate, with a mean relative percentage difference (RPD) of 12%. Additionally, to characterize the types of algae comprising the periphyton communities of the Everglades and to

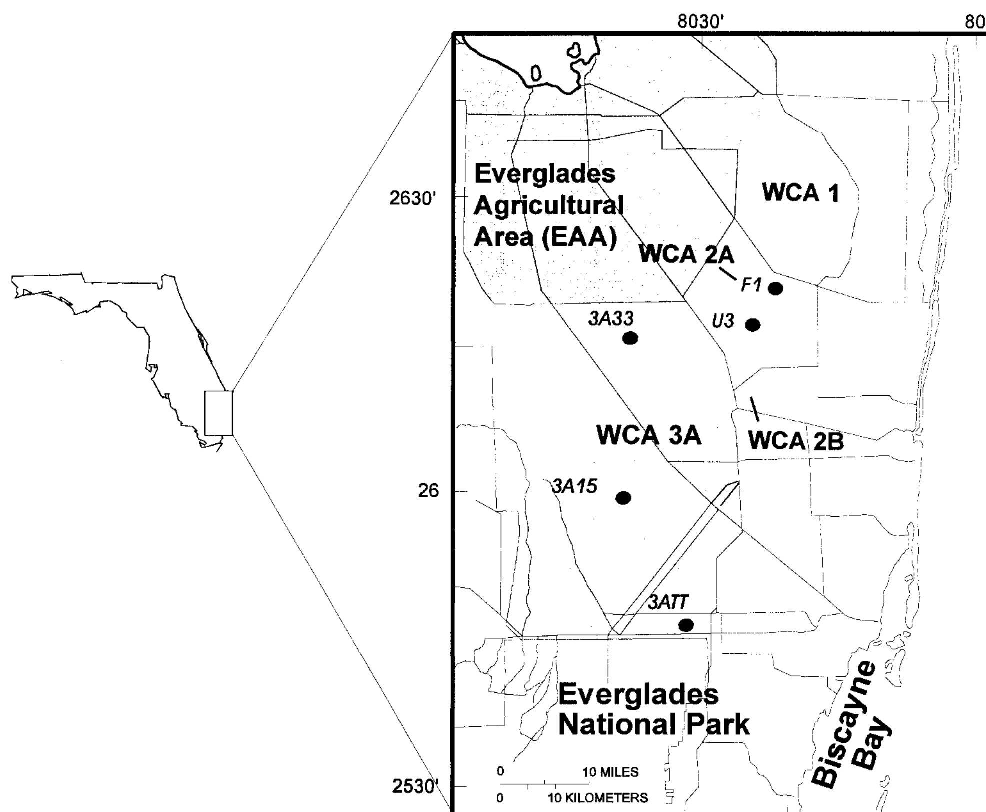


Fig. 1. Map of ACME periphyton sampling sites in the Florida Everglades.

assess relative rates of primary productivity, chlorophylls and carotenoids were determined using an acetone extraction and high-pressure liquid chromatography (HPLC) analysis (Hurley and Watras 1991; Pinckney et al. 1997). A qualitative microscopic assessment of periphyton was also made.

High specific-activity ^{203}Hg (processed through Buffalo Materials Research) was spiked into intact periphyton communities incubated in vitro to assess the potential for MeHg production (Gilmour and Riedel 1995). For these measurements, 20 g of periphyton was incubated with 20 ml of site water in 60 ml clear polycarbonate vials for 2–4 h under ambient light and temperature conditions. Polycarbonate vessels were chosen because this material has optimal light passage with minimal Hg and MeHg sorption to container walls. At the end of the incubation period, Me^{203}Hg was extracted, and its activity was counted. At the time of use, the specific activity of the ^{203}Hg ranged from roughly 15 to 35 mCi mg^{-1} (3.3–8.9 E10 Bq mg^{-1}), and the total amount of ^{203}Hg added to periphyton ranged from 2 to 3 ng g^{-1} wet weight of periphyton. Ambient total Hg concentrations in periphyton ranged from 1 to 4 ng g^{-1} wet weight.

Methylation rates estimated with Hg tracers reflect the bioavailability of the added Hg for methylation as well as the metabolic activities of methylating organisms. Thus, the spike may not have the same bioavailability as Hg in situ. To mimic ambient chemical speciation as closely as possible, solutions containing $^{203}HgNO_3$ were preincubated with site water for 1 h to allow equilibration with dissolved ligands before addition of the spike to periphyton samples. The blank for the methylation measurement is the carryover of inorganic ^{203}Hg during extraction and also includes any Me^{203}Hg created as an analytical artifact during the extraction process. The method detection limit (DL), based on three times the standard deviation of the blank, ranged from 5×10^{-4} to 2×10^{-3} d^{-1} across the sample dates. The average RPD for duplicate extractions was 26%, which was very similar to the RPD between duplicate samples (28%). Blanks and duplicates were determined for >10% of the number of samples for all extraction dates.

Initial work in December 1995 and March 1996 showed high rates of MeHg production under light and dark conditions in periphyton from WCA 2A (Fig. 2A). Methylation rates were highest in periphyton from the more eutrophic site, F1, and were higher in the dark than in the light. Note that because of large differences in rates and concentrations among sites, log scales are used in many of the figures. Dark Me^{203}Hg production in F1 periphyton exceeded the highest rates observed in Everglades surface soils using the same method (Gilmour et al. 1998). Also, methylation rates measured in both sediment and periphyton from the Everglades appeared to be at the high end of the range of rates we had measured in other aquatic ecosystems (Gilmour and Henry 1991; Gilmour et al. 1992; Gilmour and Riedel 1995; Krabbenhoft et al. 1998*a*).

The distribution of MeHg in periphyton relative to water (K_d, Fig. 2B) was highest for F1 periphyton in December 1995, perhaps reflecting relatively high rates of MeHg production within the periphyton and a strong affinity in the

Table 1. Summary of treatments for periphyton amendment experiments (Oremland and Capone 1988; Gilmour et al. 1998).

Amendment (label in Fig. 3)	Final concentration	Expected effect on periphyton methylation	Mechanism
Nutrients (NO_3/PO_4)	100 μM/10 μM	Enhancement	Relieve any nutrient limitation on bacterial and algal growth
Sulfide (N_2/HS)	50 μM	Inhibition	Decrease bioavailability of Hg for methylation
Sulfate (SO_4)	2.5 mM	Enhancement	Relieve any sulfate limitation on bacterial sulfate reduction
Molybdate (MoO_4)	20 mM	Inhibition	Specific inhibitor of sulfate reduction
DCMU	10 μM	Inhibition	Specific inhibitor of photosystem II
Cycloheximide (cyclo)	50 mg liter^{-1}	Inhibition	Broad-spectrum eukaryotic antibiotic
Chloramphenicol (chloram)	60 mg liter^{-1}	Inhibition	Broad-spectrum bacterial antibiotic

periphyton for newly formed MeHg. This is significant because it represents enrichment in MeHg at the bacterial and/or primary producer level that may potentially be consumed by invertebrates and small fish. The MeHg K_d for U3 periphyton was slightly higher in March 1996 than in December 1995. At both of the sites examined in winter 1995 and spring 1996, log K_ds reflected temporal and spatial patterns similar to methylation rates.

The types of algae comprising the periphyton differ greatly among sites within WCA 2A, driven by the nutrient gradient across this system (McCormick et al. 1996; Grimshaw et al. 1997; McCormick et al. 1998). The algal community at F1 is dominated by green filamentous algae and contains higher chlorophyll *a* (Chl *a*) and bacteriochlorophyll levels than the periphyton at U3 (Fig. 2C). Bacteriochlorophyll *a* is produced by purple sulfur bacteria (PSB), microbes that photosynthetically oxidize sulfide to sulfate. In contrast, periphyton at U3 is more visibly calcareous and is dominated by diatoms (fucoxanthin).

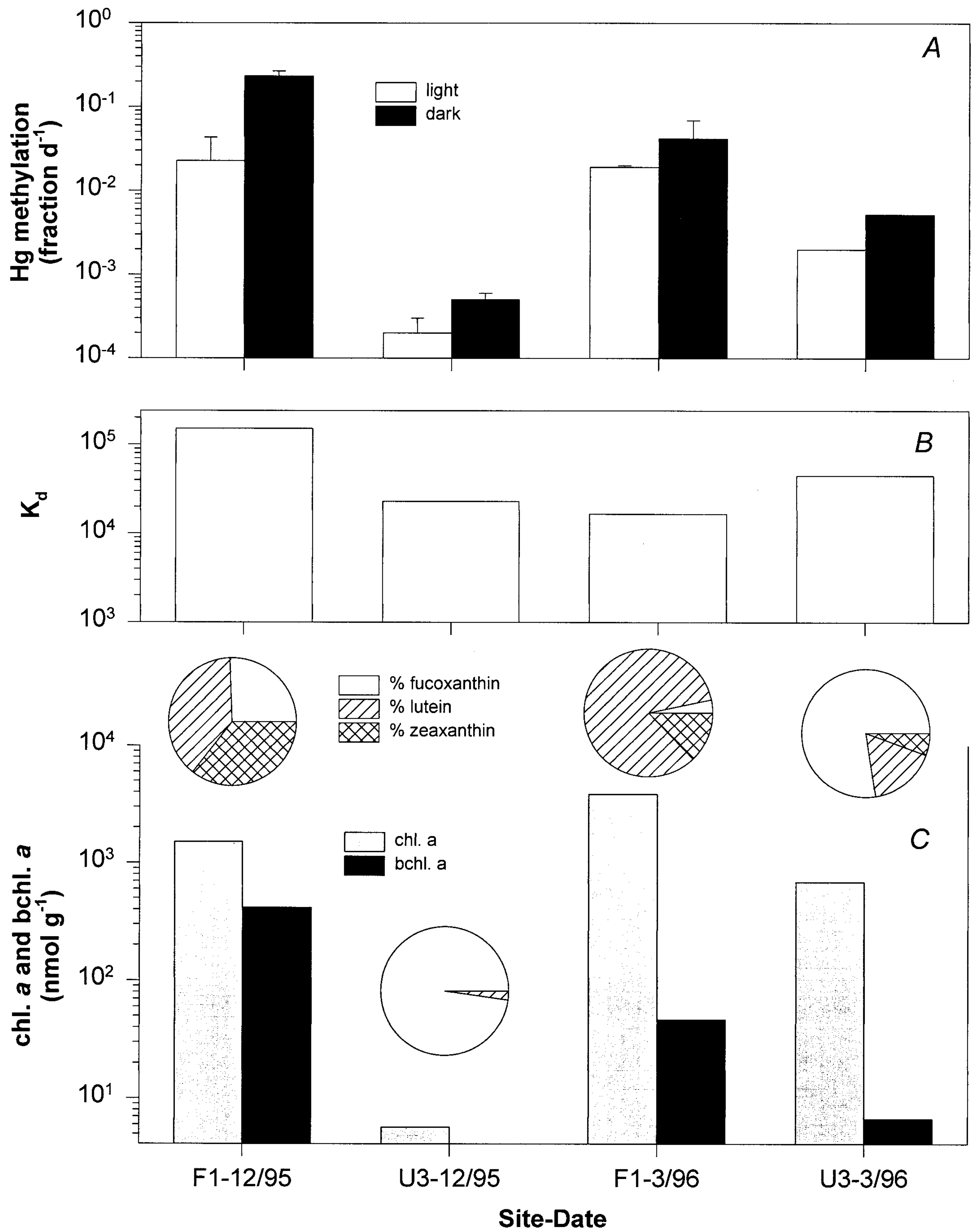
In March 1996, a number of approaches were used to determine which microbial groups were involved in Hg methylation in Everglades periphyton. Inhibitors and stimulants of various microbial processes (Table 1) were added individually to periphyton samples collected at sites F1 and U3 in March 1996, and ^{203}Hg methylation rates were measured. All amendments were buffered to pH 7, and periphyton samples were incubated with amendments for 1 h before injection of ^{203}Hg(II) to allow time for the amendments to take effect.

No Me^{203}Hg was produced in site water without periphyton (Fig. 3). For each treatment other than molybdate, methylation rates in the dark were as high as or higher than in the light. Molybdate significantly reduced methylation to about 5% of the rate in unamended periphyton controls from both sites. This is consistent with the effect of molybdate in many other studies of methylation, both in the Everglades (Gilmour et al. 1998) and in other ecosystems (Compeau and Bartha 1985; Gilmour et al. 1992). Inhibition of methylation by molybdate suggests direct involvement of SRB in the methylating process. However, because of potential chemical effects of molybdate on Hg complexation and bioavailability and because of close links between the activities of SRB and other organisms, molybdate inhibition is not sufficient to solely define the role of SRB in methylation.

Inhibitors 3-(3,4-dichlorophenyl)-1,1 dimethyl urea (DCMU), cycloheximide, and chloramphenicol all decreased methylation rates in F1 periphyton but not significantly (these inhibitors were not tested on U3 periphyton). Large differences in methylation rates among replicate bottles, especially in F1 periphyton samples, decreased our ability to detect treatment effects. Partial inhibition of methylation by DCMU, which blocks oxygenic photosynthesis through photosystem II, suggests that both anoxygenic and oxygenic photosynthesis play a role in periphyton. Mild inhibition of methylation by broad-spectrum antibiotics against bacteria and eukarya suggests that inhibition of algal and microbial activities also affects methylation rates. However, at the concentrations used in these experiments, chloramphenicol and cycloheximide were not completely inhibitory against the organisms that produce MeHg directly. We have previously found that a number of strains of Hg-methylating SRB are resistant to chloramphenicol (Henry 1992).

Direct addition of inorganic nutrients (NO_3 + PO_4) did not affect methylation rates in F1 periphyton over the time course of these experiments, but it did decrease methylation rates in both the light and dark in U3 periphyton. The lack of stimulation by inorganic N or P suggests that neither algae nor denitrifying bacteria are directly involved in methylation and/or that the activities of methylating organisms are not nutrient limited during the experimental period. However, these nutrients may stimulate organisms such as denitrifiers or blue-green algae that compete against Hg-methylating organisms. It is important to recognize that inorganic nutrients, over longer time frames, will stimulate algal growth, leading to additional organic substrate for methylating bacteria, although this effect is not likely to occur during the short time course of these experiments.

Sulfate additions did not significantly affect methylation rates relative to the unamended periphyton controls from either site, while sulfide significantly inhibited methylation in periphyton from U3 but not F1. Sulfate (added to 2.5-mM final concentration) and sulfide (added to 50-μM final con-


A
light
dark
Hg methylation (fraction d-1)
10^0
10^-1
10^-2
10^-3
10^-4
B
Kd
10^5
10^4
10^3
% fucoxanthin
% lutein
% zeaxanthin
C
chl. a
bchl. a
chl. a and bchl. a (nmol g-1)
10^4
10^3
10^2
10^1
F1-12/95
U3-12/95
F1-3/96
U3-3/96
Site-Date

centration) additions were sufficient to significantly increase concentrations in the incubation flasks. Surface-water sulfate concentrations at F1 and U3 generally ranged from 400 to 500 μM sulfate in 1995–1996, while sulfide generally ranged from 1 to 5 μM. Since methylation was blocked by molybdate, the lack of a sulfate effect may mean that SRB are involved in methylation but are not limited by sulfate at these sites. Sulfide has been shown to inhibit microbial Hg methylation in a number of ecosystems, including the Everglades (Gilmour and Henry 1991; Gilmour et al. 1998). This inhibition may arise through the formation of charged Hg–S complexes that are not available to cells (Benoit et al. 1999).

In summary, the periphyton amendment experiments are consistent with other studies that have shown the importance of SRB in methylation in aquatic sediments and wetland soils. Inhibition of methylation by DCMU and cycloheximide suggests that methylation is also tied to photosynthetic organisms, either directly or indirectly, through coupled biogeochemical cycles. Our past experience shows that sulfide removal stimulates methylation. Therefore, we hypothesize that sulfide oxidation by photosynthetic sulfur bacteria is a potential mechanism whereby photosynthesis could be coupled to Hg methylation. Nevertheless, the higher methylation rates in the dark and the incomplete inhibition of methylation by inhibitors of photosynthesis suggest that the organisms that directly produce MeHg are heterotrophs.

Similar amendment experiments have been performed on soil surface flocs from sites across the Everglades, although inhibitors of photosynthesis were not examined. An experiment from site F1 in December 1995 can be found in Gilmour et al. (1998). In general, the effects of these amendments on methylation in soil surface flocs have been similar to the effects of the same amendments to periphyton. In surface flocs, molybdate consistently blocked methylation. Inorganic nitrate and phosphate additions did not stimulate methylation, although nitrate sometimes inhibited it. Bromoethane sulfonic acid, an inhibitor of methanogenesis, often stimulated methylation. Denitrifiers and methanogens may compete with SRB for organic carbon. The effect of sulfate and sulfide on methylation in soils has varied, depending on addition levels and the ambient concentrations of both. At F1 in December 1995, surface sediments responded to sulfide and sulfate in the same way that periphyton at F1 and U3 did 3 months later in March 1996. Addition of 2.5 mM sulfate to soil cores did not stimulate methylation, while addition of 50 μM sulfide inhibited methylation. These responses suggest that SRB mediate Hg methylation in surface soils in the Everglades. We have not directly examined the role of photosynthesis in surface flocs, however. Soil surface flocs are very porous organic matter derived from periphyton and other vegetation. In some cases, periphyton mats and the sediment surface are physically indistinguishable.

In June and December 1996, we examined field distributions of Hg methylation, dissimilatory sulfate reduction, and bacteriochlorophyll *a* to test the idea that Hg methylation was linked to the photosynthetic sulfur cycle in periphyton. Sulfate reduction rates (SRRs, Fig. 4A), the concentration of reduced sulfur species (which are end products of dissimilatory sulfate reduction; Fig. 4B), and Hg methylation rates (Fig. 4C) were measured simultaneously in periphyton from three sites in WCA 2A and 3A in June 1996. Dissimilatory sulfate reduction was measured by the reduction of $^{35}SO_4$ spiked into intact periphyton communities as described above for ^{203}Hg(II). Carrier-free $^{35}SO_4$ was diluted with cold sulfate to 400 μCi μM^{-1}, and a total sulfate concentration of 100 μM, before addition to periphyton. Diluted into periphyton incubation bottles, the final concentration of the $^{35}SO_4$ addition was <1 μM. Sulfate reduction was linear in some samples through only 1–2 h; most incubations were carried out over shorter time periods. Sulfate reduction into both acid-volatile (AVS) and chromium-reducible (CRS) reduced sulfur phases was analyzed and summed (Fossing and Jorgensen 1989).

In June 1996, filamentous green algal communities from F1 and heavily decomposed, black periphyton from site 3A15 supported rapid rates of both sulfate reduction and Hg methylation (Fig. 4). Periphyton from these sites also contained higher concentrations of pyritic sulfide than did calcareous periphyton from U3 and 3A15. These measurements demonstrate the presence of active microbial sulfate reduction in photosynthetically active Everglades periphyton as well as in decaying periphyton mats. Mercury methylation and sulfate reduction generally covaried across the sites examined in June 1996 (Fig. 4). However, we feel that it is the balance between the activity of cells that produce MeHg (SRRs) and the bioavailability of Hg, which is negatively affected by the concentration of sulfide, that determines methylation rates. In this data set, for example, SRRs are similar in F1 and black 3A15 periphyton. However, the methylation rate was about 10 times lower in the 3A15 periphyton. We speculate that higher levels of sulfide in the 3A15 periphyton depressed methylation rates. Sulfide appears to accumulate in decaying periphyton once the periphyton drops out of the photosynthetic zone, as evidenced by the black color and high reduced sulfur content of this sample.

In December 1996, once again, the highest methylation rates in periphyton sampled occurred at the most eutrophic site, F1 (Fig. 5). As in December 1995 (Fig. 2), filamentous green algal mats from the eutrophic areas contained high concentrations of bacteriochlorophyll *a*. Methylation rates

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Fig. 2. Methylation, distribution coefficients, and pigment composition of Everglades periphyton collected from WCA 2A in December 1995 and March 1996. Sites and dates are presented on the x-axis. (A) Methylation expressed as fraction per day methylated on a log scale. Each bar represents the average ($n = 2$) fraction of added ^{203}Hg(II) methylated per day + RPD in intact periphyton communities. (B) Methylmercury distribution coefficient (liters kg^{-1}) expressed as K_d = (ng MeHg kg^{-1} DW) (ng MeHg liter^{-1})$^{-1}$. (C) Chlorophyll and bacteriochlorophyll concentrations (nmol g^{-1} DW; carotenoids expressed as relative percentage of the sum of fucoxanthin [diatoms], lutein [green algae], and zeaxanthin [blue-green algae]).

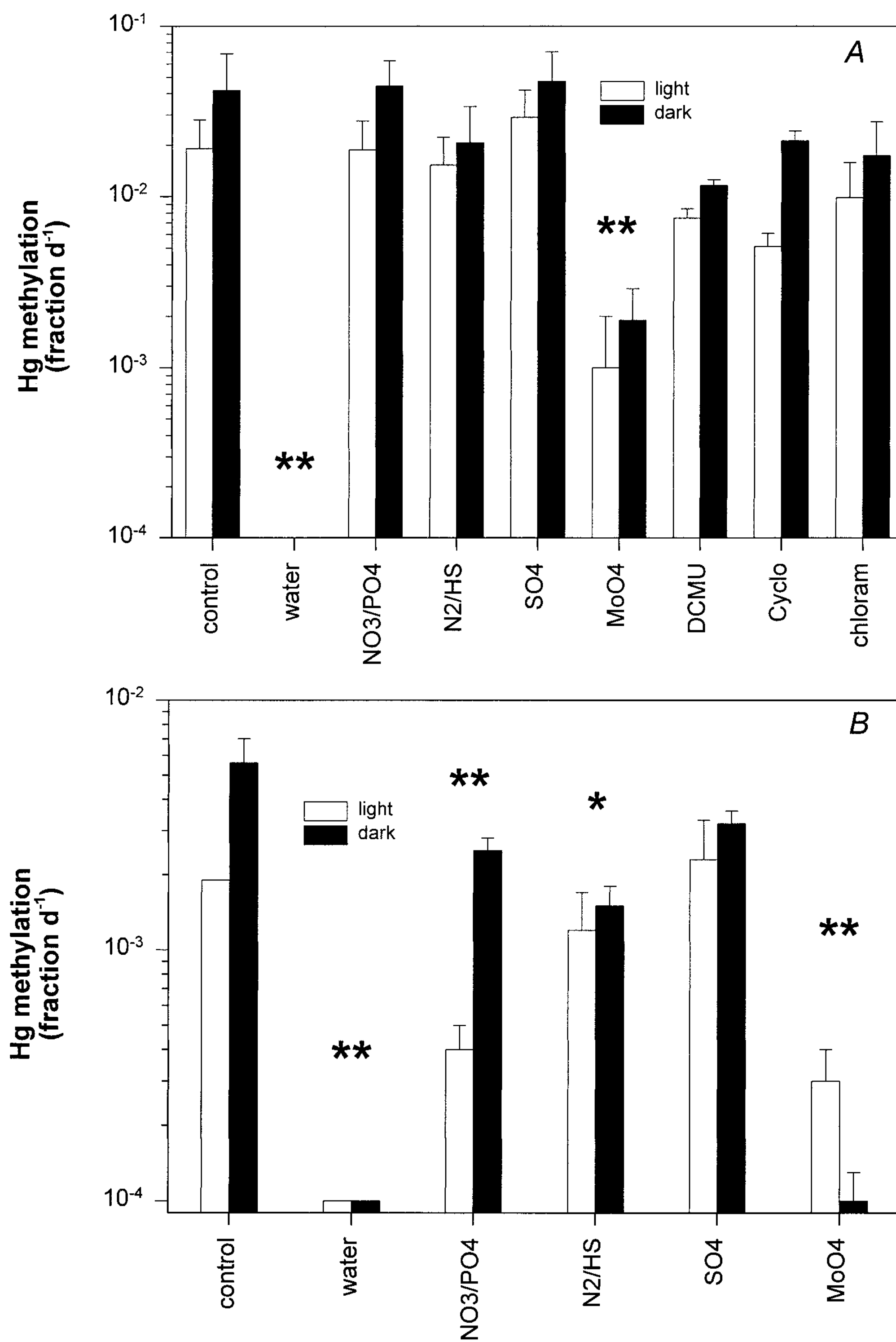

Fig. 3. Effect of amendments on $Me^{203}Hg$ production in site F1 (A) and site U3 (B) periphyton, March 1996. Each bar represents the average ($n = 2$) fraction of added $^{203}Hg(II)$ methylated per day, +RPD, in mostly intact periphyton communities incubated with an equal volume of site water in 60-ml polycarbonate vials. Controls were unamended periphyton samples; methylation rates were also measured in site water without periphyton. Methylation rates in water-only samples were at the detection limit for the analysis, 1×10^{-4} d^{-1}. Treatments significantly different from controls based on a one-tailed Dunnett's *t*-test are marked as ** for $P < 0.01$ and * for $P < 0.10$.

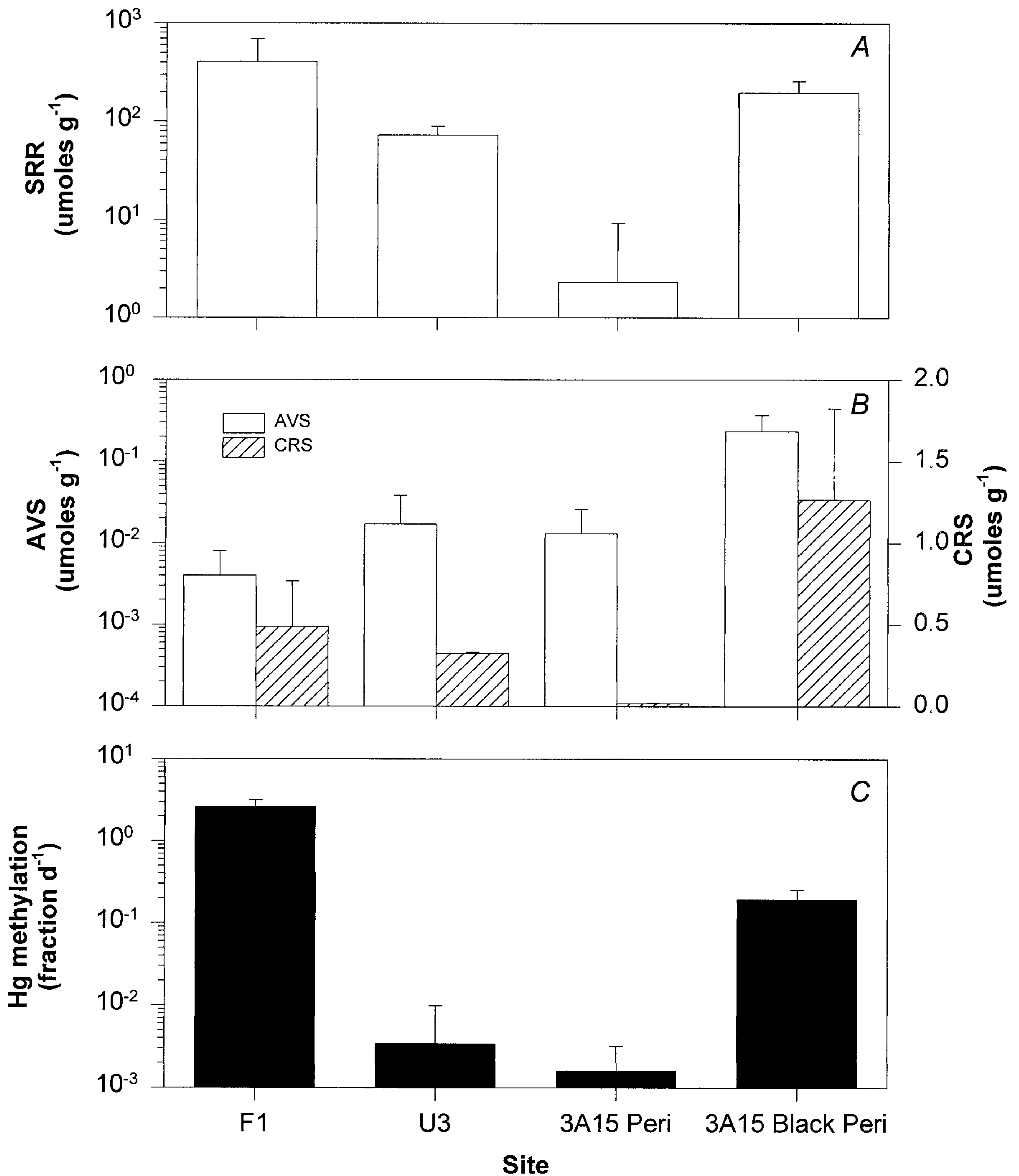

Fig. 4. (A) Dissimilatory SRRs, (B) reduced sulfur compounds, and (C) Hg methylation rates in periphyton from three sites in WCA 2A and 3A, June 1996. SRRs are expressed per gram wet periphyton.

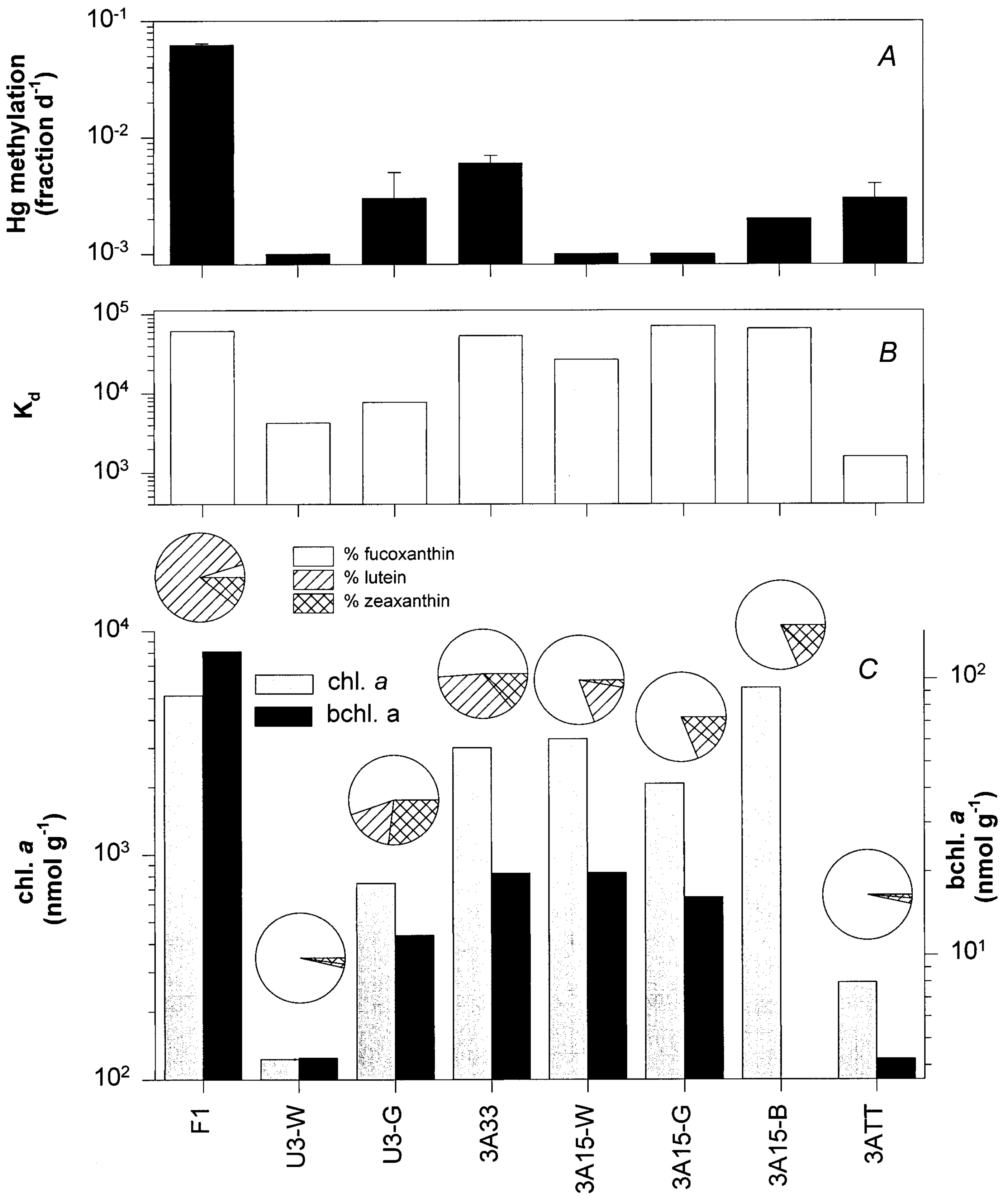

Fig. 5. (A) Mercury methylation, (B) MeHg distribution coefficients, and (C) pigment composition of Everglades periphyton collected in December 1996. Chlorophyll and bacteriochlorophyll concentrations are expressed in nmol g^{-1} dry weight; carotenoids are expressed as relative percentage of the sum of fucoxanthin (diatoms), lutein (green algae), and zeaxanthin (blue-green algae). Sites are listed from north

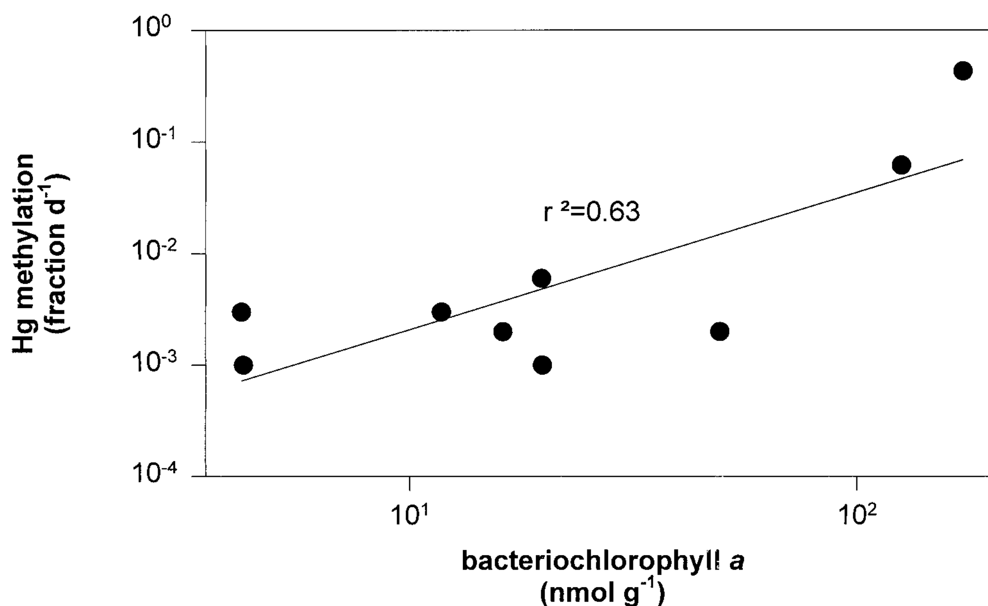

Fig. 6. Bacteriochlorophyll vs. methylation for December 1996 periphyton across the northern Everglades. Data are included for periphyton communities in Fig. 5 and from the Everglades Nutrient Removal project (Miles and Fink 1998).

were lower, and the concentration of bacteriochlorophyll *a* was less, at less eutrophic sites. In general, methylation occurred in periphyton containing substantial bacteriochlorophyll *a,* and bacteriochlorophyll *a* concentrations were well correlated with Hg methylation rates (Fig. 6). However, there were some inconsistencies. For example, 3A15 periphyton showed very low or no methylation in the periphyton, despite having relatively high levels of both Chl *a* and bacteriochlorophyll, while methylation was measured in periphyton at a southern site in WCA 3, 3ATT, despite low bacteriochlorophyll *a* levels. There were also differences in methylation rates between different types of periphyton within the same site. For instance, white calcareous periphyton from site U3 gave a lower methylation rate and had a lower MeHg distribution coefficient (as well as lower chlorophyll and bacteriochlorophyll levels) than did green calcareous periphyton from the same site. In addition, carotenoids indicated that diatoms were the dominant group of algae comprising the white calcareous periphyton, while there were green and blue-green algae present in the green calcareous algae in addition to diatoms.

The presence of both bacteriochlorophyll *a* and dissimilatory sulfate reduction shows that an active photosynthetic microbial sulfur cycle is present in some Everglades periphyton. These communities are particularly active within filamentous green mats at site F1 but are not limited to one type of Everglades periphyton. This community includes heterotrophic sulfate reducers, the presumptive Hg-methylating agents.

The north-to-south spatial distribution of methylation rates in periphyton appears to contrast with patterns of MeHg production and concentration in peat (Gilmour et al. 1998) and MeHg concentrations in biota (Cleckner et al. 1998). Methylmercury concentrations in biota (including fish), sediments, and surface waters are maximal in the Everglades in central WCA 3A (Cleckner et al. 1998; Gilmour et al. 1998; Hurley et al. 1998). One possible reason for this paradox is differences in the food quality of periphyton across the trophic gradient. The periphyton found in the more eutrophic areas is generally composed of filamentous green algae (e.g., *Spirogyra* spp. and *Mougeotia* spp.). These algae are not preferred food sources for zoobenthos, as larger size cells are harder to digest (Burns 1968). Also, the spatial coverage of the filamentous algal communities in the Everglades is much lower than the calcareous types since cattail stands are largely devoid of periphyton (McCormick et al. 1998). Another factor may be variability in the rate of photochemical MeHg decomposition across the trophic gradient, which varies from about 0 to 1% at site F1 and steadily increases southward in the system to about 10–15% d^{-1} in aqueous phase (Krabbenhoft unpublished data). Microbial demethylation in sediments also varies spatially in the Everglades (Marvin-DiPasquale and Oremland 1998). Rates of flux of MeHg from sites of production (surficial peat and periphy-

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to south and include F1 and U3 from WCA 2A and 3A33, 3A15, and 3ATT from WCA 3A. The sites shown represent a rough nutrient gradient from left to right. Two types of periphyton associated with *Utricularia* spp. at site U3 included one visibly white and more calcareous (U3-W) than a green calcareous type (U3-G). For site 3A15, the periphyton communities included one associated with *Utricularia* spp. (3A15-W), an epiphyton coating *Eleocharis* spp. (3A15-G), and a floating blue-green mat (3A15-B).

ton) to the water column and to organisms also affect bioaccumulation and are under investigation as part of the ACME project.

Based on the sum of information presented here, we conclude that some types of Everglades periphyton support an active photosynthetic microbial sulfur cycle. The cycle includes sulfide oxidation by PSB, as evidenced by bacteriochlorophyll *a,* and dissimilatory reduction of sulfate to sulfide by SRB, as measured directly using a radiotracer. This activity appears to be most prevalent in periphyton growing in eutrophic areas of the marsh. Further, Hg methylation occurs very rapidly in periphyton communities that support a photosynthetic microbial sulfur cycle, while little or no methylation occurs in photosynthetically active periphyton in which sulfur reduction and oxidation do not occur. It appears that methylation may also occur in degrading periphyton where sulfate reduction takes place but is not coupled to photosynthetic sulfide oxidation, similar to the situation in surface flocs on Everglades soils. Amendment experiments and field distributions of sulfate reduction and Hg methylation suggest that SRB are the direct methylators in Everglades periphyton, as they are in many aquatic sediments. However, the data presented here do not exclude the possibility that other organisms also participate in methylation.

The unique finding of this study is that methylation in periphyton is also coupled to photosynthesis. Photosynthetic microbial sulfide oxidation may stimulate methylation through the removal of sulfide and/or by providing substrate for SRB. Conditions in sulfur-cycling periphyton appear to be ideal for MeHg production. SRB are very active, while rapid sulfide oxidation by PSB prevents accumulation of sulfide. Sulfide is a strong inhibitor of Hg methylation in Everglades peat (Gilmour et al. 1998) and in other aquatic sediments (Gilmour and Henry 1991). Because periphyton in the Everglades is ubiquitous and integrally associated with the base of the food web as food and/or refuge, the issues of MeHg production, degradation, and accumulation in periphyton, as well as the distribution and control of these processes (e.g., sulfur supply and nutrients), will be critical to understanding the Hg problem in south Florida and other wetlands.

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References

BENOIT, J. M., C. C. GILMOUR, R. P. MASON, AND A. HEYES. 1999. Sulfide controls on mercury speciation and bioavailability in sediment pore waters. Environ. Sci. Technol. **33:** 951–957.

BLOOM, N. S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49:** 1010–1017.

BROWDER, J. A., P. J. GLEASON, AND D. R. SWIFT. 1994. Periphyton in the Everglades: Spatial variation, environmental correlates, and ecological implications, p. 379–419. *In* S. M. Davis and J. C. Ogden [eds.], Everglades: The ecosystem and its restoration. St. Lucie.

BURNS, C. W. 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particle ingested. Limnol. Oceanogr. **13:** 675–678.

CLARKSON, T. W. 1990. Human health risks from methylmercury in fish. Environ. Toxicol. Chem. **9:** 821–823.

CLECKNER, L. B., P. G. GARRISON, J. P. HURLEY, M. L. OLSON, AND D. P. KRABBENHOFT. 1998. Trophic transfer of methyl mercury in the northern Florida Everglades. Biogeochemistry **40:** 347–361.

COMPEAU, G., AND R. BARTHA. 1985. Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microbiol. **50:** 498–502.

FOSSING, H., AND B. B. JORGENSEN. 1989. Measurement of bacterial sulfate reduction in sediments—evaluation of a single-step chromium reduction method. Biogeochemistry **8:** 205–222.

GILMOUR, C. C., AND E. A. HENRY. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. **71:** 131–169.

———, ———, AND R. MITCHELL. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. **26:** 2281–2287.

———, AND G. S. RIEDEL. 1995. Measurement of Hg methylation in sediments using high specific-activity ^{203}Hg and ambient incubation. Water Air Soil Pollut. **80:** 747–756.

———, ———, M. C. EDERINGTON, J. T. BELL, J. M. BENOIT, G. A. GILL, AND M. C. STORDAL. 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry **40:** 327–345.

GRIMSHAW, H. J., AND OTHERS. 1997. Shading of periphyton com-

munities by wetland emergent macrophytes: Decoupling of algal photosynthesis from microbial nutrient retention. Arch. Hydrobiol. **139:** 17–27.

HENRY, E. A. 1992. The role of sulfate-reducing bacteria in environmental mercury methylation. Ph.D. dissertation, Harvard Univ.

HILL, W. R., A. J. STEWART, AND G. E. NAPOLITANO. 1996. Mercury speciation and bioaccumulation in lotic primary producers and primary consumers. Can. J. Fish. Aquat. Sci. **53:** 812–819.

HORVAT, M., L. LIANG, AND N. S. BLOOM. 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part II. Water. Anal. Chim. Acta **282:** 153–168.

HURLEY, J. P., AND OTHERS. 1995. Influences of watershed characteristics on mercury levels in Wisconsin rivers. Environ. Sci. Technol. **29:** 1867–1875.

———, D. P. KRABBENHOFT, L. B. CLECKNER, M. L. OLSON, G. R. AIKEN, AND P. S. RAWLIK. 1998. System controls on the aqueous distribution of mercury in the northern Florida Everglades. Biogeochemistry **40:** 293–310.

———, AND C. J. WATRAS. 1991. Identification of bacteriochlorophylls in lake waters via reverse-phase HPLC. Limnol. Oceanogr. **36:** 307–315.

KORTHALS, E. T., AND M. R. WINFREY. 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl. Environ. Microbiol. **53:** 2397–2404.

KRABBENHOFT, D. P., J. M. BENOIT, C. L. BABIARZ, J. P. HURLEY, AND A. W. ANDREN. 1995. Mercury cycling in the Allequash Creek Watershed, Northern Wisconsin. Water Air Soil Pollut. **80:** 425–433.

———, C. C. GILMOUR, J. M. BENOIT, C. L. BABIARZ, A. W. ANDREN, AND J. P. HURLEY. 1998*a*. Methyl mercury dynamics in littoral sediments of a temperate seepage lake. Can. J. Fish. Aquat. Sci. **55:** 835–844.

———, J. P. HURLEY, M. L. OLSON, AND L. B. CLECKNER. 1998*b*. Diel variability of mercury phase and species distributions in the Florida Everglades. Biogeochemistry **40:** 311–325.

MARVIN-DIPASQUALE, M. C., AND R. S. OREMLAND. 1998. Bacterial methylmercury degradation in Florida Everglades peat sediment. Environ. Sci. Technol. **32:** 2556–2563.

MASON, R. P., W. F. FITZGERALD, AND F. M. M. MOREL. 1994. The biogeochemical cycling of elemental mercury: Anthropogenic influences. Geochim. Cosmochim. Acta **58:** 3191–3198.

MCCORMICK, P. V., P. S. RAWLIK, K. LURDING, E. P. SMITH, AND F. H. SKLAR. 1996. Periphyton–water quality relationships along a nutrient gradient in the Northern Everglades. J. North Am. Benthol. Soc. **15:** 433–449.

———, R. B. E. SHUFORD, J. G. BACKUS, AND W. C. KENNEDY. 1998. Spatial and seasonal patterns of periphyton biomass and productivity in the northern Everglades, Florida, USA. Hydrobiologia **362:** 185–208.

MILES, C. J., AND L. E. FINK. 1998. Monitoring and mass budget for mercury in the Everglades nutrient removal project. Arch. Environ. Contam. Toxicol. **35:** 549–557.

OLSON, M. L., L. B. CLECKNER, J. P. HURLEY, AND D. P. KRABBENHOFT. 1997. Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades. Fresenius J. Anal. Chem. **358:** 392–396.

OREMLAND, R. S., AND D. G. CAPONE. 1988. Use of specific inhibitors in biochemistry and microbial ecology. Adv. Microb. Ecol. **10:** 285–383.

PINCKNEY, J. L., D. F. MILLIE, K. E. HOWE, H. W. PAERL, AND J. P. HURLEY. 1997. Flow scintillation counting of ^{14}C-labeled microalgal photosynthetic pigments. J. Plankton Res. **18:** 1867–1880.

RAMLAL, P. S., C. A. KELLY, J. W. M. RUDD, AND A. FURUTANI. 1993. Sites of methylmercury production in remote Canadian shield lakes. Can. J. Fish. Aquat. Sci. **50:** 972–979.

ST. LOUIS, V. L., J. W. M. RUDD, C. A. KELLY, K. G. BEATY, N. S. BLOOM, AND R. J. FLETT. 1994. The importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Can. J. Fish. Aquat. Sci. **51:** 1065–1076.

U.S. ENVIRONMENTAL PROTECTION AGENCY. 1997. Mercury study report to Congress. EPA-452/R-97-004. U.S. EPA Office of Air. VYMAZAL, J., C. C. CRAFT, AND C. J. RICHARDSON. 1994. Periphyton response to nitrogen and phosphorous additions in Florida Everglades. Algol. Stud. **73:** 75–97.

WARE, F. J., H. ROYALS, AND T. LANGE. 1990. Mercury contamination in Florida largemouth bass. Proc. Annu. Conf. Southeast Assoc. Fish Wildl. Agencies **44:** 5–12.

WINFREY, J. R., AND J. W. M. RUDD. 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. **9:** 853–869.

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