Bioaccumulation of Mercury in Riverine Periphyton

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Contents

Introduction 1
Methods 2
  Collection 2
  Processing 4
  Laboratory Analysis 4
Results 5
Acknowledgments 5
References Cited 8

Figures

1. Location map of the three detailed study units and the U.S. Geological Survey's National Water Quality Assessment Program's study units 1
2. Location maps of the eight study sites for mercury in periphyton, 2003. 3
3. Photo of a wide range of algal species found in the sediment of the St. Marys River, Fla. 5
4. Photo of a Chlorophyta (green algae) *Closterium sp.* at 400X magnification found in the sediment of the Santa Fe River, Fla. 5

Tables

1. Site information for the eight stations sampled for mercury in periphyton, 2003 2
2. Raw data of all periphyton samples 6
3. Divisional classification data of all periphyton samples 7

Bioaccumulation of Mercury in Riverine Periphyton

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Introduction

In aquatic ecosystems, algae are the primary producers and the base of the food web. To date, there has been little research on the role of benthic algae (periphyton) in the bioaccumulation of mercury (Hg) in riverine systems —a key step of the process of bioaccumulation from the physical environment (water and sediments) to higher aquatic organisms (invertebrates, fish, and others). Periphyton has been shown to have an important role in the transfer of mercury in wetlands of the Florida Everglades (Cleckner and others, 1999) and in some situations served as the host site for mercury methylation, which is the key process controlling mercury toxicity in the environment. Pickhardt and others (2002) found that algal blooms in lakes resulted in reduced bioaccumulation of mercury in algal-rich eutrophic lake systems due to decreases in the concentration of mercury per algal cell.

In 2003, the United States Geological Survey (USGS) National Water Quality Assessment (NAWQA) and Toxic Substances Hydrology (Toxics) programs initiated a study to assess mercury bioaccumulation and cycling in eight differing stream-ecosystem settings across the U.S. One aspect of this project involved a detailed examination of

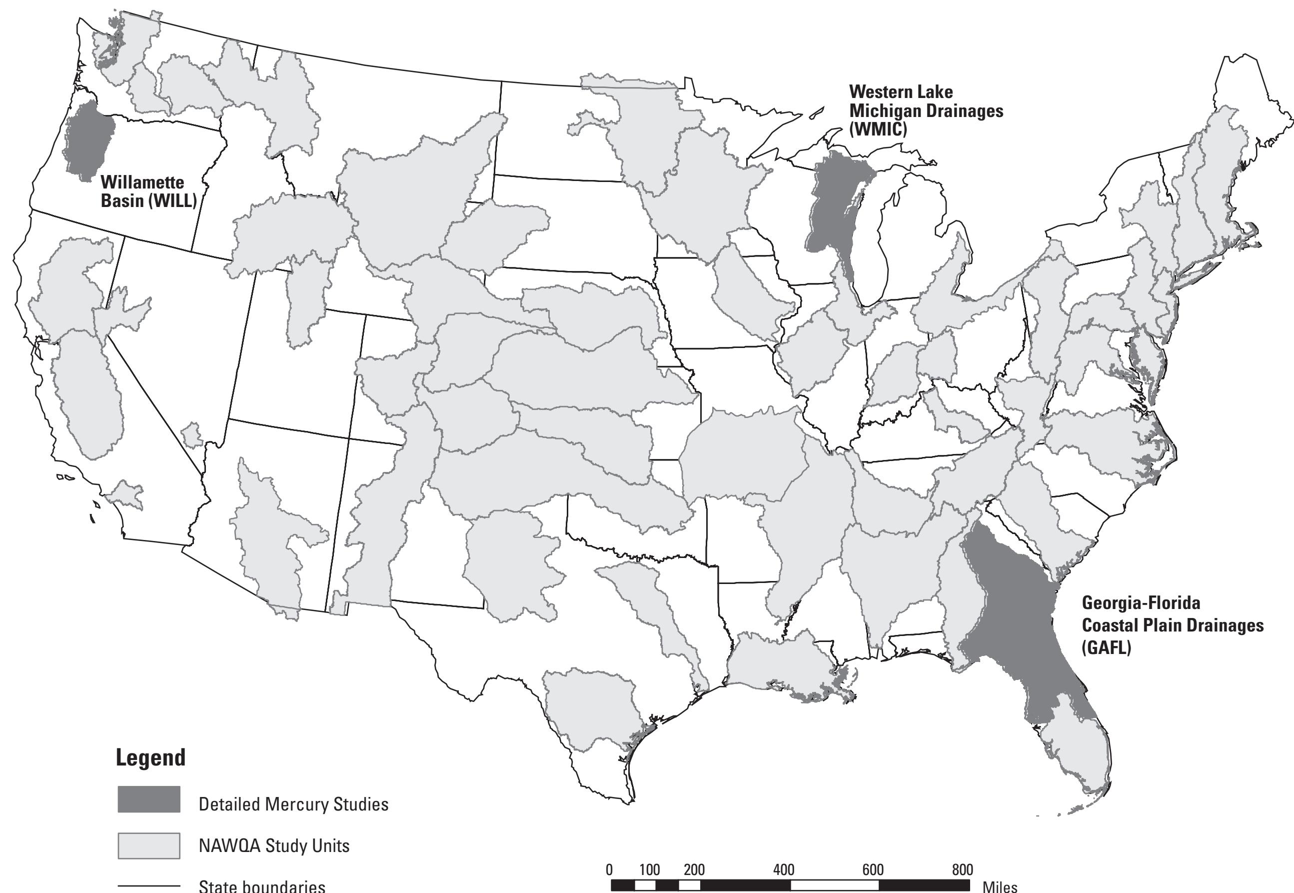


Figure 1. Location of the three detailed study units and the U.S. Geological Survey's National Water Quality Assessment Program's study units.

Table 1. Site information for the eight stations sampled for mercury in periphyton, 2003.

[d, degree; m, minute; s, second]

Study unit	USGS station ID	Station name	River code	Landscape type	Basin area (square miles)	Latitude-longitude (ddmmssdddmmss)	Percent wetland[1]
GAFL	02234998	Little Wekiva River near Longwood, FL	LW	Urban	44.5	2842070812332	4.50
GAFL	02322500	Santa Fe River near Fort White, FL	SF	Reference, non-cultivated	1020	2950550824255	18.0
GAFL	02231000	St. Marys River near MacClenny, FL	SM	Reference, non-cultivated	700	3021310820454	48.0
WILL	14206435	Beaverton Creek at SW 216th Ave, near Orenco, OR	BT	Urban	36.9	4531151225354	0.16
WILL	14161500	Lookout Creek near Blue River, OR	LO	Reference, non-cultivated	24.1	4412351221520	0.00
WMIC	04075365	Evergreen River below Evergreen Falls near Langlade, WI	EG	Reference, non-cultivated	64.5	4503570884034	9.30
WMIC	04087204	Oak Creek at South Milwaukee, WI	OC	Urban	25.0	4255300875212	8.10
WMIC	04066500	Pike River at Amberg, WI	PR	Reference, non-cultivated	255	4529490881818	18.0

[1] Land use data derived from National Land Cover Dataset using 30-meter Thematic Mapper data (Vogelmann and others, 2001).

the role of periphyton in the trophic transfer of methylmercury (MeHg) in indigenous food webs. This periphyton-mercury study was based in three NAWQA study basins chosen for the first intensive mercury project sampling; the Western Lake Michigan Drainages (WMIC), the Willamette Basin (WILL), and the Georgia-Florida Coastal Plain (GAFL), shown in figure 1. Two to three study sites in each of the three basins were chosen for the NAWQA/Toxics study to represent one urban site and one to two reference/non-cultivated (low- and high-percent wetland) sites. Table 1 lists site information for the sampled rivers, and site locations are shown in figure 2. Currently, there are no generally accepted methods for collection of periphyton for mercury or methylmercury determinations. This paper discusses the collection process and analysis for mercury in periphyton.

Methods

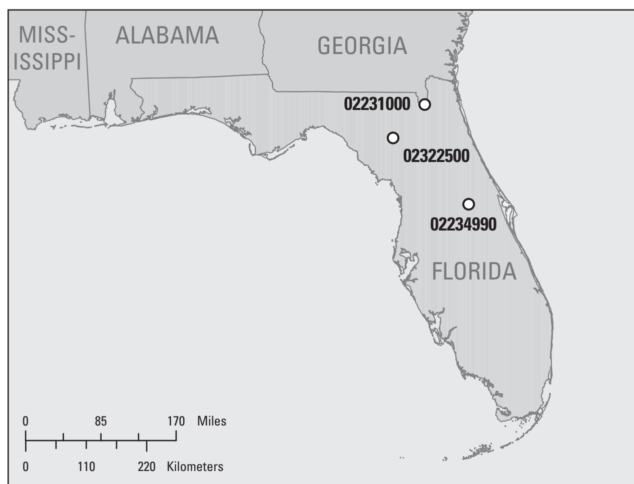
Collection

Trace-metal clean sampling techniques were used to minimize potential sample contamination (USEPA, 1996; Cleckner and others, 1998; Cleckner and others, 1999). These techniques generally serve to minimize contact between the sample and field crews that employ sampling devices and sample containers that have been stringently cleaned in acid. Prior to use in the field, all glass and Teflon® [1] equipment was cleaned by immersing in 4 normal (N) hydrochloric acid (HCl) at 65° celsius (C) for at least 48 hours and then immersed and rinsed at least three times with reagent-grade deionized water (>18 megohms (MOhm)). Equipment other than glass or Teflon® was soaked for at least four hours in a solution of reagent-grade water and Liquinox® (a non-ionic surfactant detergent). This equipment was then triple-rinsed with reagent-grade water, placed in five-percent HCl (Omnitrace) for at least four hours, and finally immersed and triple rinsed with reagent-grade water. After cleaning, all sampling equipment and sample containers were stored by double bagging in hermetically sealed plastic bags.

At each sampling site, two types of periphyton samples were collected. The (USEPA) Rapid Bioassessment Protocol (Barbour and others, 1999) recommends “single-habitat sampling should be used when biomass of periphyton will be assessed.” The single-habitat sampling targets two contrasting habitats that are estimated to be the primary periphyton habitats in the study streams: the depositional-targeted habitat (DTH) and the taxonomi-

[1] The use of firm, trade, and brand names does not constitute endorsement by the U.S. Government.

Florida sampling locations for periph ton mercur



Wisconsin sampling locations for periph ton mercur

y regon sampling locations for periph ton mercur

Figure 2. Location of the eight study sites for mercury in periphyton, 2003.

cally richest-targeted habitat (RTH). The DTH sample may be collected from fine sediment such as silt/clay or sand as appropriate. The DTH and RTH periphyton samples for this study were collected and composited from separate locations in the stream. The NAWQA single habitat sampling method (Moulton and others, 2002) was used for this study because the surface area sampled by this method is quantifiable and those two habitats are generally where periphyton growth dominates in streams.

The overall study design for this project called for seasonal comparison of mercury and methylmercury concentrations and fluxes during spring (high flow) and fall conditions (base flow). For the spring DTH sampling, three areas in each stream were sampled, typically in depositional areas with high organic carbon content in the streambed sediment. These areas were targeted for sampling due to relative abundance of fine sediments and presumably low redox conditions that would promote mercury methylation. Hem (1985) defines redox as the processes of a participating element losing or gaining orbital electrons. The sediment sampling procedure employed by this study seeks to capture the upper 0.5 centimeters (cm) of sediment by employing a Teflon® petri dish (2.54 cm diameter) that is carefully placed open-side down on the streambed sediment to enclose a 19.64 cm^2 circle of sediment. A thin sheet of Teflon® was slid under the opening to capture the sediment contained in the petri dish, which is then transferred into a 500 milliliter (mL) Teflon® jar. A more complete description of this method can be found in Porter and others (1993) and Moulton and others (2002) with the addition of trace-metal-clean techniques. For the fall DTH sampling, subsamples were collected at specific areas where methylation rates were found to have the highest methylation potential during the springtime sampling (M. Marvin-DiPasquale, U.S. Geological Survey, Menlo Park, Calif., written commun., August 11, 2003).

The RTH samples were collected from either rock cobbles (epilithic) or woody snags (epidendric) by brushing the algal growth with a stiff-bristled toothbrush-type brush into a Teflon® dish and transferring the slurry into a 500-mL Teflon® jar. Five cobble or woody snags were collected at five locations in each stream for a total of twenty-five composited samples as described in Moulton and others (2002). This method was used with the addition of trace-metal-clean techniques. Rock cobble was the preferred substrate in the WMIC and WILL basins; however, woody snags were used in the GAFL basin because of a general lack of cobble or larger sized rocks at these sites. To determine the surface area of the cobble samples, a section of aluminum foil was placed over the rock and

cut to the size and shape of the area scraped. Each sample template was then weighed to determine surface area based on a seven-point curve of mass to surface area from each roll of aluminum foil used. To determine surface area of the woody snags, the length of each snag was measured to the nearest millimeter excluding the first two centimeters from each end. These areas were not scraped to minimize contamination from handling and cutting of the snag.

Processing

Samples were processed on site, or in some instances held in a darkened cooler with wet ice for up to six hours until processing. If the DTH sample contained a large quantity of sand, the slurry was shaken vigorously for 30 seconds and immediately decanted into another 500-mL wide-mouth Teflon® bottle. Fifty milliliters of reagent-grade water was added to the original container, which was shaken again for 30 seconds. The new slurry was immediately decanted into the second 500-mL wide-mouth Teflon® bottle. This elutriation was repeated a third time so that all that remained in the original 500-mL wide-mouth Teflon® bottle were sand particles and the final sample contained little or no sand. The RTH samples contained little or no sand at the time of sampling and were processed without decanting/elutriation.

For total mercury, methylmercury, and stable isotopes, the sample was swirled and shaken to homogenize and suspend algal cells and 5 to 15 mL of the sample was placed on a 47 millimeters (mm) Whatman® quartz fiber filter (QFF) for each subsample. The subsample was filtered by vacuum filtration, using methods of Lewis and Brigham, (2005, in press). Care was taken to ensure that pressure inside the vacuum filtration chamber remained below 10 pounds per square inch (psi) so that the algal cells did not lyse due to high pressures. Each filter was then placed into a petri dish (Teflon® for mercury samples and sterile polystyrene for stable isotopes) and frozen on dry ice for shipment to the respective laboratory for analysis. The chlorophyll *a* and ash-free biomass subsamples were prepared similarly on 47-mm Whatman® glass fiber filters (GFF). GFF filters were folded in quarters, wrapped in aluminum foil, placed into a sterile polystyrene petri dish, then frozen on dry ice and shipped to the laboratory for analysis.

Two 100-mL subsamples were removed from the remaining sample for gross taxonomic identification and preserved to 5 percent (5 mL addition) with 100 percent formalin buffered to pH 7.

Laboratory Analysis

Total Hg and MeHg analyses were performed by the USGS Wisconsin District Mercury Laboratory (WDML) in Middleton, Wis. For total Hg, the frozen filters were thawed at room temperature for 20 minutes and placed in a 125-mL wide-mouth Teflon® bottle. The petri dish that contained the filter was rinsed three times with five percent bromine chloride (BrCl) into the same bottle. The volume of the bottle was then brought to 100.0 mL with 5-percent BrCl. The bottles were tightly capped, double bagged and allowed to oxidize in an oven at 50° C for five days. The oxidized samples were analyzed with USEPA Method 1631 (USEPA, 2002) using an automated mercury analysis system (Tekran® 2600) with gold trapping, thermal desorption, and cold vapor atomic fluorescence spectrometry detection.

For methylmercury, the extraction method for filtered periphyton was used, which is the same procedure developed by the WDML for the analysis of suspended solids on filters (DeWild and others, 2004). Thawed filters were placed into 125 mL distillation vessels and the petri dish that contained the filter was rinsed three times with reagent-grade water into the vessel. The volume in the vessel was brought up to 50.0 mL by weight, and 2.0 mL of a combined reagent (two parts 8 M sulfuric acid (H_2SO_4), one part 20-percent potassium chloride (KCl), and two parts copper sulfate ($CuSO_4$)) was added. The distillation vessel was capped and placed in a distillation block, and 50.0 mL of reagent-grade water was added to a receiving vessel. Nitrogen gas (N_2) was allowed to flow into the distillation vessel at 60 mL/min. The distillation block was heated to 125 ± 5° C and distillation was allowed to proceed until approximately 25 percent of the original volume remained. The volume was recorded and the solution in the receiving vessel was analyzed for methylmercury according to DeWild and others (2002).

Chlorophyll *a* and ash-free biomass were analyzed at the National Water Quality Laboratory (NWQL) in Denver, Colo., using a spectrofluorometric method described in USEPA Method 445 (Arar and Collins, 1997).

The USGS National Research Program Isotopic Tracers Laboratory in Menlo Park, Calif., analyzed periphyton samples for stable ^{13}C and ^{15}N isotopes as described in Kendall and others (2001) by determining carbon and nitrogen isotopic and elemental composition on a Carlo Erba 1500® elemental analyzer that is linked in series to a Micromass Optima® mass spectrometer.

Taxonomic identification to algal division was performed using a Bausch and Lomb compound microscope

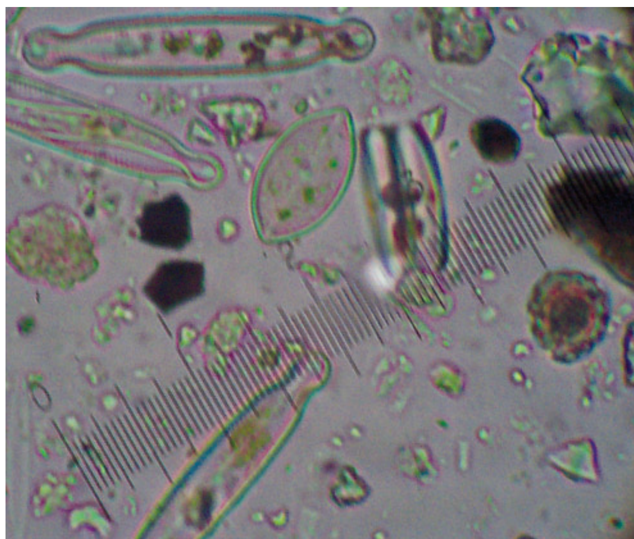


Figure 3. A wide range of algal species at 400X magnification found in the sediment of the St. Marys River, Fla.

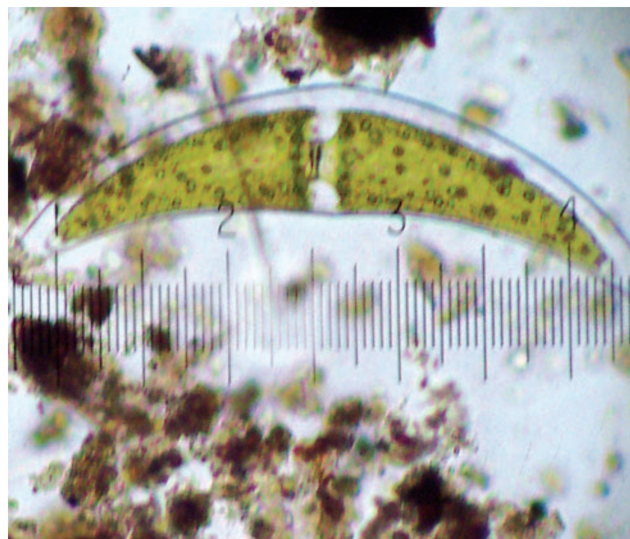


Figure 4. A Chlorophyta (green algae) *Closterium sp.* at 400X magnification found in the sediment of the Santa Fe River, Fla.

with 400X magnification. Samples were gently swirled to homogenize, and 10 wet-mount slides per sample were prepared using one milliliter aliquots of homogenized sample slurry. Each slide was viewed for identification, and one digital picture was taken for each genus of algal cells occurring more than once and for unique cells encountered, with examples of cells found in figures 3 and 4. Divisional characteristics were determined based on Prescott (1962 and 1970), and Wehr and Sheath (2003). The number of times an algal division was encountered on each slide was recorded per sample site. The divisional composition was rated as very common (≥50 percent of the total cells on the slide), common (50–25 percent), few (≤25 percent), and unique (≤1 percent).

Results

All raw periphyton data collected are given in table 2 and table 3. Quality control procedures for the collection and processing included collection of approximately 17 percent replicate samples. Replicate values for all analytical parameters were found to be within 5 percent of targeted values.

Acknowledgments

Funding for this project was provided by the U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment (USEPA/ORD/NCEA), and the U.S. Geological Survey National Water-Quality Assessment and Toxic Substances Hydrology programs. We thank Keith G. Sappington, USEPA/ORD/NCEA, for providing technical input and support for this study; and Mark E. Brigham, USGS Minnesota District, for assistance in planning and guidance throughout the project. We are grateful for the guidance from Dr. N. Earl Spangenberg, Dr. Robert A. Bell, and Dr. Bryant A. Browne, professors at the University of Wisconsin-Stevens Point, Amanda Bell's graduate committee. Special thanks to David P. Krabbenhoft, Mark L. Olson, Shane D. Olund, and John F. DeWild of the USGS Wisconsin District Mercury Laboratory staff for their dedication and direction in processing and interpretation of the data. We also recognize the help in guidance, preparation, and sample processing from Dennis A. Wentz, Lia S. Chasar, Richard L. Marella, Kurt D. Carpenter, Michelle A. Lutz, Rebecca H. Woll, Krista A. Stensvold, Jennifer L. Hogan, David O. Bratz, Mark C. Marvin-DiPasquale, Jeffery J. Steuer, and others who assisted during this project.

Table 2. Raw data of all periphyton samples. See table 1 for complete description of sites and river codes.

[First two characters of the sample code denote the river code; the next charater denotes the season (S, spring; F, fall); and the last character denotes the habitat (R, rock; S, sediment; W, wood). Number of samples, n, is 32.]

Sample code	Total mercury (nanograms per square meter)	Methylmercury (nanograms per square meter)	Biomass, ash free dry mass (grams per square meter)	Chlorophyll *a* (milligrams per square meter)	Total mercury/AFDM (nanograms per gram)	Methylmercury/ CHL A (nanograms per milligram)	Percent methyl/total mercury	Stable isotopes	
								d13C	d15N
LWFW	589.8	25.75	8.00	4.20	73.73	6.13	4.37	-28.29	10.07
LWSW	603.7	30.91	8.40	4.30	71.87	7.19	5.12	-29.28	11.15
LWFS	10,800	265.3	29.30	18.60	368.7	14.26	2.46	-27.03	5.77
LWSS	27,400	534.6	70.40	67.40	389.3	7.93	1.95	-28.02	6.99
SFFW	861.8	54.94	238.4	12.30	3.62	4.47	6.38	-27.90	4.05
SFSW	637.0	51.43	4.70	5.00	135.5	10.29	8.07	-28.78	6.22
SFFS	89,360	856.2	7.40	7.40	12,080	115.7	0.96	-27.67	4.08
SFSS	127,200	3,798	240.3	20.50	529.4	185.3	2.99	-28.20	3.44
SMFW	1,530	184.1	26.50	23.10	57.74	7.97	12.03	-28.13	3.69
SMSW	1,267	35.34	10.20	<0.1	124.1	353.4	2.79	-28.86	-0.01
SMFS	6,519	218.5	8.70	8.90	749.3	24.55	3.35	-27.23	5.74
SMSS	6,206	181.3	15.20	0.70	408.3	258.9	2.92	-27.70	1.69
BTFS	247,800	2,458	335.2	7.70	739.1	319.3	0.99	-27.68	3.58
BTSS	21,000	132.2	28.70	2.30	731.6	57.48	0.63	-27.49	-6.31
BTFR	1,024	18.54	3.30	2.60	310.3	7.13	1.81	-27.81	3.87
BTSR	556.1	13.63	1.80	2.60	308.9	5.24	2.45	-37.52	12.46
LOFS	18,750	432.3	122.8	33.60	152.7	12.87	2.31	-25.64	-1.21
LOSS	3,024	18.54	7.10	0.80	425.9	23.17	0.61	-27.13	5.93
LOFR	74.35	1.43	2.60	0.60	28.60	2.39	1.93	-18.60	-1.21
LOSR	38.13	1.33	1.00	1.40	38.13	0.95	3.48	-25.85	3.59
EGFS	27,280	3,653	53.40	41.00	510.8	89.10	13.39	-27.05	3.20
EGSS	44,770	2,857	209.0	93.40	214.2	30.59	6.38	-27.43	2.42
EGFR	2,253	191.4	19.10	40.60	117.9	4.71	8.50	-27.47	2.98
EGSR	321.0	35.23	1.80	10.00	178.3	3.52	10.98	-31.00	5.80
OCFS	50,200	665.5	82.10	59.90	611.5	11.11	1.33	-28.85	4.85
OCSS	110,000	2,589	152.3	275.0	722.2	9.42	2.35	-28.42	4.11
OCFR	3,009	77.79	25.10	77.30	119.9	1.01	2.59	-26.66	10.05
OCSR	3,163	213.0	22.30	126.0	141.8	1.69	6.73	-30.42	13.24
PRFS	39,720	1,284	277.3	61.40	143.2	20.92	3.23	-27.09	1.51
PRSS	24,580	1,751	114.2	51.70	215.3	33.87	7.12	-28.03	1.03
PRFR	1,165	73.35	12.60	25.30	92.46	2.90	6.30	-29.50	2.20
PRSR	314.2	14.73	1.20	2.40	261.8	6.14	4.69	-27.97	5.97
Maximum	**257,500**	**3,798**	**358.6**	**275.0**	**12,080**	**353.4**	**13.39**	**-18.60**	**13.24**
Minimum	**38.13**	**0.91**	**1.00**	**<0.10**	**3.62**	**0.65**	**0.61**	**-38.32**	**-6.31**
Mean	**30,070**	**684.8**	**67.73**	**30.32**	**590.0**	**53.89**	**4.17**	**-28.22**	**4.39**
Median	**3,024**	**132.2**	**15.20**	**9.03**	**215.3**	**9.42**	**2.99**	**-27.90**	**3.69**

Table 3. Divisional classification data of all periphyton samples. See table 1 for complete description of sample codes.

[First two characters of the sample code denote the river code; the next charater denotes the season (S, spring; F, fall); and the last character denotes the habitat (R, rock; S, sediment; W, wood).

Sample code	Habitat	Chrysophyta (number of cells encountered)	Cyanophyta (number of cells encountered)	Chlorophyta (number of cells encountered)	Other (number of cells encountered)	Total (number of cells encountered)
BTFR	Rock	58	148	265	2	473
BTSR	Rock	62	159	287	3	511
LOFR	Rock	49	77	152	2	280
LOSR	Rock	39	98	164	1	302
EGFR	Rock	68	168	369	10	615
EGSR	Rock	86	156	264	6	512
OCFR	Rock	66	168	359	9	602
OCSR	Rock	98	192	364	13	667
PRFR	Rock	67	148	426	5	646
PRSR	Rock	115	234	379	5	733
LWFS	Sediment	435	256	121	15	827
LWSS	Sediment	521	302	168	19	1010
SFFS	Sediment	398	188	156	20	762
SFSS	Sediment	415	234	109	11	769
SMFS	Sediment	365	219	126	16	726
SMSS	Sediment	531	354	150	22	1057
BTFS	Sediment	617	316	286	19	1238
BTSS	Sediment	486	206	111	16	819
LOFS	Sediment	346	194	125	13	678
LOSS	Sediment	289	183	213	23	708
EGFS	Sediment	582	318	194	16	1,110
EGSS	Sediment	617	349	167	22	1,155
OCFS	Sediment	423	216	194	13	846
OCSS	Sediment	359	168	168	17	712
PRFS	Sediment	522	326	124	19	991
PRSS	Sediment	456	267	138	23	884
LWFW	Wood	123	352	159	3	637
LWSW	Wood	131	289	154	4	578
SFFW	Wood	99	223	116	7	445
SFSW	Wood	111	258	136	6	511
SMFW	Wood	86	207	109	1	403
SMSW	Wood	119	291	159	8	577
LOSW	Wood	114	264	139	9	526

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