Article

Diatom responses to microenvironment structure within metaphyton mats

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Abstract

Microscale conditions within metaphyton mats affected the distribution of diatom genera. We investigated the conditions within layers of floating filamentous algal mats and changes in diatom genera over 57 days using microprobes every 2, 4, and 6 cm down through mats held in floating nets. Mats were then collected, frozen, and sliced into 2 cm layers for analysis. Filamentous algae and their diatom epiphytes were identified, counted, and analyzed for nitrogen, phosphorus, carbon, silicon, ash-free dry mass (AFDM), and chlorophyll *a*. Light intensity, temperature, dissolved oxygen, dry mass, and AFDM all fell significantly with increasing depth in the mat. Diatom coverage per filament was calculated as an epiphyte area index (EAI) and was significantly higher at the edge versus the center of the mat. The uppermost 2 cm layer showed the greatest downward trend in EAI over the sampling period. The densities of *Gomphonema*, *Cocconeis*, and *Fragilaria* were significantly positively correlated with lower light intensity and lower layers of the mat. *Cymbella/Encyonema* density was significantly correlated with higher light intensity. *Gomphonema*, *Cocconeis*, and *Nitzschia* were positively correlated with filaments with higher chlorophyll a content. *Achnanthidium*, *Cymbella/Encyonema*, and *Nitzschia* required higher levels of silicon. Diatoms with different growth habits responded similarly to measured variables. Stalk-forming *Gomphonema* and adnate *Cocconeis* both occurred in lower light areas and grew well under low nitrogen and phosphorus conditions.

Key words: diatom genera, epiphyte area index, growth habits, light, metaphyton, microscale variability, nutrients

Introduction

Diatoms provide an important food source for herbivores in aquatic ecosystems and can reach numbers sufficient to affect nutrient concentrations, especially silica, in a water column (Hall and Smol 1999, Martin-Jézéquel et al. 2000). Freshwater diatoms are often found as epiphytes in mats of metaphyton. Metaphyton comprises unattached, mat-forming filamentous algae that originate as benthic biofilms but lift off the pond bottom when sunny days produce sufficient amounts of trapped gases to float the mats to the surface (Hillebrand 1983, Wetzel 1996, Zohary et al. 1998). Once at the surface, the conditions for the filamentous algae and their attached epiphytes change drastically (Ibarra et al. 2009). The algae are no longer in contact with nutrient-rich sediments, and solar radiation and temperature rise dramatically. Uptake of carbon dioxide and nitrogen can be reduced as the algal cells experience desiccation at the surface (Ibelings and Mur 1992). The temperature at the top of a floating mat can reach lethal levels during the day, and dehydration can become a problem for exposed algae. Ultraviolet (UV) radiation can also reach levels that cause photoinhibition, degradation of photosynthetic pigments, cell bleaching, DNA damage, and even cell death in the upper layers of the mat (Hillebrand 1983, Ibelings and Mur 1992, Berry and Lembi 2000, Sinha et al. 2001, Jiang and Qiu 2005, Falkowski and Raven 2007). While the surface of a floating

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algal mat is exposed to high light conditions, the lower layer of the mat can experience low light levels, which can reduce algal growth (Graham et al. 1995). When light intensity is less than optimal for growth, many algal species need more nutrients such as nitrogen (N), phosphorus (P), and silica (SiO_2) , but this can vary with species (van Donk and Kilham 1990, Borchardt 1996). For example, many algal species require more N to produce more N-rich photosynthetic pigments in low light conditions (Rhee and Gotham 1981, Interlandi and Kilham 2001).

Because epiphytes can block sunlight and absorb nutrients from their host (Usher and Blinn 1990, Ruesink 1998), they can potentially affect the growth of metaphyton. Epiphytes might also absorb nutrients that would otherwise be released into the water column as filaments senesce (Stevenson and Stroemer 1982). How environmental conditions within metaphyton mats affect diatom epiphytes has been little studied, however. Diatoms can potentially affect water column nutrient content, especially SiO_2 as well as N and P, which diatoms store when these nutrients are in excess (van Donk and Kilham 1990, Hall et al. 2005, Lee and Tsai 2005). Diatoms can also be an important part of food webs because isopods, snails, and insect larvae use them as a food source (Scheffer 2001). Attached epiphytes may compete with host filaments for nutrients and light (Marks and Power 2001), and different species of diatoms may affect their hosts differently. For example, adnate forms, such as *Achnanthes*, may block more light and receive more P from their host filament than stalked forms such as *Gomphonema* (Burkholder and Wetzel 1990). The distribution and abundance of diatom epiphytes may also affect the amount and longevity of nuisance metaphyton mats, yet the microscale interactions of these 2 have been little studied. We investigated whether diatom communities varied among different areas of a metaphyton mat based on changes of the microenvironment within the mat and evaluated whether mat nutrient content would affect diatom genera and filament coverage. Location at the center versus the edge of a mat was hypothesised to affect diatom genera and coverage, as was depth within a mat. Sampling date was also hypothesised to affect epiphyte genera and coverage.

Study site

We chose a small, shallow, man-made pond typical of many areas of southeast Pennsylvania, located in Chester County at 39°36′54″N, 75°36′5″W, near the borough of West Chester. The pond is part of a common area in a suburban neighborhood and is surrounded by mowed, fertilized lawns. Canada geese (Branta canadensis) live around the pond at times, also adding nutrients, especially N and P (Wetzel 2001). The trophic state of the pond was characterised using Carlson's (1977) trophic state index (TSI) as eutrophic–hypereutrophic by Fairchild et al. (2005). The mean depth was 1.79 m with a maximum depth of 4.0 m (Saunders 2009).

Methods

We randomly anchored 24 floating open net bags of 300 µm mesh Nitex fabric in the northeast end of the pond in July 2005. Each net had 4 foam floats about 10 cm in length attached around the edge. A thin, clear, acetate sheet 15 cm high was then attached as a wall above the perimeter of the net to prevent the algae from spilling out during windy periods. To fill the nets, we collected benthic algae from the bottom of the pond using a 300 µm mesh long-handled dip net, weighing out 25 g samples from each net. During a 2 month period, triplicate nets were removed from the pond at weekly intervals after measuring temperature, dissolved oxygen (DO), and light every 2 cm down through the center of each of 3 nets. Temperature was determined with an Oakton Temp-JKT Acorn series thermocouple; DO was measured with a Lazar MV portable DO microelectrode (DO-166MT-1; Lazar, Los Angeles, CA, USA); and light reading measurements were taken with a Heinz Walz U-SQS/L spherical quantum micro-sensor (Heinz Walz GmbH, Pepperwell, MA, USA) attached to a Li-Cor LI250A light meter (LiCor, Lincoln, NE, USA). Thereafter, the acetate wall was carefully cut off each net, the cord anchoring the net to the pond was cut, and a solid plastic container the size of the net was placed in the pond and brought up from underneath, so that the entire net and contents were carefully lifted from the pond without disturbing the mat. A tight-fitting lid was placed on the container before it was put on ice in a cooler, returned to the lab, and immediately frozen solid. The frozen nets were removed from their plastic containers and sliced in cross-section into 2 cm layers yielding 3 circular layers; top (0–2 cm), middle (2–4 cm), and bottom (4–6 cm). Each cross-section was then subdivided into 3 wedge-shaped samples. One-half of each cross-section was used for nutrient analysis, one-fourth for chlorophyll *a* (Chl-*a*) analysis, and one-fourth for algal identifications. To obtain samples for filamentous and epiphyte algal identifications, a sample core was taken from the end of the pointed tip and opposite edge of each frozen wedge using a copper corer with an inside diameter of 0.7 cm; the resulting 137 samples were immediately stored in a scintillation vial in the freezer until identification (Saunders 2009, Saunders et al. 2012).

Each core sample was examined under a stereoscope, and filament genera were segregated and further examined under a compound light microscope. To count epiphytes, 10 filaments of each genus were separated from a sample and examined under a Nikon microscope. All live epiphytes were counted along two 100 µm randomly

chosen lengths on each filament. To identify diatom taxa to the genus level, the diatoms were cleaned using 30% hydrogen peroxide and concentrated nitric acid (Stoermer et al. 1995). The cleaned and rinsed diatoms were dried and mounted on a slide with Naprax and examined at 1000×. A minimum of 200 valves were counted and identified along 18 mm transects on the slide. Macroalgae, soft algal epiphytes, and diatoms were identified primarily using Prescott (1962), Patrick and Reimer (1966, 1975), Whitford and Schumacher (1984), Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b), and Dillard (1989a, 1989b, 1990, 1991a, 1991b, 1993, 1999).

Spectophotometric determination of Chl-*a* was performed after acetone extraction, with correction for phaeophytin *a*, as described in American Public Health Association (APHA) Method 10200 H (1998). Ash free dry (AFDM) was determined by incinerating the dried samples at 500 °C for 1 h (APHA 1998).

Calculations

An epiphyte area index (EAI) was estimated as a measure of diatom cover on metaphyton filaments, as seen through a compound microscope. A median surface area per cell (ai; median cell length × median cell width) estimated for each diatom genus i was multiplied by its abundance (n_i). Total surface areas (μm^2) for each genus were then summed to produce total epiphyte area, which was then divided by filament area, A_{fil} = 200 µm length × width to produce this unit-free metric. Only the area actually visible under the compound microscope was used for this calculation because diatoms could not be counted or identified under the filament (Saunders et al. 2012):

$$EAI = \sum_{t=1}^{I} a_i n_i / A_{fil}. \quad (1)$$

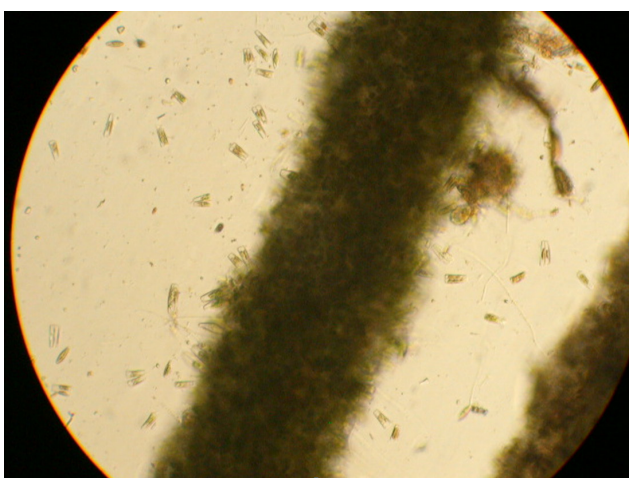

Fig. 1. Photomicrograph of a filament from one of the study nets on 25 Sep 2005 showing a heavy coverage of diatom epiphytes (100×).

An EAI value of 1.0 indicates complete coverage of an algal filament surface by diatoms, as seen under the compound microscope. EAI values >1 can occur because diatoms can form several layers (Fig. 1).

The relative frequency was calculated as the number of individuals of that genus in the total number of cleaned and mounted diatoms counted in a particular 10-filament sample (Brower et al. 1989, APHA 1998). All genera were taken into account when calculating relative frequency, including rare taxa. Diatom density was calculated along each 200 µm length of filament by counting the total number of diatoms and dividing by the filament area.

Statistical analysis

To determine if changes in environmental variables such as light and temperature were significantly different among layers of the mat or over the sampling period, a series of fully crossed, fixed-effects 2-way ANOVA procedures was performed with depth as a repeated measure for each net (because 3 measurements were taken in each net at 2, 4, and 6 cm) and with sampling date as a between-subjects variable (because nets were sampled on 8 sampling dates). We could thus evaluate 3 hypotheses: depth within a mat would have no effect on epiphyte genus; date would have no effect on epiphyte genus; and no interaction would involve effects of date and depth. The same form of statistical analysis was used to determine if the differences in nutrient content between layers and over the sampling period were significant.

Effects of date, center versus edge, and depth in the mat on diatom density and EAI were similarly assessed with a 3-way, fixed effects ANOVA, with both depth (measured 3 times in each net) and center-edge (measured twice at each depth) as repeated measures of the model, and sampling date as a between-subjects variable. This method potentially evaluates 8 hypotheses: date has no effect on diatom density or EAI; center versus edge location has no effect on diatom density or EAI; depth in the mat has no effect on diatom density or EAI; and no interaction of date, depth, or center versus edge affects diatom density or EAI. Tukey tests were performed to determine which group means were significantly different.

A series of molar nutrient ratios (total N:total P, total C:total N, total C:total P, total Si: total P, Chl-*a*:AFDM) were calculated and compared with the densities of individual diatom genera. A series of Spearman rank correlations were performed comparing diatom density and nutrient molar ratios and the environmental variables because the nutrient ratios were not normally distributed. All analyses were conducted using Statistica (StatSoft 2008).

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Table 1. The total number of individuals of each genus of diatom epiphytes counted on algal filaments from the experimental nets. Rare taxa can be found in Saunders (2009).

Genus	Number of Samples Found in, out of 137	Total Number of Individuals Counted
Gomphonema	137	34 935
Cocconeis	137	16 566
Cymbella/Encyonema	137	15 537
Achnanthidium	135	7080
Nitzschia	132	4119
Epithemia	134	1453
Fragilaria	112	1248

Results

Pithophora was the most common algal filament in all samples and had heavy diatom coverage (Fig. 1). *Gomphonema* was the most common diatom genus and was found in all samples (Table 1). *Cocconeis* and *Cymbella/Encyonema* were also found in all samples but not as abundantly. Rare genera present in <1% of the samples were dropped from any further analysis but are listed in Saunders (2009).

Overall mean EAI per filament was significantly higher ($F_{1,13}$ = 21.0, p < 0.001) at the edge of the mat compared with the center of the mat (Fig. 2), but the values did not vary greatly among different depths at the center of the mat over the sampling period (Fig. 3a). The topmost 2 cm layer usually had the lowest EAI at the edge of the mat (Fig. 3b) and overall (Fig. 3c).

The densities of some diatom genera showed significant variation with date, depth, and center versus edge of the mat (Table 2), although there was no significant interaction between date, depth, and center versus edge. *Achnanthidium* is the only genus to have a significant interaction with date and depth, showing an increase in density over the sampling period in the 2 and 4 cm layers. Both *Gomphonema* and *Cocconeis* densities were significantly lower in the topmost 2 cm layer of the mat. *Gomphonema*, *Cymbella/Encyonema*, *Epithemia*, and *Fragilaria* had significantly higher densities at the edge versus the center of the mat.

The nutrient concentrations at each depth in the mat over the sampling period are the result of dried samples of filament with attached epiphytes (Fig. 4a–d). The upper 2 cm layer tended to have the highest values for N, carbon (C), and Si over the sampling period whereas the 4 cm layer had higher values for P. The 6 cm layer had the lowest values for N, C, and often P, but both the 4 and 6 cm layers were lower in Si than the topmost 2 cm layer. The average values of molar nutrient ratios C:N, N:P, and C:P at each depth over the sampling period (Table 3) ranged from 10.5 to 814.9.

Chl-*a*:AFDM (Fig. 5) tended to decline during the sampling period. Mean light intensity over the sampling period was highest in the 2 cm layer and similar in the 4 and 6 cm layers (Fig. 6). Density of several diatom genera showed similar significant correlations with nutrients and environmental variables (Table 4). Overall, *Gomphonema, Fragilaria, Cocconeis*, and *Nitzschia* (GFCN) tended to fall near each other along a number of gradients of environmental and nutrient variables and ratios based on Spearman rank correlation coefficients, as did *Achnanthidium, Epithemia*, and *Cymbella/Encyonema* (AEC/E; Table 5). GFCN had negative Spearman rank correlations with date, N, C, AFDM, and ratios of C:N, C:P, N:P, light:P, and light:Si, whereas AEC/E showed positive correlations. GFCN had positive Spearman rank correlations with Chl-*a* and Chl-*a*:AFDM compared with the negative correlations shown by AEC/E. GFCN had positive correlations with depth, but so did *Epithemia*, a genera that also joined GFCN with a negative correlation to percentage light. All the genera had a positive correlation with Si:N, and all but *Cocconeis* had a positive correlation with Si:P. All but *Nitzschia* had a negative correlation with percentage saturation of oxygen. *Gomphonema, Nitzschia*, and *Cymbella/Encyonema* had positive correlations with temperature whereas all others had negative correlations. *Gomphonema* and *Cocconeis* both had negative correlations with P and Si whereas all others had positive correlations, except *Achnanthidium*, which also had a negative correlation to P.

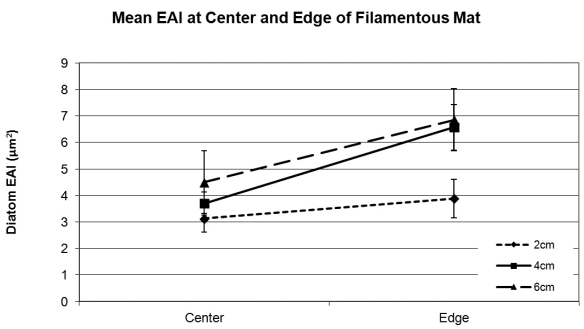

Fig. 2. Mean epiphyte area index (EAI, ±SE) on the algal filaments from experimental nets. EAI at the edge of the algal mat was significantly (p = 0.001) higher than at the center of the mat. The 2 cm layer had the lowest EAI overall.

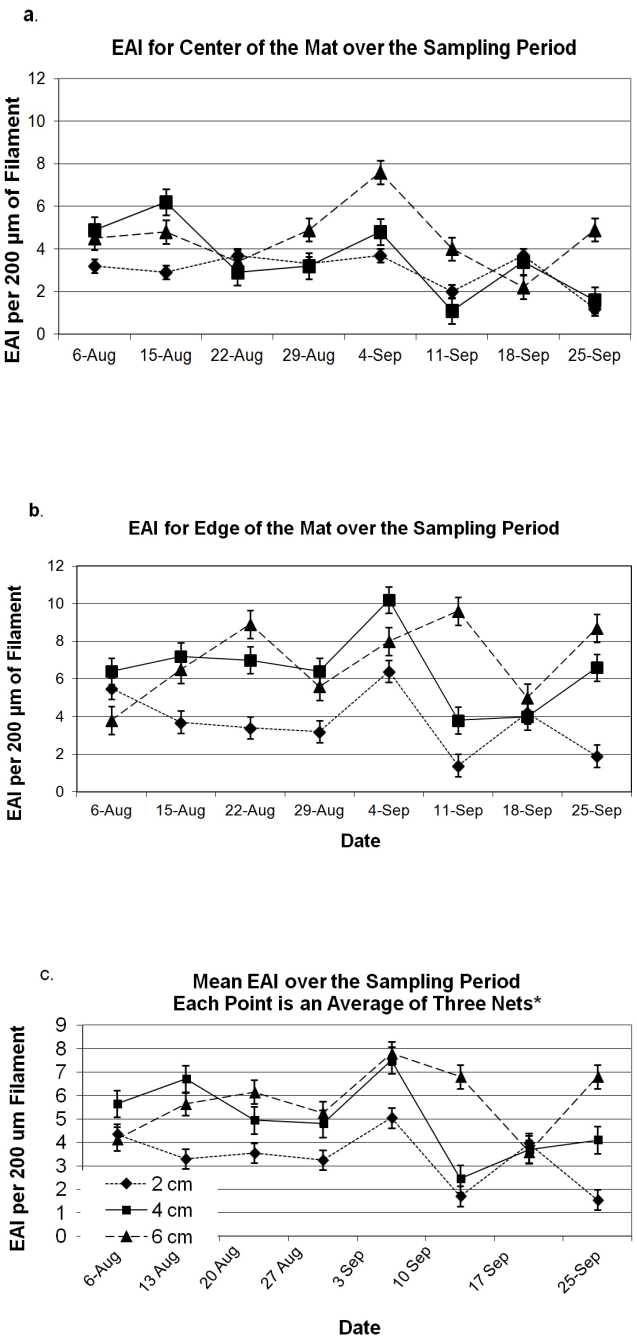

Fig. 3. Mean EAI at (a) different depths for the center of the algal mat; (b) different depths for the edge of the algal mat; and (c) center and edge at different depths of the algal mats. Filaments were collected from experimental nets anchored in the study pond in Chester County, PA, in 2005. Each point is an average (±SE) of 3 nets except the last day, when only one net was found.

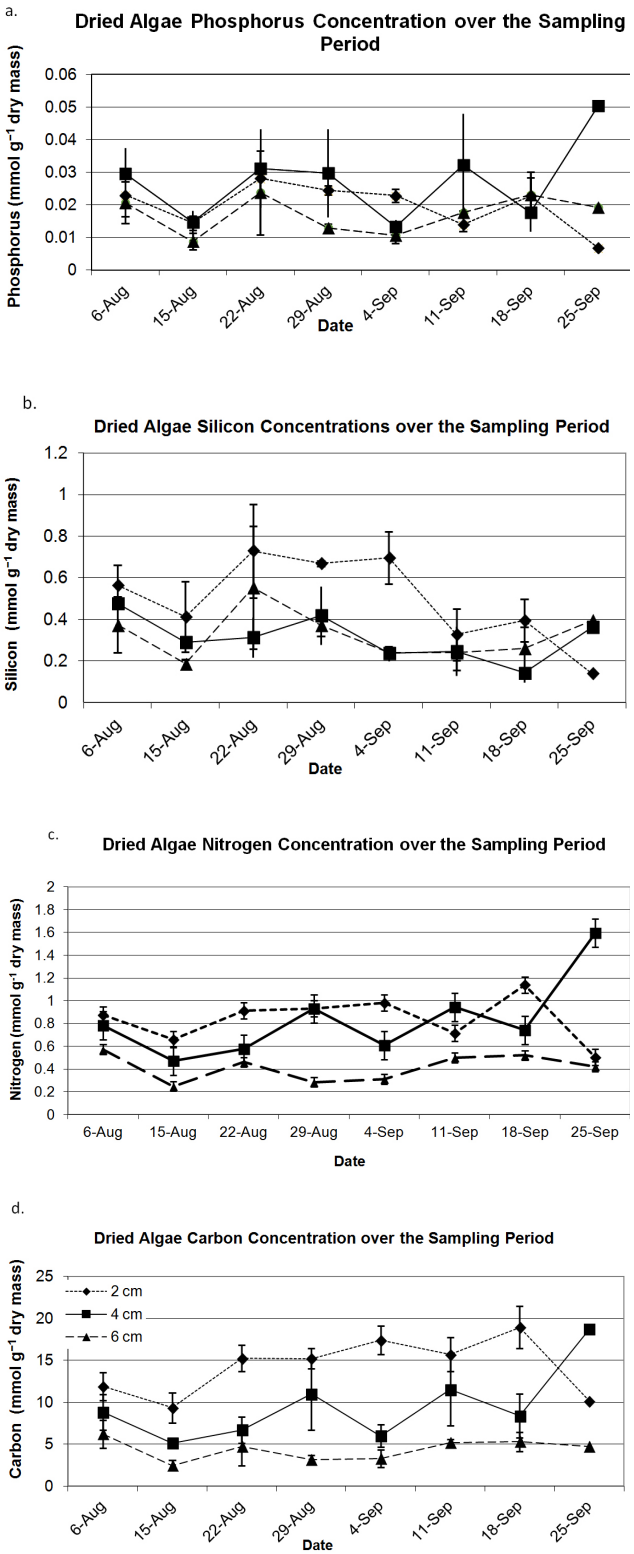

Fig. 4. Nutrient concentrations for (a) phosphorus, (b) silicon, (c) nitrogen, and (d) carbon over the sampling period at various depths within the algal mat within the experimental nets. All data points are an average (±SE) of 3 nets except the last day, when only one net was found.

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Table 2. Results of 3-way ANOVA comparing diatom densities to date and location within the filamentous mat from experimental nets. Significant values ($p < 0.05$) are marked*. No interactions between date, depth, or center-edge were significant except the one listed for Achnanthidium.

Genus and Source of Variation	df	mean squares	*F*	*P*
Gomphonema				
Date	7	116 868.6	1.09	0.43
Depth in Mat	2	0.3	8.13	0.002*
Center/Edge	1	18 5913.7	6.46	0.03*
Cocconeis				
Date	7	5955.9	1.08	0.43
Depth in Mat	2	44 583.0	9.64	0.001*
Center/Edge	1	12 505.0	2.10	0.17
Cymbella/Encyonema				
Date	7	15 071.0	2.60	0.07
Depth in Mat	2	17 316.6	1.81	0.19
Center/Edge	1	118 678.1	8.51	0.01*
Achnanthidium				
Date	7	9430.9	2.08	0.13
Depth in Mat	2	1870.3	1.64	0.21
Center/Edge	1	8092.2	4.19	0.06
Date with Depth	14	3012.8	2.64	0.02*
Nitzschia				
Date	7	1464.6	1.45	0.27
Depth in Mat	2	440.1	0.58	0.57
Center/Edge	1	2604.4	4.00	0.07
Epithemia				
Date	7	185.7	0.64	0.72
Depth in Mat	2	47.9	0.30	0.75
Center/Edge	1	478.0	5.32	0.04*
Fragilaria				
Date	7	549.8	0.87	0.56
Depth in Mat	2	1601.4	2.22	0.13
Center/Edge	1	1590.4	5.21	0.04*

Discussion

Epiphyte area index

The overall EAI varied within a mat with significantly higher values at the edge as opposed to the center of the mat (Fig. 2), most likely due to the higher levels of light at the edge of the mat. Light effects are also probably responsible for the similar EAI at the center of the mat for all layers over the sampling period (Fig. 3a), with the 2 and 4 cm layers often the lowest (Hudon and Bourget 1983). The EAI at the edge of the mat was usually the lowest in the 2 cm layer (Fig. 3b), which received more damaging UV, was at a higher temperature, and was more prone to desiccation (Hillebrand 1983, Usher and Blinn 1990, Ibelings and Mur 1992, Berry and Lembi 2000, Falkowski and Raven 2007), and diatoms tend to be sensitive to UV radiation (Weidman et al. 2005). Overall, the light intensity decreased down through the layers of the mat; however, the 6 cm layer did receive some ambient light from underneath because it was at the bottom of the mat, and therefore the 4 and 6 cm layers had similar light intensities (Saunders 2009). The 4 cm layer often received the least light at the center of the mat, rendering it a less optimal location for a photosynthetic diatom (Fig. 2).

Table 3. Average molar nutrient ratios at each depth within the metaphyton mats over the sampling period.

Depth in Mat	C:N	N:P	C:P
2 cm	17.1	46.5	814.9
4 cm	11,3	32,1	360.4
6 cm	10.5	24.9	261.9

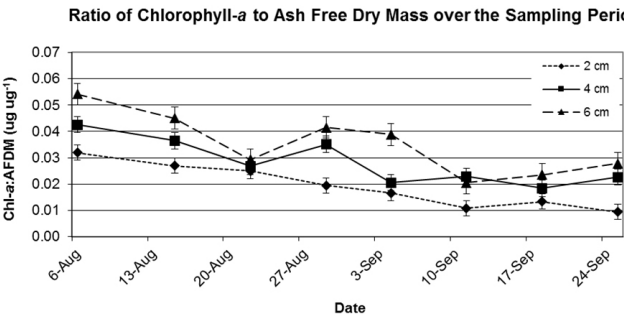

Fig. 5. Ratio of Chl-*a* to ash free dry mass over the sampling period at various depths in the algal mat within the experimental nets. All data points are an average (±SE) of 3 nets except the last day, when only one net was found.

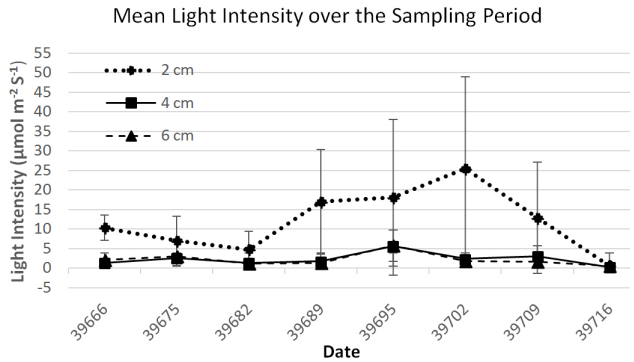

Fig. 6. Mean light intensity over the sampling period at various depths within the algal mat within the experimental nets from the study pond in Chester County, PA, 2005. All data points are an average (±SD) of 3 nets except the last day, when only one net was found.

Table 4. Spearman rank correlation coefficients between diatom density per 200 µm of algal filament, and particulate nutrient ratios, each nutrient alone, and environmental field measurements from experimental nets anchored in the study pond in Chester County, PA, in 2005. Significant values are marked. All ratios are molar ratios except Chl-*a*:AFDM, which is in micrograms.

	Gomphonema	*Cocconeis*	*Cymbella/Encyonema*	*Achnanthidium*	*Nitzschia*	*Epithemia*	*Fragilaria*
C:N	−0.63***	−0.35*	0.05	0.03	−0.20	−0.11	−0.26^{+}
C:P	−0.52***	−0.36*	0.05	−0.04	−0.36*	−0.14	−0.43**
N:P	−0.38**	−0.30*	0.02	−0.06	−0.36*	−0.15	−0.42**
Si:P	0.15	−0.05	0.34*	0.26^{+}	0.25^{+}	0.05	0.06
Si:N	0.38*	0.17	0.28^{+}	0.28^{+}	0.47***	0.06	0.32*
Chl-*a*: AFDM	0.38*	0.24^{+}	−0.10	−0.10	0.29+	−0.07	0.13
light:P	−0.37*	−0.26^{+}	0.20	0.18	−0.15	−0.09	−0.29*
light:N	−0.36*	−0.25^{+}	0.22	0.20	−0.09	−0.10	−0.26^{+}
light:Si	−0.46***	−0.30*	0.15	0.13	−0.21	−0.10	−0.32*
light:C	−0.33*	−0.24^{+}	0.22	0.19	−0.09	−0.11	−0.26^{+}
N	−0.33*	−0.44**	0.14	−0.05	−0.10	0.16	−0.19
C	−0.52***	−0.47***	0.13	−0.04	−0.19	0.08	−0.27^{+}
P	−0.13	−0.22	0.12	−0.03	0.09	0.16	0.04
Si	−0.04	−0.21	0.33*	0.13	0.29^{+}	0.20	0.13
Chl-*a*	0.26^{+}	0.05	−0.21	−0.20	0.21	−0.10	−0.06
AFDM	−0.44**	−0.34*	−0.07	−0.08	−0.34*	−0.02	−0.33*
% light	−0.37*	−0.32*	0.25^{+}	0.13	−0.08	−0.04	−0.29^{+}
temp.	0.11	−0.12	0.07	−0.10	0.25^{+}	−0.11	−0.11
% sat. O_2	−0.01	−0.03	−0.02	−0.09	0.01	−0.12	−0.26^{+}
depth	0.42**	0.45***	−0.18	−0.04	0.08	0.09	0.38*
date	−0.33*	−0.02	−0.00	0.18	−0.40**	0.16	−0.02

$^{+}p < 0.05$, $*p < 0.01$, $**p < 0.001$ $***p < 0.0001$. Number of samples per correlation is 66.

Table 5. Location of diatom genera along gradients of nutrient and environmental variables, and ratios based on Spearman rank correlation coefficients. Significant correlations are marked *. Genera that tended to have similar responses are highlighted in the same color.

	High Coeff. (+) Low Coeff.
date	ACH > EPI > CYEN > FRG, COC > GMP* > NTZ*
depth	COC* > GMP* > FRG* > EPI > NTZ > ACH > CYEN
temp.	NTZ* > GMP > CYEN > ACH > FRG, EPI > COC
% light	CYEN* > ACH > EPI > NTZ > FRG* > COC* > GMP*
% sat. O_2	NTZ > GMP > CYEN > COC > ACH > EPI > FRG*
N	EPI > CYEN > ACH > NTZ > FRG > GMP* > COC*
C	CYEN > EPI > ACH > NTZ > FRG* > COC* > GMP*
P	EPI > CYEN > NTZ > FRG > ACH > GMP > COC
Si	CYEN* > NTZ* > EPI > FRG, ACH > GMP > COC
Chl-*a*	GMP* > NTZ > COC > FRG > EPI > ACH > CYEN
AFDM	EPI > CYEN > ACH > FRG* > NTZ*, COC* > GMP*
C:N	CYEN > ACH > EPI > NTZ > FRG* > COC* > GMP*
C:P	CYEN > ACH > EPI > NTZ*, COC* > FRG* > GMP*
N:P	CYEN > ACH > EPI > COC* > NTZ* > GMP* > FRG*
Si:P	CYEN* > ACH* > NTZ* > GMP > FRG > EPI > COC
Si:N	NTZ* > GMP* > FRG* > CYEN*, ACH* > COC > EPI
Chl-*a*: AFDM	GMP* > NTZ* > COC* > FRG > EPI > CYEN, ACH
light:P	CYEN >ACH > EPI > NTZ > COC* > FRG* > GMP*
light:N	CYEN* > ACH > NTZ > EPI > COC* > FRG* > GMP*
light:Si	CYEN > ACH > EPI > NTZ > COC* > FRG* > GMP*

Diatom genera: GMP = *Gomphonema*; COC = *Cocconeis*; CYEN = *Cymbella/Encyonema*; ACH = *Achnanthidium*; FRG = *Fragilaria*; EPI = *Epithemia*; NTZ = *Nitzschia*

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Location of epiphyte genera within a mat

The genera of diatoms found at the center versus the edge of the mat and in the different layers of the mat varied. *Gomphonema*, *Cocconeis*, and *Fragilaria* appeared most often in the lower layers with lower light levels whereas *Cymbella/Encyonema* and *Achnanthidium* occurred in areas with higher light levels. When averaged over the sampling period, all genera occurred more often at the edge versus the center of the mat except *Cocconeis*, which was more common at the center of the mat. *Gomphonema*, *Cocconeis*, and *Fragilaria* densities were highest in the 6 cm layer of the mat, and their significant negative Spearman rank correlations with percentage light indicate a tolerance to lower light levels. Goldsborough (1993, 1994) found *Gomphonema* and *Epithemia* in areas of lower light under duckweed (*Lemna*) mats as opposed to areas outside the mat with higher light intensity. By comparison, *Cymbella/Encyonema* had the highest density in the 2 cm layer and a significant positive Spearman rank correlation with percentage light, suggesting it does not tolerate low light levels well and may prefer higher light environments, or it is outcompeted at low levels of light. Overall, the pattern of significant Spearman rank correlations was similar for GFCN, which all seemed able to tolerate lower levels of light, N, P, and C (Table 5). Hudon and Bourget (1983) found species of *Cocconeis* and *Gomphonema* in the lower stratum of the communities they studied. *Fragilaria* and *Nitzschia* were not as tolerant of low P or Si levels as *Gomphonema* and *Cocconeis* (Cymbola et al. 2008, Cuvin-Aralar 2004). The growth habits of these 4 diatoms (GFCN) are quite different from each other, with *Gomphonema*, growing on a mucilage stalk that can rise above adnate diatoms such as *Cocconeis*. *Fragilaria* is usually colonial and attaches on the valve face, as does *Nitzschia*, but *Nitzschia* was the only motile genus found in this study (Cox 1996, Wehr and Sheath 2003). An adnate form is likely tolerant of low light because it is often shaded, whereas a stalked form, normally less shaded, would benefit from any light available at any level in the mat.

Cymbella/Encyonema and *Nitzschia* had significantly positive responses to higher Si levels. *Achnanthidium* also seemed to correlate with higher Si levels, but the response was not strong, and this genus had no other significant responses to any of the measured variables other than date with depth. *Epithemia* had no significant response to any of the measured variables, possibly indicating that either the variables to which these 2 genera respond were not measured or that the genera were poor competitors in relation to the other genera present and were unable to occupy the optimal location for growth conditions.

As filaments die and decay, Chl-*a* decreases more rapidly than AFDM, so this ratio decreases as filaments senesce (Wetzel and Likens 2000). Over the sampling period, the Chl-*a*:AFDM ratio did decline (Fig. 5), indicating filament senescence. The Si content of the dried samples did not show the same decline (Fig. 4c), however, indicating that the diatom community was active, especially in the 4 and 6 cm layers. The silica content of the pond water inflow has been measured as high as 28.1 mg L^{-1} (Anderson 2004), but in our study the water column silica content varied from 1.43 to 7.55 mg L^{-1} (Saunders 2009), indicating a high intake of silica by diatoms in the pond. The heavy diatom coverage on the filaments in this study (Fig. 1) indicates the prolific growth of diatoms in this pond and a high usage of silica. GFCN were positively correlated with higher Chl-*a* content. The significant positive correlations to Chl-*a* or Chl-*a*:AFDM and significant negative correlations to AFDM for *Gomphonema*, *Cocconeis*, and *Nitzschia* could indicate that these genera prefer filaments in better condition. There was a significant tendency for *Gomphonema* density to decline over the sampling period; as the filaments progressively decomposed, *Gomphonema* found less preferable habitat.

Cocconeis and *Fragilaria* also showed significant negative correlations with AFDM but showed no significant correlations with Chl-*a*, so they may not have a preference for actively growing filaments. *Nitzschia* however, significantly declined with date but was significantly positively related to temperature and Si. *Nitzschia* may have declined throughout the sampling period due to declining water temperatures and Si concentrations, and, as the only motile genus found, it may have left the mat because of these changes.

Nutrient stoichiometry affects community composition and growth of individual species. Nutrient ratios can indicate which nutrients are limiting. For example, a molar C:N ratio >14.6 can indicate a severe N shortage, between 8.3 and 14.6 a moderate deficiency, and <8.3 no N deficiency (Kilham 1990, Wetzel 2001). A C:P ratio >369 and N:P >32 indicate P limitation (Kahlert 1998, Liess and Hillebrand 2006). High light intensity along with lower nutrient levels would increase the C:N and C:P ratios (Sterner et al. 1997). Cell decomposition can also increase C:N and C:P ratios because C-rich cell walls break down more slowly than N and P cytoplasmic compounds (Sterner and Elser 2002). The percentage of C in living algal cells has been found to be low (~8.0%) in periphyton, however, and the majority of the C seems to come from cellular exudates, microorganisms, and dead cellular material (Frost et al. 2005). Addition of C to an experimental lake caused no change in the C:P ratio of epilithic algae in studies done by Frost and Elser (2002), so nutrient ratios must be used

with caution. Nutrient ratios do indicate food quality, however, because consumers need to maintain particular C:N:P ratios (Sterner and Elser 2002) and are therefore important in food web interactions and in determining community structure (Kilham 1990). The 2 cm layer had the highest nutrient ratios of any layer, indicating nutrient deficiencies for both N and P (Table 3), most likely due to high light intensity and decomposition of cells. The 4 and 6 cm layers did not fall into the deficient category, although the 4 cm layer was close. As light is attenuated by layers of the mat, cell damage decreases.

This study illustrates that metaphyton mats contain microscale environments that affect the attached epiphyte community. As conditions within the mat changed with depth and time, the attached diatom genera also changed. These communities can be complex and affect nutrient content of the water column in shallow lakes and ponds.

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References

[APHA] American Public Health Association. 1998. Standard methods for the examination of water and wastewater, 20th ed. Washington (DC).

Anderson JN. 2004. Watershed effects on nutrients in shallow ponds [master's thesis]. [West Chester (PA)]: West Chester University.

Berry H, Lembi CA. 2000. Effects of temperature and irradiance on the seasonal variation of a *Spirogyra* (Chlorophyta) population in a Midwestern lake (U.S.A.). J Phycol. 36:841–854.

Borchardt MA. 1996. Nutrients. In: Stevenson RJ, Bothwell ML, Lowe L, editors. Algal ecology: freshwater benthic ecosystems. San Diego (CA): Academic Press. p. 183–227.

Brower J, Zar J, von Ende C. 1989. General ecology, field and laboratory methods. Dubuque (IA): Brown & Co.

Burkholder JM, Wetzel RG. 1990. Epiphytic alkaline phosphatase activity on natural and artificial plants in a P-limited lake: re-evaluation of the role of macrophytes as a phosphorus source for epiphytes. Limnol Oceanogr. 35:736–746.

Carlson RE. 1977. A trophic state index for lakes. Limnol Oceanogr. 22:361–369.

Cox EJ. 1996. Identification of freshwater diatoms from live material. London (UK): Chapman and Hall.

Cuvin-Aralar M, Ulfert F, Becker K, Aralar EV. 2004. Effects of low nitrogen-phosphorus ratios in the phytoplankton community in Laguna de Bay, a shallow eutrophic lake in the Philippines. Aquat Ecol. 38:387–401.

Cymbola J, Ogdahl M, Steinman AD. 2008. Phytoplankton response to light and internal phosphorus loading from sediment release. Freshwater Biol. 53:2530–2542.

Dillard GE. 1989a. Freshwater algae of the southeastern United States Part 1: Chlorophyceae: Volvocales, Tetrasporales and Chlorococcales. Berlin (Germany): J. Cramer.

Dillard GE. 1989b. Freshwater algae of the southeastern United States: Chlorophyceae: Ulotrichales, Microsporales, Cylindrocapsales, Sphaeropleales, Chaetophorales, Cladophorales, Schizogoniales, Siphonales and Oedogoniales. Berlin (Germany): J. Cramer. p. 248.

Dillard GE. 1990. Freshwater algae of the southeastern United States: Chlorophyceae: Zygnematales: Zygnemataceae, Mesotaeniaceae and Desmidiaceae. Berlin (Germany): p. 276.

Dillard GE. 1991a. Freshwater algae of the southeastern United States, Part 5, Section 3: Chlorophyceae, Zygnematales, Desmidiaceae. Berlin (Germany): J. Cramer. p. 310.

Dillard GE. 1991b. Freshwater algae of the southeastern United States: Chlorophyceae: Zygnematales: Desmidiaceae. Berlin (Germany): J. Cramer. p. 231.

Dillard GE. 1993. Freshwater algae of the southeastern United States: Chlorophyceace: Zygnematales: Desmidiaceae, Berlin (Germany): J. Cramer. p. 166.

Dillard GE. 1999. Common freshwater algae of the United States: an illustrated key to the genera (excluding the diatoms). Berlin (Germany): Gebrüder Borntraeger. p. 173.

Fairchild GW, Anderson JN, Velinsky DJ. 2005. The trophic state 'chain of relationships' in ponds: does size matter? Hydrobiologia. 539:35–46.

Falkowski PG, Raven JA. 2007. Aquatic photosynthesis. 2nd ed. Princeton (NJ): Princeton University Press.

Frost PC, Elser JJ. 2002. Effects of light and nutrients on the net accumulation and elemental composition of epilithon in boreal lakes. Freshwater Biol. 47:173–183.

Frost PC, Hillebrand H, Kahlert M. 2005. Low algal carbon content and its effect on the C:P stoichiometry of periphyton. Freshwater Biol. 50:1800–1807.

Goldsborough LG. 1993. Diatom ecology in the phyllosphere of the common duckweed (*Lemna minor* L.). Hydrobiologia. 269/270:463–471.

Goldsborough LG. 1994. Heterogeneous spatial distribution of periphytic diatoms on vertical artificial substrata. J N Am Benthol Soc. 13:223–236.

Graham JM, Lembi CA, Adrian HL, Spencer DF. 1995. Physiological responses to temperature and irradiance in *Spirogyra* (Zygnematales, Charophyceae). J Phycol. 31:531–540.

Hall SR, Smith VH, Lytle DA, Leibold MA. 2005. Constraints on primary producer N:P stoichiometry along N:P supply ratio gradients. Ecology. 86:1894–1904.

Hall RI, Smol JP. 1999. Diatoms as indicators of lake eutrophication. In: Stroermer EF, Smol JP, editors. The diatoms: applications for the environmental and earth sciences. Cambridge (UK): Cambridge University Press.

Hillebrand H. 1983. Development and dynamics of floating clusters of filamentous algae. In: Wetzel RG, editor. Periphyton of freshwater

 DOI: 10.5268/IW-6.2.802

ecosystems. The Hague (Netherlands): Dr. W. Junk. p. 31–39.
Hudon C, Bourget E. 1983. The effect of light on the vertical structure of epibenthic diatom communities. Bot Mar. 26:317–330.
Ibarra CR, Tavera R, Novelo E. 2009. Diversity and structure of periphyton and metaphyton diatom communities in a tropical wetland in Mexico. Rev Mex Biodivers. 80:763–769.
Ibelings BW, Mur LR. 1992. Microprofiles of photosynthesis and oxygen concentration in Microcystis sp. scums. FEMS Microbiol Ecol. 86:195–203.
Interlandi SJ, Kilham SS. 2001. Limiting resources and the regulation of diversity in phytoplankton communities. Ecology. 82:1270–1282.
Jiang H, Qiu B. 2005. Photosynthetic adaptation of a bloom-forming cyanobacterium *Microcystis aeruginosa* (Cyanophyceae) to prolonged UV-B exposure. J Phycol. 41:983–992.
Kahlert M. 1998. C:N:P ratios of freshwater benthic algae. Arch Hydrobiol Beih Egebn Limnol. 51:105–114.
Kilham SS. 1990. Relationship of phytoplankton and nutrients to stoichiometric measures. In: Tilzer MM, Serruya-Brock C, editors. Large lakes: ecological structure and function. New York (NY): Springer-Verlag. p. 403–414.
Krammer K, Lange-Bertalot H. 1986. Bacillariophyceae. 1. Teil: Naviculaceae. Jena (Germany): VEB Gustav Fisher Verlag. p. 876.
Krammer K, Lange-Bertalot H. 1988. Bacillariophyceae. 2. Teil: Epithemiaceae, Bacillariaceae, Surirellaceae. Jena (Germany): VEB Gustav Fisher Verlag. p. 610.
Krammer K, Lange-Bertalot H. 1991a. Bacillariophyceae. 3. Teil: Centrales, Fragilariaceae, Eunotiaceae, Achnanthaceae. Jena (Germany): VEB Gustav Fisher Verlag. p. 598.
Krammer K, Lange-Bertalot H. 1991b. Bacillariophyceae. 1. Teil: Achnanthaceae, Kritische Erganzungen zu *Navicula* (Lineolatae) und *Gomphonema*. Jena (Germany): VEB Gustav Fisher Verlag. p. 437.
Lee TS, Tsai PF. 2005. The effects of phosphite on phosphate starvation responses of *Ulva lactuca* (Ulvales, Chlorophyta). J Phycol. 41:975–982.
Liess A, Hillebrand H. 2006. Role of nutrient supply in grazer-periphyton interactions: reciprocal influences of periphyton and grazer nutrient stoichiometry. J N Am Benthol Soc. 25:632–642.
Marks JC, Power ME. 2001. Nutrient induced changes in the species composition of epiphytes on *Cladophora glomerata* Kütz (Chlorophyta). Hydrobiologia. 450:187–196.
Martin-Jézéquel V, Hildebrand M, Brzezinski MA. 2000. Silicon metabolism in diatoms: implications for growth. J Phycol. 36:821–840.
Patrick R, Reimer CW. 1966. Diatoms of the United States, Vol. I. Philadelphia (PA): Academy of Natural Sciences of Philadelphia, USA. Monograph 13. 688 p.
Patrick R, Reimer CW. 1975. Diatoms of the United States, Vol. II. Part 1. Philadelphia (PA): Academy of Natural Sciences of Philadelphia, USA. Monograph 13. 213 p.
Prescott GW. 1962. Algae of the Western Great Lakes Area. Dubuque (IA): W.M.C. Brown, USA. 977 p.
Rhee GY, Gotham IJ. 1981. The effect of environmental factors on phytoplankton growth: temperature and the interactions of temperature with nutrient limitation. Limnol Oceanogr. 26:635–648.
Ruesink JL. 1998. Diatom epiphytes on *Odonthalia floccosa*: the importance of extent and timing. J Phycol. 34:29–38.
Saunders LL. 2009. Metaphyton mat conditions and their effects on filamentous algal communities and their diatom epiphytes; [dissertation]. [Philadelphia (PA)]: Drexel University.
Saunders LL, Kilham SS, Fairchild GW, Verb R. 2012. Effects of small-scale environmental variation on metaphyton condition and community composition. Freshwater Biol. 57:1884–1895.
Scheffer, M. 2001. Ecology of shallow lakes. Kluwer Academic Pub. Dordrecht.
Sinha RP, Klisch M, Gröniger A, Häder DP. 2001. Responses of aquatic algae and cyanobacteria to solar UV-B. Plant Ecol. 154:221–236.
StatSoft. 2008. Statistica version 8.0. Tulsa (OK). Available from: www.statsoft.com
Sterner RW, Elser JJ, Fee EJ, Guildford SJ, Chrzanowski TH. 1997. The light: nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. Am Nat. 150:663–681.
Sterner RW, Elser JJ. 2002. Ecological stoichiometry. Princeton (NJ): Princeton University Press.
Stevenson RJ, Stoermer EF. 1982. Seasonal abundance patterns of diatoms on *Cladophora* in Lake Huron. J Great Lakes Res. 8:169–183.
Stoermer EF, Edlund MB, Pilskaln CH, Schelske CL. 1995. Siliceous microfossil distribution in the surficial sediments of Lake Baikal. J Paleolimnol. 14:69–82.
Usher HD, Blinn DW. 1990. Influence of various exposure periods on the biomass and chlorophyll *a* of *Cladophora glomerata* (Chlorophyta). J Phycol. 26:244–249.
van Donk E, Kilham SS. 1990. Temperature effects on silicon- and phosphorus-limited growth and competitive interactions among three diatoms. J Phycol. 26:40–50.
Wehr JD, Sheath RG. 2003. Freshwater algae of North America: ecology and classification. New York (NY): Academic Press.
Weidman RP, Turner MA, Goldsborough LG. 2005. Limitations on the effects of ultraviolet radiation on benthic algae in a clear boreal forest lake. J N Am Benthol Soc. 24:820–831.
Wetzel RG. 1996. Benthic algae and nutrient cycling in lentic freshwater ecosystems. In: Stevenson RJ, Bothwell ML, Lowe ML, editors. Algal ecology: freshwater benthic ecosystems. San Diego (CA): Academic Press. p. 641–667.
Wetzel RG. 2001. Limnology: lake and river ecosystems. San Diego (CA): Academic Press.
Wetzel RG, Likens GE. 2000. Limnological analyses. 3rd ed. New York (NY): Springer. p. 97–98.
Whitford LA, Schumacher GJ. 1984. A manual of fresh-water Algae. Raleigh (NC): Sparks Press, USA. 337 p.
Zohary T, Fishbein T, Kaplan B, Pollingher U. 1998. Phytoplankton–metaphyton seasonal dynamics in a newly created subtropical wetland lake. Wetl Ecol Manage. 6:133–142.