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Journal of Freshwater Ecology

ISSN: 0270-5060 (Print) 2156-6941 (Online) Journal homepage: https://www.tandfonline.com/loi/tjfe20

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To cite this article: Molly R. Grimmett & Jefferson G. Lebkuecher (2017) Composition of algae assemblages in middle Tennessee streams and correlations of composition to trophic state, Journal of Freshwater Ecology, 32:1, 363-389, DOI: 10.1080/02705060.2017.1314228

To link to this article: https://doi.org/10.1080/02705060.2017.1314228

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Published online: 18 Apr 2017.

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JOURNAL OF FRESHWATER ECOLOGY, 2017
VOL. 32, NO. 1, 363–389
https://doi.org/10.1080/02705060.2017.1314228

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Composition of algae assemblages in middle Tennessee streams and correlations of composition to trophic state

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ABSTRACT

Evaluations of the composition and biomass of photoautotrophic periphyton, nutrient concentrations of water, and concentrations of total phosphorous of benthic organic matter at eight stream sites in Middle Tennessee were used to: (1) document the composition of soft-algae and diatom assemblages, (2) assess the trophic state of the stream sites, (3) correlate trophic state to percent composition of soft-algae taxa, and (4) construct biotic indices using soft-algae taxa to help monitor trophic state. The concentration of total phosphorous of benthic organic matter was a more accurate indicator of the trophic state of the stream sites we studied relative to nutrient concentrations of water as demonstrated by correlation coefficients for nutrient concentrations to benthic concentrations of chlorophyll *a*, ash-free dry mass of benthic organic matter, and biotic indices which denote trophic state using diatom and soft-algae taxa. The algae trophic index developed using soft-algae taxa abundance-weighted averages of benthic concentrations of chlorophyll *a* correlates significantly to all of the benthic characteristics used to denote trophic state. This index is the first to utilize periphyton characteristics, as opposed to nutrient concentrations of water, to assign trophic-indicator values to soft-algae taxa in lotic systems and does not require a computer program to compute.

ARTICLE HISTORY
Received 23 December 2016
Accepted 27 March 2017

KEYWORDS
Periphyton; soft-algae; diatoms; trophic state; biotic index; stream monitoring

Introduction

Algae are a major component of the trophic base of most shallow lotic systems and assemblage composition and functional integrity are key indicators of water quality (Stancheva & Sheath 2016). The compositions of soft-algae assemblages of the vast majority of streams in the Interior Plateau Level III Ecoregion (Griffith et al. 1997; USGS 2016) are unknown. This lack of basic knowledge of a major component of the trophic base limits the ability of watershed managers to measure and monitor the impacts of poor quality water. This work documents the composition of algae assemblages essential to monitor the effects of water quality in eight stream reaches in seven different watersheds in the central region of the Interior Plateau Level III Ecoregion. Relationships between periphyton characteristics and percent composition of soft-algae taxa at sites with varying degrees of anthropogenic impact were used to assess the effects of trophic state on the soft-algae composition of the stream sites.

Estimation of the biomass of photoautotrophic periphyton by measurements of the concentration of benthic chlorophyll (chl) *a* is one of the most common methods to assess the trophic state of streams (Biggs 2000). Dodds et al. (1998) suggested classification of temperate streams with

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concentrations of benthic chl *a* ≤ 20 mg·m^{-2} as oligotrophic and concentrations >70 mg·m^{-2} as eutrophic. Measurements of the concentration of chl *a* alone may not be adequate to demonstrate trophic state because the concentration of chl *a* is influenced by many abiotic and biotic characters (Kurle & Cardinale 2011). Nutrient concentrations of water samples, ash-free dry weight of benthic organic matter, and biotic indices are often used to supplement chl *a* concentration as indicators of trophic state.

Diatom composition is the constituent of photoautotrophic periphyton most widely used to assess trophic state because more autecological information exists for diatoms relative to soft-algae taxa (Rimet 2012). The structure of diatom assemblages often reflects the impacts of trophic state and siltation and thus can be used to support proposed best management practices (Smucker & Vis 2009). Only a few indices have been developed which utilize soft-algae taxa to evaluate the trophic state of streams (Gutowski et al. 2004; Schaumburg et al. 2004; Schneider & Lindstrøm 2011; Fetscher et al. 2014; Lebkuecher et al. 2015). The deficiency of the use of soft-algae assemblages as indicators of trophic state is due largely to the fact that the autecology of most soft-algae taxa is poorly understood or unknown (Passy & Larson 2011). More assessments of the relationship of nutrient concentrations to the abundance of soft-algae taxa are needed to better understand the impact of eutrophication on lotic systems (Stancheva & Sheath 2016).

A majority of streams in the central region of the Interior Plateau level III Ecoregion are nutrient-impaired due largely to nonpoint-source pollution (Lebkuecher et al. 2011). The cumulative effects of erosion, agricultural runoff, poorly functioning sewage systems, and urban development result in poor quality water in most watersheds. Our objectives were to document the diversity and composition of photoautotrophic periphyton at eight stream sites in Middle Tennessee, evaluate the trophic state of the stream sites, and examine the relationships of percent composition of soft-algae taxa to characteristics used to denote trophic state. The objectives were met by: (1) determinations of the composition of diatom and soft-algae assemblages, (2) evaluations of the nutrient concentrations of water and characteristics of the benthic communities including biomass, concentrations of chl *a*, and concentrations of total phosphorous of benthic organic matter, and (3) development of biotic indices based on soft-algae taxa abundance-weighted averages of benthic characteristics as trophic-indicator values.

Methods

Sampling site locations and dates

Eight stream sites were sampled in Middle Tennessee located in the central region of the Interior Plateau Level III Ecoregion of the United States (Appendix 1). The geologic base of the ecoregion is limestone and includes some chert, shale, siltstone, sandstone, and dolomite. The forests are Western Mesophytic and consist largely of *Quercus* and *Carya* species (Griffith et al. 1997; USGS 2016). Stream sites were sampled in May and again in August of the same year to determine the composition of soft-algae and diatom taxa during spring and summer. Four stream sites were sampled in 2015 and four stream sites were sampled in 2016. The four sites sampled in 2015 were sampled on 1 May 2015 or 2 May 2015 and again on 15 August 2015 or 16 August 2015. The four stream sites sampled in 2016 were sampled on 8 May 2016 and again on 1 August 2016 or 11 August 2016. Benthic characteristics including pigment concentrations of photoautotrophic periphyton, ash-free dry mass of benthic organic matter, and concentrations of total phosphorus of benthic organic matter were determined from samples collected August 2015 and August 2016 on the same dates samples were collected to determine algae composition in August 2015 and August 2016.

The choice of stream sites sampled reflects the attempt to pick sites ranging from severely nutrient-impaired to unimpaired based on visual assessments and listings by United States

Environmental Protection Agency (USEPA 2016). Of the stream sites sampled in 2015, the Suggs Creek site, located in Nashville Tennessee, and the Trace Creek site, located in Waverly, Tennessee, are listed as nutrient-impaired. The Suggs Creek site appeared hypereutrophic with a visibly obvious high concentration of photoautotrophic periphyton. The Flynn and Hurricane Creek sites are both located in rural watersheds less affected by anthropogenic activity relative to most watersheds in Middle Tennessee. Although the Flynn and Hurricane Creek sites are listed as nutrient-unimpaired reference sites (TDEC 2009, 2016), reference stream sites in Middle Tennessee are not considered truly oligotrophic relative to stream sites in regions of the world with little anthropogenic activity. Of the stream sites sampled in 2016, the Jones Creek site, located 5 km downstream of the Jones Creek Wastewater Treatment Plant, and the McAdoo Creek site, located near Clarksville, TN, are listed as nutrient-impaired. The Marrow Bone and Will Hall Creek sites are not listed as nutrient-impaired or unimpaired by USEPA (2016) and appear relatively nutrient-unimpaired as judged visibly by the relatively low biomass of photoautotrophic periphyton.

Sampling site morphological characteristics

Two transects from the opposing banks and 5 m apart were established at each site. Transect widths and stream depths at 1/3 intervals between the banks of each transect were measured. Stream velocity was determined as the time required for a density-neutral object to travel 5 m downstream. Stream discharge was calculated using the equation from Robins and Crawford (1954):

$$\text{Discharge} = \text{Width} \times \text{Depth} \times \text{Velocity} \times 0.9$$

The percent of benthic substrates smaller than very course gravel was estimated visually in four replicate plots established with 0.25 m^2 wire frames placed 1.25 m apart at midstream of each stream site. Canopy angle was estimated visually as the angle between the tops of the vegetation or topography on each bank at midstream. Stream site morphological characteristics were determined to provide more detail of the abiotic characteristics of sampling sites (Appendix 2).

Sampling cobbles to determine benthic characteristics and algae composition

Cobble sampling occurred in the established 5 m reaches at depths between 0.07 and 0.37 m and stream velocities between 0.15 and 0.67 $m{\cdot}s^{-1}$ (Appendix 2). Four midstream plots in each reach were established with 0.25 m^2 wire frames placed 1.25 m apart. Cobbles nearest to the plot center between 12 and 18 cm diameter with most of the surface area for periphyton growth parallel to flow were removed. Four cobbles, one from each of the four plots, were used to determine the percent composition of soft-algae and diatom taxa. Algae were removed from cobbles in the field using a single-edge razor blade and scrub brush, preserved in 1% glutaraldehyde adjusted to pH 7.0 with NaOH, and concentrated by settling. Two additional cobbles were collected from each of the four plots per stream reach sampled August 2015 and August 2016. One cobble was used to determine pigment concentrations of photoautotrophic periphyton and ash-free dry mass of benthic organic matter. One cobble was used to determine the concentration of total phosphorous of benthic organic matter. These cobbles were placed in self-sealable plastic bags and transported to the lab on ice in darkness.

Pigment concentrations of periphyton and ash-free dry mass of benthic organic matter

One cobble was placed in a glass pan containing 0.1 L of 90% acetone and periphyton removed with a single-edged razor blade and scrub brush. Ten milliliters aliquots of periphyton suspended in acetone were placed in a mortar, ground with a pinch of sand and a pestle for 2 min, and filtered through Whatman no. 1 filter-paper circles. Optical density (OD) of the supernatant was determined at 664 nm to determine the concentration of chlorophyll (chl) *a*, then at 665 nm following acidification with 0.1 N HCl to determine the concentration of pheophytin *a*. Concentrations of chl *a* and pheophytin *a* were calculated as described by Eaton et al. (2005).

Periphyton removed from cobble was dried by allowing the acetone to evaporate at 25 °C and ash-free dry mass determined as described by Eaton et al. (2005). Ash-free dry weights were increased by the proportion of the periphyton removed to determine pigment concentrations.

The surface area of cobble from which periphyton was removed was calculated by covering the upper surface of cobble with aluminum foil, weighing the foil, and extrapolating weight to surface area (Hauer & Lamberti 2006). Means of periphyton characteristics were compared using Tukey–Kramer Honestly Significant Difference Tests preceded by Analysis of Variance Tests (Zar 2007). Assay means were considered significantly different if they differed at the experiment-wise error rate of alpha = 0.05.

Composition of soft-algae assemblages

Large filamentous algae were cut with scissors such that well-mixed aliquots of the sample could be obtained. Wet mounts on a ruled microscope slide (NeoSci, Nashua, New Hampshire) with a 16 mm^2 grid divided into eight 2 mm^2 squares were used to determine percent composition as described by Woelkerling et al. (1976) and Schoen (1988). Soft-algae within a 2 mm^2 square were observed at 100 X, 400 X, and 1000 X magnification and identified to the lowest taxon possible. Taxa were recorded as units. A unit was considered one cell of unicellular taxa, one colony of colonial taxa, and each 10 μm length of filamentous taxa. Taxa were enumerated until at least 800 units counted, or for samples with very little soft-algae relative to diatoms, until at least 20 wet mounts were observed. Primary taxonomic references used to identify soft-algae taxa included Cocke (1967), Prescott (1982), Whitford and Schumacher (1984), Anagnostidis and Komárek (1988), and John et al. (2011). The percent of soft-algae units and diatom units at each site was estimated by counting the number of soft-algae units and diatom units in 2 mm^2 squares of the ruled microscope slide until at least 1000 units were counted.

Composition of diatom assemblages

Frustule preparation for permanent mounts followed the methods of Carr et al. (1986). Organic debris and intracellular material were removed by placing concentrated frustules in 2.5% sodium hypochlorite for 1 h. Aliquots of cleaned frustules (50 μL) were pipetted onto glass cover slips, dried at 50 °C, and mounted on glass microscope slides with Permount mounting medium. All valves in the field of view at 1000 X magnification were identified and tallied until a minimum of 200 valves from each stream site were identified, the minimum number required to calculate the pollution tolerance index of diatom assemblages (KDOW 2002). Primary taxonomic references used to identify diatom taxa included Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1998), and Ponader and Potapova (2007). The permanent mounts are maintained in the Austin Peay State University Herbarium in Clarksville, Tennessee.

Shannon diversity index, evenness, and percent similarity

Shannon diversity index (H') and evenness (J) of soft-algae and diatom assemblages were calculated by the equations of Shannon and Weaver (1949):

$$H' = -\Sigma(P_i \ln P_i)$$
$$J = H'/\ln S$$

where P_i = abundance of species i and S = richness (number of taxa). Percent similarities of diatom and soft-algae assemblages associated with cobble were calculated as the sum of the lower of the two percent composition values for each taxon common to two sites.

Diatom indices

The pollution tolerance index of diatom assemblages (PTI; KDOW 2002) was calculated as

$$\mathrm{PTI} = [\Sigma_{j\,=\,1\mathrm{sp.}} n_j t_j]/N$$

where n_j = number of individuals of taxon j, t_j = eutrophication-tolerance value (1–4) of taxon j, and N = total number of individuals assigned a eutrophication-tolerance value and tallied to calculate the index. The PTI ranges from 1 (all taxa very tolerant to eutrophic conditions) to 4 (all taxa very intolerant of eutrophic conditions). PTI values ≤ 2.6 correspond to eutrophic conditions (Lebkuecher et al. 2011).

The organic pollution index (OPI) is the percentage of diatoms tolerant of organic pollution listed in Kelly (1998). OPI values of ≤ 20 indicate the absence of significant organic pollution, 21–40 infers some organic pollution present, and values >40 suggest a significant impact of organic pollution (Kelly 1998). The siltation index (SI) is the percentage of motile diatoms (Bahls 1993). Motile diatoms are able to avoid being buried and are tolerant of sedimentation. The SI is calculated as percentage of the motile diatoms *Navicula senu lato, Nitzschia senu lato,* and *Surirella* (Bahls 1993).

Soft-algae assemblage metrics and indices

The relationship of the trophic state of the stream sites with the percent composition of each soft-algae taxon sampled in August 2015 and August 2016 was assessed by calculating the abundance-weighted average (A-WA) for: (1) concentrations of chl *a,* (2) ash-free dry mass of benthic organic matter, and (3) concentration of total phosphorous of benthic organic matter. A-WA of a stream characteristic for a taxon is the average value of a characteristic weighted by the abundance of the taxon at each site and was calculated as

$$\text{A-WA}_j = [\Sigma_{j=1\ \mathrm{taxon}} n_j v]/N$$

where A-WA$_j$ is the abundance-weighted average of a stream characteristic for taxon$_j$, n_j = number of taxon units j sampled at a site, v = value for the characteristic of a site, and N = total number of taxon units j at all of the sampling sites used to calculate A-WA$_j$. Taxa more abundant at sites with greater values for a stream site characteristic will have a greater value for the A-WA.

Three variations of the algae trophic index (ATI) were calculated to assess the impact of the trophic state of a stream site on the structure of soft-algae assemblages. An ATI is calculated as

$$\mathrm{ATI} = [\Sigma_{j=1\ \mathrm{taxon}} n_j ti_j]/N$$

where n_j = number of taxon units j sampled at a site, ti_j = trophic-indicator value for taxon j, and N = total number of taxon units at the sampling site used to calculate the index. The three variations of the ATI differed by the stream site characteristic used to calculate the trophic-indicator values. The three trophic-indicator values utilized were A-WA of concentration of chl a, A-WA of ash-free dry mass of benthic organic matter, and A-WA of the concentration of total phosphorous of benthic organic matter. Taxa not identified to species were excluded from index calculations.

Nutrient concentrations of water samples and benthic organic matter

Concentrations of nutrients in water were determined from one water sample collected at mid-stream and 5 cm below the surface on the same day that cobbles were sampled. We acknowledge that nutrient concentrations of water may vary from day to day and that evaluations of water samples collected on different days are optimal, however, limited funding restricted the number of nutrient analyses to 32 (analyses of four different nutrients from one water sample collected at each of the eight sites). Concentrations of total phosphorous of benthic organic matter were determined from samples scraped from cobbles, desiccated for 24 h at 50 °C, and ashed at 500 °C for 2 h. Concentrations of soluble reactive phosphorus, $NO_2 + NO_3$ nitrogen, and total nitrogen of water samples and concentrations of total phosphorous of benthic organic matter were determined following the methods of Eaton et al. (2005) using a Lachat QuickChem 8000 Flow Injection Analyzer (Lachat Instruments, 5600 Lindbergh Dr., Loveland, Colorado 80538). The water samples for determinations of concentrations of SRP and $NO_2 + NO_3$ nitrogen were filtered through nitrocellulose membranes (0.45 μm pore size, 47 mm diameter, Advantec MFS Inc.) using a vacuum filtration system. Concentrations of SRP and $NO_2 + NO_3$ nitrogen were determined by the ascorbic-acid method and cadmium-reduction method, respectively. Concentrations of total nitrogen of water samples were determined by the persulfate digestion and cadmium-reduction method. Concentrations of total phosphorous of ashed benthic organics were determined by the persulfate digestion and ascorbic-acid method (Eaton et al. 2005).

Results

The two sites most impacted by eutrophication are the Suggs Creek site, sampled August 2015 and located in Nashville, TN, and the Jones Creek site, sampled August 2016 and located 5 km downstream of the Jones Creek Wastewater Treatment Plant as indicated by the concentrations of benthic chlorophyll (chl) *a* (Table 1). The concentrations of chl *a* at both the Suggs and Jones Creek sites are above nuisance levels ($\geq$100 $mg \cdot m^{-2}$; Dodds et al. 1998). The oligotrophic concentrations of chl *a* at the Hurricane Creek site and the low mesotrophic concentrations of chl *a* at the Flynn Creek site are consistent with the listings of these sites as nutrient-unimpaired. Photoautotrophic periphyton was in excellent physiological condition as indicated by the low concentrations of pheophytin *a* relative to concentrations of chl *a* and the corresponding OD ratios of OD664 to OD665 $\geq$ 1.5 at all sites other than the photoautotrophic periphyton sampled at the Suggs Creek site (Table 1). Shading from the very high concentration of benthic organic matter measured as ash-free-dry mass at the Suggs Creek site may contribute to the poor physiological condition of the photoautotrophic periphyton at the site.

The high concentrations of total phosphorous of benthic organic matter at the Suggs and Jones Creek sites determined in August 2015 and August 2016 reflect the nutrient-impaired habitats of the sites (Table 2). The low concentrations of total phosphorous of benthic organic matter at the Flynn and Hurricane Creek sites are consistent with these sites listed as nutrient-unimpaired

Table 1. Characteristics of photoautotrophic periphyton and ash-free dry mass of benthic organic matter collected August 2015 and August 2016. Mean characteristics ± SE of stream sites evaluated the same year are significantly different at the experiment-wise error rate of alpha = 0.05 if they do not share the same letter.

Characteristic	Suggs 2015	Trace 2015	Flynn 2015	Hurricane 2015	Jones 2016	McAdoo 2016	M. Bone 2016	Will Hall 2016
Chlorophyll *a* ($mg \cdot m^{-2}$)	136.1 ± 22.6^{A}	56.9 ± 4.7^{B}	28.4 ± 6.1^{B}	14.1 ± 2.0^{B}	217.2 ± 32.5^{A}	52.4 ± 19.1^{B}	70.9 ± 6.7^{B}	47.8 ± 6.5^{B}
Pheophytin *a* ($g \cdot m^{-2}$)	100.3 ± 8.0^{A}	10.5 ± 3.3^{B}	4.7 ± 2.1^{B}	2.8 ± 0.7^{B}	25.7 ± 8.7^{A}	22.4 ± 7.7^{A}	12.4 ± 2.3^{A}	14.2 ± 1.5^{A}
OD664/OD665	1.4 ± 0.02^{A}	1.57 ± 0.03^{B}	1.60 ± 0.02^{B}	1.59 ± 0.01^{B}	1.62 ± 0.03^{A}	1.50 ± 0.01^{B}	1.60 ± 0.02^{A}	1.53 ± 0.03^{AB}
Ash-free dry mass of benthic organic matter ($g \cdot m^{-2}$)	52.6 ± 9.1^{A}	9.2 ± 2.2^{B}	2.3 ± 0.6^{B}	1.0 ± 0.2^{B}	17.2 ± 1.1^{A}	12.6 ± 2.3^{AB}	13.7 ± 2.4^{AB}	9.1 ± 1.5^{B}

Table 2. Concentrations of total phosphorous of benthic organic matter, soluble reactive phosphorous of water, NO_2 + NO_3 of water, and total nitrogen of water at stream sites sampled in August 2015 and August 2016.

Nutrient	Suggs 2015	Trace 2015	Flynn 2015	Hurricane 2015	Jones 2016	McAdoo 2016	M. Bone 2016	Will Hall 2016
Total phosphorous of benthic organic matter (mg·m^{-2})	74.7	7.9	2.9	1.4	35.6	6.3	14.1	8.3
Soluble reactive phosphorous (μg·L^{-1} water)	6	8	38	8	197	9	7	7
NO_2 + NO_3 (μg·L^{-1} water)	38	222	750	238	2944	1098	122	72
Total nitrogen (μg·L^{-1} water)	491	303	1034	297	4205	1192	284	262

(TDEC 2016). Only the Jones Creek site had water concentrations for soluble reactive phosphorous, NO_2 + NO_3 nitrogen, and total nitrogen above the mesotrophic-eutrophic threshold (Dodds et al. 1998). The very high nutrient concentrations of water at the Jones Creek site most likely reflect continuous input from the Jones Creek Wastewater Treatment Plant located 5 km upstream. The low nutrient concentrations of water at the Suggs Creek site, designated as hypereutrophic by the concentration of chl *a*, are most likely due to the high nutrient demand by the nuisance-level concentration of periphyton at the site.

Concentrations of chl *a* and the ash-free dry mass of benthic organic matter of sites sampled in August 2015 were significantly correlated with the concentration of total phosphorous of benthic organic matter but not to the concentrations of nutrients of water samples (Table 3). These results indicate that the concentration of total phosphorous of benthic organic matter was a more accurate indicator of the trophic state of the sites sampled in August 2015 relative to the water concentration of soluble reactive phosphorous, NO_2 + NO_3, and total nitrogen. The eutrophic concentrations of chl *a* coupled with the high concentration of soluble reactive phosphorous of water at the Jones Creek site located downstream of the wastewater treatment plant (Tables 1 and 2) contribute to the significant correlation of the concentration of chl *a* to the concentration of soluble reactive phosphorous of water samples for sites sampled in August 2016.

Diatoms were the most abundant algae relative to soft-algae at every site sampled in May 2015 and May 2016, while soft-algae were most abundant at every site sampled in August 2015 and August 2016 (Table 4). These seasonal differences for diatom and soft-algae abundances were significant as determined by Student's *t*-tests for both May (n = 8, $p < 0.001$) and August (n = 8, $p <$ 0.001). Percent composition of cyanobacteria was significantly lower at sites sampled May 2015 and May 2016 ($\bar{x}$ = 16.4 ± 5.0) relative to sites sampled August 2015 and August 2016 ($\bar{x}$ = 54 ± 10.7) as determined by a Student's *t*-test (n = 8, p = 0.01). Percent composition of Chlorophyta at sites sampled May 2015 and May 2016 ($\bar{x}$ = 9.5 ± 2.2) was not significantly different from sites sampled

Table 3. Pearson's correlation coefficients for concentrations of chlorophyll *a* and ash-free dry mass of benthic organic matter to concentrations of total phosphorous of benthic organic matter and nutrient concentrations of water samples at sites sampled August 2015 and at sites sampled August 2016. Pearson's correlation coefficients are followed by the significance of probability at the 95% confidence level in parentheses.

	Total phosphorous of benthic organic matter (mg·m^{-2})	Soluble reactive phosphorous (μg.L^{-1} water)	NO_2 + NO_3 (μg·L^{-1} water)	Total nitrogen (μg.L^{-1} water)
August 2015 (Suggs, Trace, Flynn, Hurricane)				
Chlorophyll *a* (mg·m^{-2})	0.97 (0.03)	−0.43 (0.57)	−0.62 (0.37)	−0.13 (0.87)
Ash-free dry mass of benthic organic matter (g·m^{-2})	1.0 (0.00)	−0.52 (0.47)	−0.64 (0.36)	−0.12 (0.87)
August 2016 (Jones, McAdoo, M. Bone, Will Hall)				
Chlorophyll *a* (mg·m^{-2})	0.99 (0.01)	0.99 (0.01)	0.92 (0.08)	−0.28 (0.72)
Ash-free dry mass of benthic organics matter (g·m^{-2})	0.85 (0.14)	0.81 (0.18)	0.83 (0.17)	−0.09 (0.91)

Table 4. Percent composition of algae groups sampled May 2015, August 2015, May 2016, and August 2016.

	Suggs		Trace		Flynn		Hurricane	
2015	May	August	May	August	May	August	May	August
Bacillariophyceae (diatoms)	72.9	18.5	68.8	6.1	75.6	24.0	49.3	10.8
Soft-algae	27.1	81.5	31.2	93.9	24.4	76.0	50.7	89.2
Cyanobacteria	8.3	3.7	22.5	92.9	12.1	72.5	49.8	86.1
Chlorophyta	16.5	68.6	8.5	1.0	12.3	2.7	0.9	3.2
Ochrophyta (other than diatoms)	1.2	8.8	0.1			0.5		
Rhodophyta	0.1							
	Jones		McAdoo		M. Bone		Will Hall	
2016	May	August	May	August	May	August	May	August
Bacillariophyceae (diatoms)	75.3	33.8	78.6	44.0	78.2	36.8	90.8	26.7
Soft-algae	24.7	66.2	21.4	56.0	21.8	63.2	9.2	73.3
Cyanobacteria	10.0	28.4	8.1	47.8	11.7	39.3	8.8	62.0
Chlorophyta	14.9	27.8	13.0	9.3	10.0	20.5	< 0.1	9.6
Ochrophyta (other than diatoms)			<0.1		<0.1			
Rhodophyta		9.9	1.4		<0.1	3.4		1.7
Cryptophyta				0.7	<0.1			
Euglenophyta								0.3

August 2015 and August 2016 ($\bar{x} = 17.8 \pm 8.0$) as determined by a Student's t-test ($n = 8$, $p = 0.33$). These results are consistent with numerous studies of stony streams from Europe, Japan, and North America which illustrate temperature is a dominant factor controlling temporal changes in the abundance of algal groups. Diatoms dominate in the winter and often continue to be the major component of algal assemblages in spring given they are generally more abundant in cool water (Allan & Castillo 2009). Chlorophyta and cyanobacteria become more abundant during the late spring with cyanobacteria often becoming the most abundant algal group in the summer given they are typically more abundant at temperatures >30 °C (DeNicola 1996).

The 232 algae taxa identified at the sites we studied illustrate the large diversity of algae taxa in the central region of the Interior Plateau Level III Ecoregion. We identified 114 diatom taxa (Appendix 3). The two most abundant diatom taxa sampled (Table 5), *Achnanthidium rivulare* Potapova and Ponader (26.2% of all sites and dates) and *Achnanthidium minutissimum* (Kütz.) Czarn. (8.9% of all sites and dates), are common in the southeastern United States (Ponader & Potapova 2007). The relatively low percent composition of *A. rivulare* at the most eutrophic sites, Suggs and Jones Creek, relative to the other sites is consistent with the conclusions of Ponader and Potapova (2007) that *A. rivulare* is less abundant in stream reaches with poor quality water. The composition of diatom assemblages differed between the sites as little as 24% between the Trace and Hurricane Creek sites in May 2015 to as much as 83% between the Jones and Marrow Bone Creek sites in May 2016 (Table 6). The greater percent similarity for the Trace and Hurricane Creek sites in May 2016 results from the greater dominance of *A. rivulare* at the two sites (Table 5).

Values for the pollution tolerance index of diatom assemblages (PTI) illustrate the trophic state of the stream sites (Table 7). Values for the PTI were lowest, which indicate most eutrophic for the Suggs Creek site among sites sampled in 2015 and for the Jones Creek site among sites sampled in 2016. Values for the SI <60 and values for the OPI <40 for stream sites other than the Jones Creek site imply that only the composition of diatom assemblages at the Jones Creek site may have been substantially impacted by siltation and organic pollution. Values for the Shannon diversity index and evenness for diatom assemblages sampled in August 2015 and August 2016 were not correlated with the trophic state of the sites as indicated by nonsignificant Pearson's correlation coefficients to concentrations of chl *a*, total phosphorous of benthic organics, and ash-free dry mass of benthic organics (data not shown).

We identified 128 taxa of soft-algae (Appendix 4). The two most abundant soft-algae taxa sampled (Table 8), the filamentous chlorophyte *Cladophora glomerata* (17.9% of all sites and dates) and

Table 5. Most abundant diatom taxa at stream sites sampled in May 2015, August 2015, May 2016, and August 2016. Numbers in parentheses represent percent composition.

May 2015			
Suggs	Trace	Flynn	Hurricane
A. rivulare (14.9)	*A. rivulare* (66.3)	*A. rivulare* (46.8)	*A. rivulare* (72.9)
C. pediculus (13.9)	*N. palea* (16.1)	*D. vulgaris* (10.6)	*C. placentula* (4.1)
C. placentula (10.5)	*C. affinis* (3.0)	*M. varians* (6.0)	*A. minutissimum* (2.6)
August 2015			
A. rivulare (15.0)	*A. rivulare* (20.5)	*A. rivulare* (23.9)	*A. rivulare* (35.8)
N. viridula (9.2)	*C. affinis* (17.7)	*P. curtissimum* (8.2)	*C. placentula* (13.2)
A. minutissimum (8.7)	*E. appalachianum* (16.3)	*A. minutissima* (6.9)	*A. minutissimum* (11.5)
May 2016			
Jones	McAdoo	M. Bone	Will Hall
N. reichardtiana (14.7)	*A. minutissimum* (20.4)	*A. rivulare* (69.9)	*A. minutissimum* (20.4)
N. inconspicua (10.4)	*C. affinis* (8.3)	*C. affinis* (9.4)	*C. affinis* (15.4)
S. seminulum (6.6)	*A. rivulare* (7.0)	*A. minutissimum* (6.4)	*A. latecephalum* (12.2)
August 2016			
N. minima (18.4)	*A. rivulare* (10.0)	*E. appalachianum* (16.4)	*A. minutissimum* (18.6)
N. amphibia (11.1)	*N. minima* (7.5)	*A. minutissimum* (15.2)	*A. rivulare* (13.6)
N. inconspicua (6.8)	*A. purpusilla* (7.1)	*C. affinis* (13.2)	*C. placentula* (7.4)

the filamentous cyanobacterium *Phormidium diguetii* (Gomont) Anagn. & Komárek (14.3% of all sites and dates), are widespread and abundant in the eastern United States (Prescott 1982; Whitford and Schumaker 1984). The dominance of *C. glomerata* at the most eutrophic sites is consistent with numerous studies which cite *C. glomerata* as an indicator of eutrophic conditions of both lentic and lotic systems (Mackie 2013; Fetcher et al. 2014; Stancheva & Sheath 2016). *Phormidium diguetii* is tolerant of eutrophic conditions in lentic systems (Mackie 2013), yet was not more abundant at the most eutrophic sites in this study. Other taxa cited as tolerant of eutrophic, lentic water and not substantially more abundant at the eutrophic sites sampled in this study include *Phormidium tenue* (C. Agardh) Anagn. and Komárek and *Stigeoclonium tenue* (Ag.) Kütz. This result is consistent with the suggestion that factors other than trophic state may be more important in lotic systems relative to lentic systems as determinants of the abundance of soft-algae taxa (Leland & Porter 2000).

The composition of soft-algae assemblages differed between sites as much as 98% between the Suggs Creek site and both the Trace and Flynn Creek sites sampled in August 2015 (Table 9). The greater similarity of composition between the assemblages at the Suggs and Flynn Creek sites (64%)

Table 6. Percent similarity of diatom assemblages between the different sites sampled May 2015, between the different sites sampled August 2015, between the different sites sampled May 2016, and between the different sites sampled August 2016.

May 2015			
	Suggs	Trace	Flynn
Trace	30		
Flynn	44	55	
Hurricane	37	76	59
August 2015			
Trace	46		
Flynn	49	47	
Hurricane	46	67	50
May 2016			
	Jones	McAdoo	M. Bone
McAdoo	41		
Marrow Bone	17	28	
Will Hall	28	56	38
August 2016			
McAdoo	33		
Marrow Bone	40	25	
Will Hall	35	38	45

Table 7. Indices and metrics of diatom assemblages at sites sampled in May 2015, August 2015, May 2016, and August 2016.

	Suggs		Trace		Flynn		Hurricane	
2015	May	August	May	August	May	August	May	August
Pollution tolerance index	2.53	2.37	2.63	2.54	2.79	2.87	2.94	2.94
Siltation index	31	42	20	7	13	12	7	11
Organic pollution index	20	25	19	3	7	9	4	9
Shannon diversity index	3.1	3.2	1.4	2.3	2.3	2.9	1.5	2.3
Evenness	0.84	0.86	0.47	0.69	0.64	0.83	0.41	0.69
Taxon richness	40	40	19	28	35	37	34	26
Genus richness	16	16	11	17	16	17	15	13
	Jones		McAdoo		M. Bone		Will Hall	
2016	May	August	May	August	May	August	May	August
Pollution tolerance index	2.16	1.95	2.60	2.31	3.07	2.72	3.03	2.66
Siltation index	67	60	34	51	2	19	14	20
Organic pollution Index	52	41	19	27	2	14	5	13
Shannon diversity Index	3.1	3.3	3.2	3.5	1.2	2.8	2.8	3.1
Evenness	0.87	0.84	0.83	0.83	0.43	0.84	0.75	0.84
Taxon richness	36	51	46	48	17	35	40	45
Genus richness	15	18	19	12	10	13	18	21

and the Jones and Marrow Bone Creek sites (61%) results from similar percent compositions for *C. glomerata* (Table 8). The low percent similarity of soft-algae and diatom taxa of all sites between May and August sampling dates (Table 10) is consistent with data from other studies which demonstrate composition of algae taxa may vary dramatically between seasons or years (Brown et al. 2008). The change of soft-algae composition among all sites from May to August was almost double the change of diatom composition and was significantly greater as determined by a Student's *t*-test ($p = 0.001$). We do not know the factors responsible for the differences in percent composition between sites or for the changes in percent composition between sampling dates.

Table 8. Most abundant soft-algae taxa at sites sampled May 2015, August 2015, May 2016, and August 2016. Numbers in parentheses represent percent composition.

May 2015			
Suggs	Trace	Flynn	Hurricane
C. glomerata (60.9)	*C. glomerata* (22.7)	*C. glomerata* (49.3)	*P. retzii* (75.7)
L. foveolarum (10.0)	*P. diguetii* (18.0)	*H. kossinskajae* (24.0)	*L. foveolarum* (10.0)
Vaucheria sp. (7.3)	*P. autumnale* (16.9)	*P. retzii* (13.5)	*P. diguetii* (3.4)
H. kossinskajae (6.7)	*P. angustissimum* (14.5)	*L. foveolarum* (5.8)	*L.angustissimum* (1.4), *O. subtilissima* (1.4)
August 2015			
Spirogyra sp. (28.1)	*P. diguetii* (46.0)	*P. diguetii* (31.8)	*P. diguetii* (63.4)
C. glomerata (27.4)	*H. juliana* (34.1)	*G. pleuroccapsoides* (17.2)	*P. retzii* (9.6)
Oedogonium sp. (21.3)	*L. martensiana* (5.5)	*L. foveolarum* (8.5)	*P. fragile* (6.9)
Vaucheria sp. (10.8)	*Phormidium* sp. (2.2)	*L. angustissimum* (5.9)	*O. subbrevis* (5.3)
May 2016			
Jones	McAdoo	M. Bone	Will Hall
C. glomerata (58.1)	*C. glomerata* (42.3)	*Spirogyra* sp. (19.8), *S. tenue* (19.8)	*L. foveolarum* (40.9)
L. foveolarum (13.6)	*Oedogonium* sp. (13.0)	*L. foveolarum* (16.2)	*L. angustissimum* (19.5)
P. tenue (4.2)	*A. hermannii* (6.7)	*P. diguetti* (13.6)	*O. limosa* (10.1)
P. autumnale (3.8), *P. diguetti* (3.8)	*P. tenue* (5.4)	*L. angustissimum* (6.0)	*S. major* (8.1)
August 2016			
C. glomerata (17.5)	*P. diguetii* (19.2)	*Oedogonium* sp. (31.0)	*H. juliana* (28.5)
A. hermannii (15.0)	*L. nostocrum* (11.5)	*P. diguetti* (22.5)	*Phormidium* sp. (13.6)
E. rivularis (10.2)	*K. constrictum* (9.1)	*L. foveolarum* (11.5)	*L. angustissimum* (9.1)

Table 9. Percent similarity of soft-algae assemblages between the different sites sampled May 2015, between the different sites sampled August 2015, between the different sites sampled May 2016, and between the different sites sampled August 2016.

May 2015			
	Suggs	Trace	Flynn
Trace	28		
Flynn	64	31	
Hurricane	15	14	23
August 2015			
Trace	2		
Flynn	2	43	
Hurricane	4	50	39
May 2016			
	Jones	McAdoo	M. Bone
Big McAdoo	61		
Marrow Bone	25	19	
Will Hall	20	13	24
August 2016			
Big McAdoo	15		
Marrow Bone	22	40	
Will Hall	21	20	32

Table 10. Percent similarity of soft-algae and diatom assemblages between the same sites sampled May 2015 and again August 2015, between the same sites sampled May 2016 and again August 2016, and mean ± SE percent similarity of all sites sampled May and again August.

Assemblage	Suggs 2015	Trace 2015	Flynn 2015	Hurricane 2015	Jones 2016	McAdoo 2016	M. Bone 2016	Will Hall 2016	Mean ± SE % similarity
Soft-algae	35	21	12	18	27	21	38	16	24 ± 3
Diatom	61	31	40	51	43	35	29	44	42 ± 4

The values for the Shannon diversity index for soft-algae assemblages were lowest for the Hurricane Creek site sampled May 2015 and August 2015 (Table 11) due to the dominance of *Phormidium retzii* (C. Agardh) Gomont at the site during May 2015 and *P. diguetii* during August 2015 (Table 8). Values for the Shannon diversity index and evenness for soft-algae assemblages sampled in August 2015 and August 2016 did not correspond with the trophic state of the sites as indicated by nonsignificant Pearson's correlation coefficients to

Table 11. Shannon diversity index and metrics for soft-algae assemblages sampled May 2015, August 2015, May 2016, and August 2016.

	Suggs		Trace		Flynn		Hurricane	
2015	May	August	May	August	May	August	May	August
Shannon diversity index	1.5	1.8	2.2	1.5	1.5	2.5	1.1	1.4
Evenness	0.46	0.49	0.72	0.46	0.51	0.70	0.35	0.52
Taxon richness	20	39	21	26	19	36	24	15
Genus richness	16	29	13	20	14	21	13	11

	Jones		McAdoo		M. Bone		Will Hall	
2016	May	Aug.	May	Aug.	May	Aug.	May	Aug.
Shannon diversity index	1.7	2.8	2.3	2.5	2.5	2.1	2.0	2.4
Evenness	0.53	0.79	0.63	0.73	0.71	0.68	0.69	0.76
Taxon richness	26	29	37	29	32	22	18	23
Genus richness	17	21	23	16	22	15	12	18

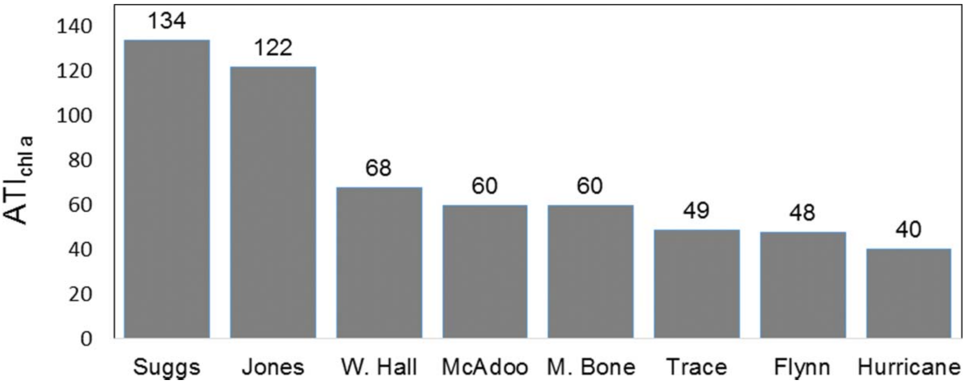

Figure 1. Values for the algae trophic index using abundance-weighted average of chlorophyll *a* concentrations ($ATI_{chl\ a}$) for soft-algae taxa as the trophic-indicator values of stream sites sampled August 2015 and August 2016.

concentrations of chl *a*, total phosphorous of benthic organics, and ash-free dry mass of benthic organics (data not shown). This result coupled with the nonsignificant effect of trophic state on the Shannon diversity index for diatom assemblages sampled in August 2015 and August 2016 supports earlier conclusions that high values for the Shannon diversity index may not imply good quality habitats in aquatic environments (Carlisle et al. 2008; Lebkuecher et al. 2015).

The A-WA of chl *a* for soft-algae taxa (Appendix 5) serves as trophic-indicator values. Soft-algae taxa with greater values for the A-WA of chl *a* ($A\text{-}WA_{chl\ a}$) are more abundant at sites with greater concentrations of chl *a* and thus are more abundant at the relatively nutrient-rich stream sites. Taxa which occur at more than one site and with a low standard deviation (SD) of the A-WA, and thus a low SD to A-WA ratio, are interpreted as potential indicators of trophic state (Stancheva et al. 2012). There are no set guidelines for the use of A-WA and SD data to designate taxa as indicators of habitat quality (Stancheva et al. 2012). For our data, we consider taxa with an $A\text{-}WA_{chl\ a}$ in the upper and lower tertiles ($A\text{-}WA_{chl\ a} \geq 132.5$ and $A\text{-}WA_{chl\ a} \leq 52.4$, respectively) with a SD to A-WA ratio below the mean SD to A-WA ratio ($\bar{x} = 0.41$) as potential indicators of the most nutrient-impacted and least nutrient-impacted sites, respectively. Using these criteria and excluding taxa that occurred at only one site, taxa designated as potential indicators of nutrient-rich sites include only *C. glomerata*, *Dactylococcopsis raphidioides* Hansg., *Gloeocapsopsis cyanea* (Krieg) Komárek and Anagn., and *Vaucheria* sp. Taxa designated as potential indicators of relatively nutrient-unimpacted sites include only *Chaetopeltis orbicularis* Berthold, *Aphanocapsa elachista* West and West, and *Oscillatoria subtilissima* Kütz. and De Toni.

The ATI using A-WA of chl *a* concentrations for soft-algae taxa as the trophic-indicator values ($ATI_{chl\ a}$; Figure 1) accurately denotes the trophic state of the stream sites (Table 12). The $ATI_{chl\ a}$ is significantly correlated with concentrations of total phosphorous of benthic organic matter, ash-free dry mass of benthic organic matter, concentrations of chl *a*, and PTI. The ATI using A-WA of ash-free dry mass of benthic organic matter (Figure 2) and the ATI using A-WA of total phosphorous concentration of benthic organic matter (Figure 3) as the trophic-indicator values did not correspond significantly to all of the periphyton characteristics used to indicate the trophic state of the stream sites (Table 12). None of the biotic indices were significantly correlated to water concentrations of soluble reactive phosphorous (Table 12), $NO_2 + NO_3$ (data not shown) or total nitrogen (data not shown).

Table 12. Pearson's correlation coefficients for indices of algae assemblages sampled August 2015 and August 2016 to other site characteristics followed by the significance of probability at the 95% confidence level in parentheses. The ATI using soft-taxa abundance-weighted averages of concentrations of chlorophyll (chl) *a* as the trophic-indicator values is abbreviated $\mathrm{ATI}_{\mathrm{chl}\ a}$. The ATI using abundance-weighted averages of ash-free dry mass of benthic organic matter as the trophic-indicator values is abbreviated $\mathrm{ATI}_{\mathrm{AFDM}}$. The ATI using abundance-weighted averages of the concentration of total phosphorous of benthic organic matter as the trophic-indicator values is abbreviated $\mathrm{ATI}_{\mathrm{TP}}$.

	Total phosphorous of benthic organics (mg/m^2)	Ash-free dry mass of benthic organics (g·m^{-2})	Chl *a* (mg·m^{-2})	Pollution tolerance index of diatom assemblages	Soluble reactive phosphorous of water (µg·L^{-1} water)
$\mathrm{ATI}_{\mathrm{chl}\ a}$	0.93 (0.001)	0.85 (0.01)	0.89 (0.003)	−0.75 (0.03)	0.51 (0.20)
$\mathrm{ATI}_{\mathrm{AFDM}}$	0.98 (<0.0001)	0.61 (0.11)	−0.48 (0.23)	0.00 (0.99)	−0.15 (0.71)
$\mathrm{ATI}_{\mathrm{TP}}$	0.99 (<0.0001)	0.98 (< 0.0001)	0.65 (0.08)	−0.49 (0.21)	0.16 (0.71)
Pollution tolerance index of diatom assemblages	−0.54 (0.17)	−0.49 (0.22)	−0.86 (0.006)		−0.48 (0.23)

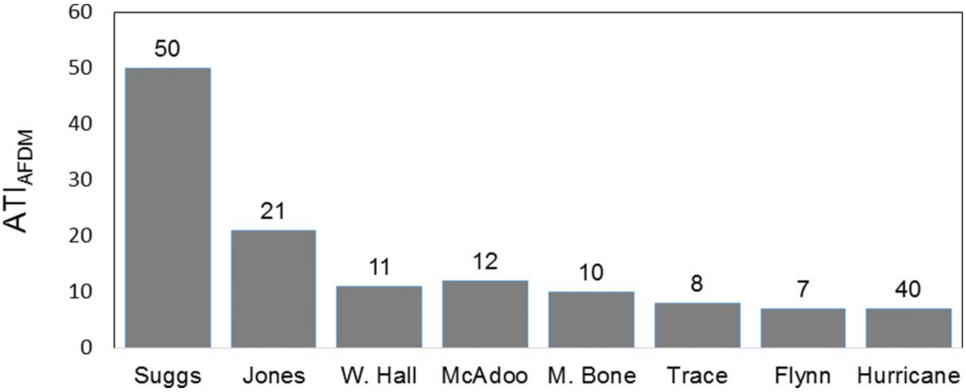

Figure 2. Values for the algae trophic index using abundance-weighted average of ash-free dry mass of benthic organic matter ($\mathrm{ATI}_{\mathrm{AFDM}}$) for soft-algae taxa as the trophic-indicator values of stream sites sampled August 2015 and August 2016.

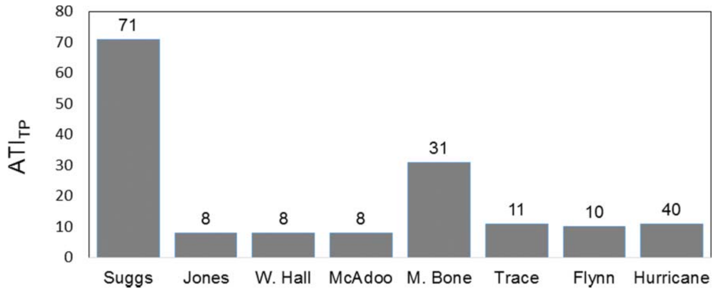

Figure 3. Values for the algae trophic index using abundance-weighted average of the concentrations total phosphorous of benthic organic matter ($\mathrm{ATI}_{\mathrm{TP}}$) for soft-algae taxa as the trophic-indicator values of stream sites sampled August 2015 and August 2016.

Discussion

The nuisance levels of benthic algae at the Suggs and Jones Creek sites illustrate a negative impact of eutrophication. Estimates of biomass alone do not advance our limited understanding of the effects of nutrient concentration on the autecology of soft-algae. Analyses of the relationships of nutrient concentration and the structure of soft-algae assemblages are essential to improve our understanding of the effect of eutrophication on periphyton communities (Stancheva et al. 2012). This study advances our understanding of the effects of trophic state on the composition of photoautotrophic assemblages by evaluating the percent composition of soft-algae taxa at sites with differing trophic states.

Several characteristics of soft-algae most likely contribute to the scarcity of data correlating nutrient concentration to abundance for most soft-algae taxa in lotic systems. The physical characteristics of a stream site often have a greater impact on the structure of soft-algae assemblages relative to diatom assemblages. Soft-algae taxa may be more affected by intermittent changes of water velocity due to their greater diversity of surface area relative to diatoms (Whitton 2012). The greater phylogenetic diversity for soft-algae relative to diatoms most likely contributes to greater differences of ecological interactions. The study by Lebkuecher et al. (2015) of three mesotrophic sites and one hypereutrophic site in Sulphur Fork Creek in Middle Tennessee is the only other research we are aware of in which the changes of percent composition of both diatoms and soft-algae taxa from spring to summer were evaluated at the same sites in a lotic system. The similarity of percent composition of diatoms from spring to summer was much more consistent, ranging from 58% to 65%, relative to the similarity of percent composition of soft-algae taxa which ranged from 30% to 85%. Our results demonstrate an almost twofold greater difference in the change of soft-algae composition from spring to summer relative to diatom composition (Table 10) which, if occurs in other temperate streams, may contribute to the difficulty of assigning cosmopolitan trophic-state optima to many soft-algae taxa.

The few taxa designated as reliable indicators of trophic state by this study strengthen the conclusions of earlier studies that trophic state may not be the most important factor affecting percent composition for many soft-algae species of lotic assemblages. Lebkuecher et al. (2015) demonstrated that only 4 of 125 soft-algae taxa sampled from Sulphur Fork Creek in Middle Tennessee were significantly correlated to $\log_{10}$-transformed concentrations of soluble reactive phosphorous of water samples. No soft-algae taxa out of 221 taxa sampled streams of Washington and Idaho were designated as eutrophic indicators using a computer regression program to model $\log_{10}$-transformed concentrations of soluble reactive phosphorous of water samples to percent composition of soft-algae taxa (Munn et al. 2002). Stancheva et al. (2012) designated 7 out of 180 soft-algae taxa as indicators of trophic state in streams of southern California streams using abundance-weighted averages of total phosphorous concentrations of water.

Ecoregion-specific assessments may be required to evaluate the impact of eutrophication on soft-algae assemblages (NAWQA 2005). Soft-algae taxa often found in nutrient-impaired streams of North America are cited as intolerant of eutrophication in Europe (Porter 2008). Rott and Schneider (2014) reported that the optimum water concentration of total phosphorus was significantly different for 16 of 21 soft-algae taxa in Norway relative to Austria. Regression analysis indicated that the positions of many optima relative to each other were stable across Norway and Austria. Rott and Schneider (2014) concluded that this result supports suggestions that identifying lotic soft-algae taxa for use as cosmopolitan trophic-indicators may be possible. Our study reveals the impact of trophic state on the percent composition of soft-algae taxa needed to understand and monitor the effects of eutrophication on soft-algae assemblages in the central region of the Interior Plateau Level III Ecoregion of the United States. More studies which correlate trophic state to percent composition of soft-algae taxa may help to lead to the designation of some soft-algae taxa as universal indicators of trophic state as has been done for many diatom taxa (Danielson et al 2011).

Governments worldwide require periodic determinations of the chemical constituents of water to monitor the trophic state of rivers and streams (Whitton 2013). The concentration of soluble reactive phosphorus of water samples is a primary criterion for assigning trophic state to reaches in rivers because soluble reactive phosphorus is the form of phosphorous available to photoautotrophs and limits primary production of most algae in most rivers (Moss et al. 2013). Chemical analyses of water samples may not accurately reflect trophic state because pulses of nutrient enrichment may be missed during sampling or concentrations of nutrients of water samples may be low due to high nutrient demand (Dodds 2006). The United States Geological Survey National Water-Quality Assessment (NAWQA) program ranked soft-algae taxa by eutrophication-tolerance based on abundance-weighted averages of $\log_{10}$-transformed concentrations of soluble reactive phosphorous of water samples at sites across the United States (NAWQA 2005). NAWQA (2005) concluded that the rankings of many taxa are not accurate. The low nutrient concentrations of water samples collected at the eutrophic sites during our study, other than the Jones Creek site downstream of the wastewater treatment plant, support the suggestion by Dodds (2006) that nutrient concentrations of water may underestimate the trophic state of eutrophic sites.

The five indices developed previously which utilize soft-algae taxa exclusively to evaluate trophic state of stream sites that we are aware of (Gutowski et al. 2004; Schamburg et al. 2004; Schneider & Lindstrøm 2011; Fetscher et al. 2014; Lebkuecher et al. 2015) are not applicable for the evaluation of the trophic state of stream sites in the Interior Plateau Level III Ecoregion. These indices are designed to assess trophic state of lotic systems in countries other than the United States (Gutowski et al. 2004; Schaumburg et al. 2004; Schneider & Lindstrøm 2011), or southern California (Fetscher et al. 2014), or are based on trophic-indicator values which are not useful at stream sites other than those used to calculate the values (Lebkuecher et al. 2015). The index developed by Gutowski et al. (2004) and Schaumburg et al. (2004) to evaluate the water quality of German rivers is based on 74 and 51 taxa, respectively, and very few of these taxa were present at the sites we sampled in Middle Tennessee. The periphyton index of trophic status (PIT; Schneider & Lindstrøm 2011) was developed to evaluate the trophic state of Nordic rivers. Few taxa sampled by Schneider and Lindstrøm (2011) are present at the sites we sampled in Middle Tennessee and the maximum concentration of total phosphorus of water samples for which the index was developed is below the concentration of many eutrophic streams in other regions (Whitton 2013).

Fetscher et al. (2014) used a computer program to calculate values for indices using diatom taxa, soft-algae taxa, and both diatom and soft-algae taxa to evaluate the water quality of southern California streams. The indicator values for taxa were established using numerous criteria specifically to assess the water quality of southern California streams and included landscape features, land use, and water chemistry parameters. The index using soft-algae taxa corresponds to nutrient concentrations of the stream sites and demonstrates that the structure of soft-algae assemblages can be affected by trophic state. Lebkuecher et al. (2015) developed an index utilizing Pearson's correlation coefficients for $\log_{10}$-transformed concentrations of soluble reactive phosphorous of water samples to percent composition of soft-algae taxa as trophic-indicator values to assess the trophic state of sites in Sulphur Fork Creek located in Middle Tennessee. The index accurately denotes the trophic state of the two mesotrophic sites and one hypereutrophic site sampled because the water at the hypereutrophic site has a consistently high concentration of soluble reactive phosphorous since it is immediately downstream of a wastewater treatment plant. Because the trophic-indicator values are based on the concentration of soluble reactive phosphorous of water, which may be low at eutrophic sites not immediately downstream from a consistent source of soluble reactive phosphorous, the trophic-indicator values cannot be expanded to include additional taxa from different streams.

Our results support the long standing consensus that benthic concentrations of chl *a* (Dodds et al. 1998) and the composition of diatom assemblages (Kelly & Whitton 1995) are often effective indicators of the trophic state of stream sites. Use of several measurements to assess trophic state is optimal given any one measurement may not be accurate. Diatoms such as *Cocconeis placentula* Ehrenb. and *Rhoicosphenia curvata* (Kütz.) Grun. have oligotrophic to mesotrophic trophic values for the pollution tolerance index of diatom assemblages (KDOW 2002). Both of these taxa are epilithic and epiphytic on large, filamentous algae and may be more abundant at eutrophic sites dominated by large filamentous algae (Leland & Porter 2000). The ATI using abundance-weighted averages of concentrations of chl *a* for soft-algae taxa as trophic-state indicators ($\mathrm{ATI}_{\mathrm{chl}\ a}$) accurately denotes the trophic state of the sites we studied and provides a valuable methodology supplement to monitor the trophic state of the sites. The $\mathrm{ATI}_{\mathrm{chl}\ a}$ has several positive features. Use of all taxa of the assemblage to calculate the index values avoids subjective exclusion of less common taxa or taxa not considered as strong indicators of trophic state. The range of the index emulates concentrations of chl *a* at nutrient-unimpaired and nutrient-impaired sites and thus is easy to interpret. This index is the first to utilize periphyton characteristics, as opposed to nutrient concentrations of water, to assign trophic-indicator values to soft-algae taxa and does not require a computer program to compute.

Acknowledgments

This work was supported by the Tennessee Healthy Watershed Initiative (grant number 32701-02096), a collaboration of the Tennessee Department of Environment and Conservation Division of Water Resources, Tennessee Valley Authority, Tennessee Chapter of The Nature Conservancy, and the West Tennessee River Basin Authority. Funding was also provided by the Department of Biology at Austin Peay State University and The Center for Field Biology at Austin Peay State University. We thank Drs Jennifer Greenwood, Susanne Schneider, and two anonymous reviewers for reviewing the manuscript and offering suggestions for improvement.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Tennessee Healthy Watershed Initiative [grant number 32701-02096]; Department of Biology at Austin Peay State University; The Center for Field Biology at Austin Peay State University.

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References

Allan JD, Castillo MM. 2009. Stream ecology: structure and function of running waters. Rotterdam: Springer.

Anagnostidis K, Komárek J. 1988. Modern approach to the classification system of cyanophytes 3 - Oscillatoriales. Arch Hydrobiol Suppl. 80:327–472.

Bahls LL. 1993. Periphyton bioassessment methods for Montana streams. Helana (MT): Water Quality Bureau, Department of Health and Environmental Sciences.

Biggs BJF. 2000. Eutrophication of streams and rivers: dissolved nutrient-chlorophyll relationships for benthic algae. J N Am Benthol Soc. 19:17–31.

Brown LR, May JT, Hunsaker CT. 2008. Species composition and habitat associations on benthic algae assemblages in headwater streams of the Sierra Nevada, California. West N Am Naturalist. 68:194–209.

Carlisle DM, Hawkins CP, Meader MR, Potapova M, Falcone J. 2008. Biological assessments of Appalachian streams based on predictive models for fish, macroinvertebrates and diatom assemblages. J N Am Benthol Soc. 27:16–37.

Carr JM, Hergenrader JL, Troelstrup NH Jr. 1986. A simple, inexpensive method for cleaning diatoms. Trans Am Micro Soc. 105:152–157.

Cocke EC. 1967. The Myxophyceae of North Carolina. Ann Arbor (MI): Edwards Brothers.

Danielson TJ, Loftin CS, Tsomides L, DiFranco JL, Connors B. 2011. Algal bioassessment metrics for wadeable streams and rivers of Maine, USA. J N Am Benthol Soc. 30:1033–1048.

DeNicola DM. 1996 Periphyton responses to temperature at different ecological levels. In: Stevenson RJ, Bothwell ML, Lowe RL, editors. Algal ecology: freshwater benthic ecosystems. San Deigo (CA): Academic Press; p. 149–181.

Dodds WK. 2006. Eutrophication and trophic state in rivers and streams. Limnol Oceanogr. 51:671–680.

Dodds WK, Jones JR, Welch EB. 1998. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorous. Water Resour. 32:1455–1462.

Eaton AD, Clesceri LS, Rice EW, Greenberg AE. 2005. Standard methods for the examination of water and wastewater. 21st ed. Washington (DC): American Public Health Association.

Fetscher AE, Stancheva RS, Kociolek JP, Sheath RG, Stein ED, Mazor RD, Ode PR. 2014. Development and comparisons of stream indices of biotic integrity using diatoms vs. non diatom algae vs. a combination. J Appl Phychol. 26:433–450.

Griffith GE, Omernik JM, Azevedo SH. 1997. Ecoregions of Tennessee. EPA/600/R97/022. NHREEL. Corvallis (OR): United States Environmental Protection Agency, Western Ecological Division.

Gutowski A, Foerster J, Schaumburg J. 2004. The use of benthic algae, excluding diatoms and Charales, for the assessment of the ecological status of running fresh waters: a case history from Germany. Oceanol Hydrobiol St. 33:3–15.

Hauer R, Lamberti GA. 2006. Methods in stream ecology. 2nd ed. Maryland Heights (MO): Academic Press.

John DM, Whitton BM, Brook AJ. 2011. The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae. 2nd ed. Cambridge: Cambridge University Press.

Kentucky Department of Water (KDOW). 2002. Methods for assessing biological integrity of surface waters in Kentucky. Frankfort (KY): Department for Environmental Protection, Division of Water. Available from: http://water.ky.gov/Pages/SurfaceWaterSOP.aspx

Kelly MG. 1998. Use of the diatom trophic index to monitor eutrophication in rivers. Water Res. 32:236–242.

Kelly MG, Whitton BA. 1995. The trophic diatom index: a new index for monitoring eutrophication in rivers. J Appl Phycol. 7:433–444.

Krammer K, Lange-Bertalot H. 1998. Bacillariophyceae. 2. Teil: Bacillariaceae, Epithemiaceae, Surirellaceae. In: Ettl H, Gerloff J, Heynig H, Mollenhauer D, editors. Süsswasserflora von Mitteleuropa, Band 2/2. Jena: VEB Gustav Fischer Verlag; p. 596.

Kurle CM, Cardinale BJ. 2011. Ecological factors associated with the strength of trophic cascades. Oikos. 120:1897–1908.

Lebkuecher JG, Rainey SM, Williams CB, Hall AJ. 2011. Impacts of nonpoint-source pollution on the structure of diatom assemblages, whole-stream oxygen metabolism, and growth of *Selenastrum capricornutum* in the Red River Watershed of North-Central Tennessee. Castanea. 76:279–292.

Lebkuecher JG, Tuttle EN, Johnson JL,Willis NKS. 2015. Use of algae to assess the trophic state of a stream in middle Tennessee. J Freshw Ecol. 30:346–379.

Leland HV, Porter SV. 2000. Distribution of benthic algae in the upper Illinois River basin in relation to geology and land use. Freshw Biol. 44:279–301.

Mackie GL. 2013. Applied aquatic ecosystem concepts. Dubuque (IA): Kendall Hunt.

Moss B, Jeppesen E, Sondergaard M, Lauridsen TL, Liu ZW. 2013. Nitrogen, macrophytes, shallow lakes and nutrient limitation: resolution of a current controversy? Hydrobiologia. 710:3–21.

Munn MD, Black RW, Gruber SG. 2002. Response of benthic algae to environmental gradients in an agriculturally dominated landscape. J N Benthol Soc. 21:221–237.

NAWQA. 2005. Relationships of soft-bodied algae to water-quality and habitat characteristics in U.S. rivers: analysis of the National Water-Quality Assessment (NAWQA) Program data set. Report No. 05-08. Benjamin Franklin Parkway (PA): The Academy of natural Science. Patrick Center for Environmental Research, Phycology Section; p. 19103—1195.

Passy SI, Larson CA. 2011. Succession in stream biofilms is an environmentally driven gradient of stress tolerance. Microb Ecol. 62:414–424.

Patrick R, Reimer CW. 1966. The diatoms of the United States. Volume 1. Monographs Acad Nat Sci Philadelphia. 13:1–688.

Patrick R, Reimer CW. 1975. The diatoms of the United States. Volume 2. Monographs Acad Nat Sci Philadelphia. 13:1–213.

Ponader KC, Potapova MG. 2007. Diatoms from the genus *Achnanthidium* in flowing waters of the Appalachian Mountains (North America): ecology, distribution and taxonomic status. Limnologica. 37:227–241.

Porter SD. 2008. Algal attributes: an autecological classification of algal taxa collected by the national water-quality assessment program. US Geological Survey Data Series 329. Available from: http://pubs.usgs.gov/ds/ds329/

Prescott GW. 1982. Algae of the western great lakes area. Koenigstein: Otto Koeltz Science Publishers.

Rimet F. 2012. Recent views on river pollution and diatoms. Hydrobiologia. 683:1–24.

Rott E, Schneider SC. 2014. A comparison of ecological optima of soft-bodied benthic algae in Norwegian and Austrian rivers and consequences for river monitoring in Europe. Sci Total Environ. 475:180–186.

Schaumburg J, Schranz C, Foerster J, Gutowski A, Hofmann G, Meilinger P, Schneider S, Schmedtje U. 2004. Ecological classification of macrophytes and phtobenthos for rivers in Germany according to Water Framework Directive. Limnologica. 34:283–301.

Schneider S, Lindstrøm EA. 2011. The periphyton index of trophic status PIT: a new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers. Hydrobiologia. 665:143–155.

Schoen S. 1988. Cell counting. In: Lobban CS, Chapman DJ, Kremer BP, editors. Experimental phycology. a laboratory manual. Cambridge (MA): Cambridge University Press; p. 16–22.

Shannon CE, Weaver W. 1949. The mathematical theory of communication. Urbana (IL): University of Illinois Press.

Smucker NJ, Vis ML. 2009. Use of diatoms to assess agricultural and coal mining impacts on streams and a multiassembage case study. J N Am Benthol Soc. 28:659–675.

Stancheva R, Fetcher AE, Sheath RG. 2012. A novel quantification method for stream- inhabiting, non-diatom benthic algae, and its application in bioassessment. Hydrobiologia. 684:225–239.

Stancheva R, Sheath RG. 2016. Benthic soft-bodied algae as bioindicators of stream water quality. Knowl Manag Aquat Ecosyst. 414:1–16.

TDEC. 2009. Habitat quality of least-impacted streams in Tennessee. Nashville (TN): Tennessee Department of Environment and Conservation, Division of Water Pollution Control. Available from: http://www.tennessee.gov/assets/entities/environment/attachments/Habitat_Guidelines.pdf

TDEC. 2016. The status of water quality in Tennessee. Nashville (TN): Tennessee Department of Environment and Conservation, Division of Water Pollution Control. Available from: http://tn.gov/environment/article/wr-wq-water-quality-reports-publications

USEPA. 2016. Tennessee impaired waters. Washington (DC): Environmental Protection Agency. Available from: https://iaspub.epa.gov/tmdl_waters10/attains_impaired_waters.control?p_state=TN

USGS. 2016. Interior plateau. Reston (VA): United States Geological Survey. Available from: https://landcovertrends.usgs.gov/east/eco71Report.html

Whitford LA, Schumacher GJ. 1984. A manual of fresh-water algae. Raleigh (NC): Sparks Press.

Whitton BA. 2012. Ecology of Cyanobacteria II. Their diversity in space and time. New York (NY): Springer.

Whitton BA. 2013. Use of benthic algae and bryophytes for monitoring rivers. J Ecol Environ. 36:95–100.

Woelkerling WJ, Kowal RR, Gough SB. 1976. Sedgwick-rafter cell counts: a procedural analysis. Hydrobiologia. 48:95–107.

Zar JH. 2007. Biostatistical analysis. 5th ed. Englewood Cliffs (NJ): Printice Hall.

Appendices

Appendix 1. Streams sampled, year streams sampled, and locations of stream sites sampled

Stream name	Year sampled	Watershed	Location of sampling site
Suggs	2015	Stones River	10 km W of Nashville, TN. 100 m upstream of Hwy 171 bridge. 36° 08′ N, 86° 31′ W.
Trace	2015	Kentucky Lake	Waverly, TN. 300 m upstream of bridge on E. Main St. 36° 05′ N, 87° 48', W.
Flynn	2015	Cordell Hull	10 km N of Baxter, TN. Flynn Creek Rd across from Flatt Cemetery. 36° 18' W, 85° 41' N.
Hurricane	2015	Lower Duck	5 km S of McEwen, TN. 50 m downstream of bridge at intersection of Hurricane Creek Rd and Little Hurricane Creek Rd. 36° 03′ N, 87° 36′ W.
Jones	2016	Harpeth River	4 km NE of Dickson, TN. 50 m upstream of bridge on Jones Creek Rd. 36° 06′ N, 87° 19′ W.
McAdoo	2016	Lake Barkley	10 km SW of Clarksville, TN. 20 m downstream of bridge on Gholson Rd. 36° 28′ N, 87 ° 17′ W.
Marrow Bone	2016	Cheatham Lake	4 km E of Ashland City, TN. 0.2 km N on Marrow Bone Rd from the junction of Marrow Bone Rd and Little Marrow Bone Rd. 36° 14' N, 87° 0.05' W.
Will Hall	2016	Harpeth River	4.5 km E of Dickson, TN. 50 m upstream of Four Mile Campground off Jackson Hill Rd in Montgomery Bell State Park. 36° 06′ N, 87° 18′ W.

Appendix 2. Morphological characteristics (mean ± SE) of stream sites sampled in 2015 and 2016

Characteristic	Suggs 2015	Trace 2015	Flynn 2015	Hurricane 2015	Jones 2016	McAdoo 2016	M. Bone 2016	Will Hall 2016
Discharge (m^{3}·s^{-1})	0.25 ± 0.01	0.34 ± 0.13	0.99 ± 0.06	0.46 ± 0.04	0.50 ± 0.00	0.34 ± 0.03	0.13 ± 0.00	0.26 ± 0.03
Width (m)	16.5 ± 1.5	8.8 ± 0.3	9.3 ± 0.3	6.6 ± 0.6	8.8 ± 1.2	17.0 ± 0.5	13.9 ± 0.4	5.9 ± 0.3
Depth (m)	0.10 ± 0.01	0.13 ± 0.03	0.2 ± 0.03	0.20 ± 0.01	0.37 ± 0.11	0.14 ± 0.04	0.07 ± 0.00	0.27 ± 0.06
Velocity (m·s^{-1})	0.17 ± 0.01	0.33 ± 0.13	0.60 ± 0.04	0.39 ± 0.03	0.17 ± 0.00	0.20 ± 0.00	0.15 ± 0.00	0.18 ± 0.02
Benthic substrate <64 mm (%)	10 ± 2	60 ± 7	14 ± 2	35 ± 17	6 ± 5	4 ± 1	20 ± 7	0 ± 0
Estimated canopy angle (degrees)	120	40	10	60	40	40	60	0

Appendix 3. Percent composition of diatom taxa listed in alphabetical order at stream sites sampled May 2015, August 2015, May 2016, and August 2016

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Achnanthes exigua var. *constricta* Boyer										0.5						
Achnanthes pinnata Hust	1.5				0.9		0.4			0.5						0.8
Achnanthidium deflexa Reimer					0.5		0.7				0.4					
Achnanthidium eutrophilum Lange-Bert.										0.5				0.4		
Achnanthidium gracillimum Lange-Bert.							0.4							1.6		
Achnanthidium latecephalum Kobayasi									0.5				5.6	2.8	12.2	0.8
Achnanthidium minutissimum (Kütz.) Czarn.	7.0	8.7	1.0	15.3	3.2	3.2	2.6	11.5	4.7	1.0	20.4	2.9	6.4	15.2	20.4	18.6
Achnanthidium rivulare Potapova and Ponander	14.9	15.0	66.3	20.5	46.8	23.9	72.9	35.8	5.7	2.4	7.0	10.0	69.9	4.0	10.9	13.6
Achnanthidium sp.	0.5	1.9			0.5				1.4	0.5				1.2		0.4
Amphipleura pellucida Kütz.																0.8
Amphora minutissima W. Sm.						6.9						1.7		0.4		1.2
Amphora montana Krasske												0.8	0.4	0.4		
Amphora perpusilla Grun.	1.0				0.9	1.2	0.4	1.8	5.2	4.3	5.7	7.1		0.8	0.5	3.3
Amphora sp.															0.5	
Amphora veneta Kütz.						0.4				0.5		1.2			0.5	
Bacillaria paradoxa Gmelin										4.8				0.4		7.0
Cocconeis pediculus Ehrenb.	13.9	1.9	1.0	0.5	0.5		0.7		4.3						0.5	
Cocconeis placentula Ehrenb.	10.5	5.3	1.5	11.6		6.1	4.1	13.2	1.9	1.4	3.9		0.8	4.4	1.4	7.4
Cocconeis placentula var. *euglypta* Ehrenb.	4.6		1.5	4.2		1.2	1.5	4.0		2.9	0.4		0.4	2.4	0.5	2.1
Cocconeis placentula var. *lineata* Ehrenb.	2.6			0.5		1.2	0.4	3.1	0.5	3.4				3.6	0.9	2.5
Craticula halophila (Grun.) G.D. Mann		0.5		0.5	0.5				0.5	0.5	1.7				0.9	
Cyclotella meneghiniana Kütz.		1.9	0.5	0.5							0.4			0.4	0.5	0.8
Cyclotella pseudostelligera Kütz.																0.4
Cyclotella stelligera Kütz.															3.6	
Cymbella affinis Kütz	4.6	6.3	3.0	17.7	0.5	6.1	1.9	7.5	1.9	4.3	8.3		9.4	13.2	12.4	2.5
Cymbella sp.												0.4				
Cymbella tumida (Bréb.) Van Heurck							0.4			0.5		0.4			1.4	
Diatoma vulgaris Bory	7.8				10.6	0.4	2.6		0.5		1.7				0.9	
Encyonema appalachianum Potapova	7.8	2.9		16.3		0.4	0.7	7.1		5.3	1.3		2.6	16.4	7.7	
Encyonema minutum (Hilse) Mann							0.4		0.4			2.2				0.9
Encyonema prostra-tum (Berk.) Kütz.										0.5						
Encyonema silesiacum (Bleisch) Mann					0.5		0.4	0.4					0.4			0.8
Epithemia adnate (Kütz.) Bréb.																0.4
Epithemia sp.														0.4		
Eunotia lunaris Grun.							0.4									

(continued)

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Fragilaria vaucheriae (Kütz.) Peters													0.4			
Frustulia vulgaris (Thwaites) De Toni											0.4					
Gomphoneis olivacea (Horn.) Daws.	2.6	0.5		0.5	4.6	1.6		0.4	0.5		0.4				0.9	
Gomphonema angustatum (Kütz.) Rabenh.					0.5							0.4				
Gomphonema brasiliense Grun.	2.6			2.8		3.2	0.4	0.9		0.5	1.3			8.0	0.5	
Gomphonema gracile Ehrenb.	4.6					1.6				0.5		0.4				
Gomphonema minutum Ag.	9.0	1.9			1.4	1.6			0.9		2.2					
Gomphonema parvulum (Kütz.) Kütz.		3.4				0.8						2.1		0.4		0.8
Gomphonema pseudoaugur Lange-Bert.						1.2					0.9	0.4	0.4			
Gomphonema pumilum (Grun.) Reich. and Lange-Bert		0.9	0.5			1.6						0.8				
Gomphonema sp.	0.5					0.8					0.4				0.5	
Gomphonema tergestinum Frickle										0.5						
Gomphonema truncatum Ehrenb.										0.5		0.8	0.4			
Gyrosigma scalproides (Rabenh.) Cleve												3.3				
Hippodonta capitata (Ehrenb.) Lange-Bert.		0.5														
Karayeva clevei var. *rostrata* Hust.						0.4										
Melosira varians Ag.	0.5		1.0		6.0		0.7				2.6					0.8
Navicula accomida (Hust.) D.G. Mann		0.5						0.4				0.4		0.4		
Navicula capitatoradiata Germ.		0.5	1.0	0.5			0.4	0.4	2.4	2.9	4.8	1.2	0.4	0.8	3.2	1.2
Navicula cryptotenella Lange-Bert.	6.5	0.5			0.5		0.7	0.4	1.5	0.5	3.5			0.8	0.5	0.4
Navicula cryptocephala Kütz.		1.9					0.4				0.4	2.5			0.5	
Navicula elginensis Greg.												0.4				
Navicula gregaria Donk.												0.4		0.4		
Navicula lanceolata (Ag.) Ehrenb.	0.5	0.9					0.4			0.5	0.4	0.4				
Navicula menisculus Schum.		0.5							0.5			1.2		0.8		0.4
Navicula menisculus var. *upsaliensis* (Grun.) Grun.												0.8				0.4
Navicula minima Grun	1.5	4.4		1.9	1.8	2.8			2.4	18.4	6.1	7.5		7.6	0.5	2.9
Navicula radiosa var. *tenella* (Breb.) Grun.						0.4			0.5						0.5	
Navicula reichardtiana Lange-Bert.	7.0	0.9	2.5		0.5	0.8	0.4		14.7	0.5	1.3	2.9		0.4	1.4	
Navicuala reinhardii Grun.											0.4					
Navicula rhynchocephala Kütz.	0.5			0.5											0.5	0.8
Navicula sp. (<12 μm length)									0.5	0.5	0.4					
Navicula sp. (>12 μm length)	0.5	2.9			0.5		0.4			1.0		1.2		1.2	1.8	0.4
Navicula subminuscula Mang.		1.4				1.2	0.7			2.9	2.2	1.2				0.4
Navicula subrotundata Hust.										1.9	1.3	4.5				3.3
Navicula tripunctata (O.F. Müll.) Bory	1.5		0.5	0.5	0.5	0.8	1.1	0.4	5.2	0.5	3.0	0.8			0.9	
Navicula trivialis Lange-Bert.		1.9										0.4			0.5	
Navicula viridula (Kütz.) Ehrenb.		9.2		0.5	0.5					0.5	0.4	5.4		0.4		
Nitzschia amphibia Grun.		1.9		0.5	0.5	2.0	0.4		3.8	11.1	0.4			1.2		0.8
Nitzschia capitellata Hust.	3.5	7.7	0.5			0.8		1.3	4.3	2.4	0.4	3.7	0.4	1.6	0.5	0.8
Nitzschia disputata (Kütz.)	1.5								0.5							

(*continued*)

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Nitzschia dissipata (Kütz.) Grun.					3.2					0.5	1.7	3.7			0.9	1.2
Nitzschia dissipata var. *media* (Hantz.) Grun.	0.5											1.2				
Nitzschia fonticola Grun.		0.5			0.5					0.5						
Nitzschia frustulum (Kütz.) Grun.				0.5			0.7			1.0	0.9	0.4				
NItzschia gracilis Hantz.	0.5											0.8				0.4
Nitzschia inconspicua Grun.	1.5	0.5		0.5		1.2	1.1	0.4	10.4	6.8	0.4	1.2		0.4		
Nitzschia linearis (Ag.) W. Sm.		0.5								0.5						
Nitzschia microcephala Grun.											0.4					
Nitzschia minuta Bleisch					0.9		0.4		0.5	0.5		0.4				0.4
Nitzschia palea (Kütz.) W. Sm.	2.5	1.4	16.1	0.5	2.8		0.4	3.1	3.8	2.9	0.9	0.4	0.4	0.4	0.5	0.4
Nitzschia perminuta (Grun.) M. Perag	1.0															
Nitzschia sinuata var. *tabellaria* Grun.												0.4			0.5	1.2
Nitzschia recta Hantz.		0.9														
Nitzschia sociabilis Hust.	1.0	0.5						0.4	0.5			2.5				
Nitzschia sp.		0.9	1.0		0.5	0.8		1.8	3.8	1.4	1.3	0.4			0.9	0.8
Nitzschia tubicola Grun.	1.0	0.5			0.5			1.8	4.7	1.0	0.9		0.8			
Pinnularia borealis var. *borealis* Ehrenb.				0.5	0.5											
Planothidium lanceolatum Bréb.	1.0	1.4		0.5	0.9	2.8	0.4					0.8				0.8
Planothidium lanceolatum var. *dubia* Grun.	0.5				3.2	2.8						1.2				0.4
Psammothidium curtissimum (Carter) Aboal			0.5	0.5		8.9	0.4	0.9	0.9	1.4	0.4	5.0			0.5	3.3
Psammothidium levanderi Hust. L. Bukht. and Round					0.5											
Psammothidium subatomoides Hust.					0.9											1.2
Psammothidium sp.			0.5		0.9				1.4							
Reimeria sinuata (Greg.) Kociolek & Stoermer		2.4	0.5	0.5		6.1	0.4	1.3	1.4	1.0	2.6		1.1	4.0	1.8	0.8
Rhoicosphenia curvata (Kütz.) Grun.	1.5	3.4		0.5		2.4		0.4	0.9	1.4						
Rhopalodia gibba (Ehrenb.) O. Müll.												1.7				
Sellaphora pupula Kütz.															0.5	
Sellaphora seminulum (Grun.) D. G. Mann.						0.8			6.6	1.4	2.2	2.1		2.8		2.5
Staurosirella leptostauron (Ehrenb.) D.M. Williams																0.8
Stephanodiscus parvus Stoermer & Hankansson										0.5						
Stephanodiscus sp.					0.9					0.5					2.7	
Surirella brebissonii Lange-Bert. and Krammer	1.5									0.5	0.4					
Surirella linearis W. Sm.																0.4
Surirella ovalis Breb											0.4					
Surirella ovata var. *pinnata* (W. Sm.) Brun.	0.5											0.8				
Synedra delicatissima W. Sm.				0.5												
Synedra rumpens Kütz.								0.4								0.4
Synedra ulna (Nitz.) Ehrenb.	3.0		0.5		2.3	0.8			0.5	0.5	0.4			0.4	0.5	
Thalassiosira weissflogii (Grun.) G. Fryxell and Hasle				0.5												

Appendix 4. Percent composition of soft-algae taxa listed in alphabetical order by phylum at sites sampled in May 2015, August 2015, May 2016, and August 2016

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Chlorophyta																
Carteria globulosa Pascher												0.2				
Chaetopeltis orbicularis Berthold						0.6		0.2								
Characium ambiguum H. Jaeger		0.1														
Chlamydomonas angulosa Dill												0.2				
Chlamydomonas cienkowskii Schmidle		0.1														
Chlamydomonas globosa Snow.		0.1		0.1								0.2				
Chlamydomonas gloeogama Korschikov					0.1							0.2				
Chlamydomonas patellaria Whitford		0.1		0.1							0.1	0.4	0.1			
Chlamydomonas sp.	0.2	0.1							0.2		0.1		0.1			
Cladophora glomerata (L.) Kütz.	60.9	27.4	22.7	0.6	49.3		0.8	3.4	58.1	17.5	42.3					
Closterium acerosum (Schrank) Ehrenb.										0.3	0.1	0.2				
Closterium leibleinni Kütz.													0.1			
Closterium moniliferum (Bory) Ehrenb.		0.1							0.1	0.3					0.7	0.5
Closterium setaceum Ehrenb.											0.1					
Closterium sp.	0.1											0.2				
Cosmarium sp.									0.2	0.3						
Coelastrum microporum Nägeli						0.1										
Cosmarium galeritium Nordst.		0.1														
Desmidium baileyi (Ralfs) Nordst.					0.8											
Entocladia polymorpha (G.S. West) G.M. Sm.									0.1							
Eudorina elegans Ehrenb.																0.5
Geminella ellipsoidea (Prescott) Smith													2.0			
Gloeocystis gigas (Kütz.) Langerh.						0.1					0.1				1.3	
Gloeocystis sp.		0.1														
Gloeocystis vesiculosa Nägeli				0.3	0.1	2.8			1.3	8.2	1.6	2.6	0.4	1.5	2.0	5.4
Hydrodictyon reticulatum (L.) Lagerh.		7.2														
Mougeotia sp.			1.1													
Oedogonium sp.		21.3			0.2					10.0	13.0		2.1	31.0		6.8
Oocystis lacustris Chodat.			0.1													
Pandorina morum (Müller) Bory		0.1														
Protoderma viride Kütz.							0.2									
Rhizoclonium hieroglyphicum (C. Agardh) Kütz.												4.6				
Scenedesmus dimorphus (Turp.) Kütz.			0.1							0.3	0.1					
Scenedesmus sp.										0.3					0.7	

(*continued*)

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Selenastrum capricornutum Printz		0.1					0.1		0.1							
Spirogyra sp.		28.1			0.2								19.8			
Stigeoclonium tenue (C.A. Ag.) Kütz.			2.3									4.6	19.8			
Stigeoclonium sp.		0.1														
Tetraselmis cordiformis (Carter) Stein												0.2				
Ulothrix cylindricum Prescott										5.0						
Ulothrix sp.							0.8									
Ulothrix subtilissima Rabenh.													1.5			
Ulothrix tenerrima Kütz.			1.1								3.1					
Ulothrix variabilis Kütz.		0.1														
Cyanobacteria																
Aphanocapsa elachista West and West				0.1		0.4										
Aphanocapsa pulchra (Kütz.) Rabenhorst		0.1														
Aphanothece castagnei (de Breb.) Rabenh.				0.1		0.4			0.2							
Aphanothece nidulans Richter									0.8	0.3	0.7			0.1	1.3	0.5
Borzia periklei Anag.										0.3						0.5
Borzia sp.										0.3						
Borzia trilocularis Cohn.	0.1	0.1		0.2	0.1	1.0	0.2				0.1	0.2			0.7	
Calothrix sp.				0.6												
Calothrix stellaris Bornet and Flahault						0.1										
Chamaesiphon incrustans Grun.	1.8															
Chroococcus minimus (Keissler) Lemmerman						0.1										
Chroococcus minor (Kütz.) Nägeli	3.0	0.1	0.6	0.1		1.4	0.6		0.2	2.5	0.1		0.5	0.2		1.8
Chroococcus minutus Kütz.						0.5				0.3			0.1			
Chroococcus sp.		0.1				0.6	0.1	0.1								
Dactylococcopsis acicularis Lemmerman											0.2					
Dactylococcopsis raphidioides Hansg.		0.1	0.1							0.3						
Dactylococcopsis smithii Chodat and Chodat											0.1					
Entophysalis rivularis Kütz.		0.1		0.2	0.1	2.4	0.5	0.1	1.3	10.2	2.6	2.0	0.5	0.6	0.7	5.0
Gloeocapsa aeruginosa (Carm.) Kütz.									0.7						0.7	0.5
Gloeocapsa sp.											0.1				0.7	
Gloeocapsopsis cyanea (Krieg) Komárek and Anagn.						0.2			0.1	4.0	0.4				0.7	
Gloeocapsopsis pleuroccapsoides (Novacek) Komárek and Anagn.		0.1		0.1		17.2			0.7	2.2	0.6		0.2		6.0	0.5
Gloeocapsopsis sp.							0.1				0.1					
Gloeothece sp.		0.1														
Heteroleibleinia kossinskajae (Elenkin) Anagn. and Komárek	6.7			0.6	24.0			2.8	1.1			9.1	3.0	3.6		0.5
Homeothrix crustaceae Woron.						1.2										
Homeothrix juliana (Bornet and Flahault) Kirchner				34.1		3.1							2.0	7.0		28.5
Jaaginema pseudogeminatum (G. Schmid) Anagn. and Komárek								0.1								

(*continued*)

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Komvophoron constrictum (Szafer) Anagn. and Komárek	0.1	0.1								5.5		9.1				
Komvophoron munitum (Skuja) Anagn. and Komarek		0.1		0.1	0.3	0.4					1.7	3.3	0.9	0.1	1.3	
Komvophoron schmidlei (Jaag.) Anagn. and Komárek											0.6	6.4		2.3		
Leibleinia nordgaardii (Wille) Anagn. and Komárek	1.2				0.8											
Leptolyngbya angustissimum (West and West) Anagn. and Komárek	0.5		14.5		2.4	5.9	1.4		0.5		4.2	5.8	6.0	1.2	19.5	9.1
Leptolyngbya foveolarum (Mont.) Anagn. and Komárek	10.0			2.1	5.8	8.5	10.0	2.9	13.6	4.0	2.2	5.5	16.2	11.5	40.9	1.8
Leptolyngbya nostocrum (Bomont.) Anagn. and Komárek											0.9	11.5	1.0			
Lyngbya major Menegh.												3.7		5.4		6.8
Lyngbya martensiana Menegh.			0.8	5.5										0.8		9.1
Lyngbya nana Tilden	0.6		2.8													
Leptolyngbya ochracea (Thur. and Gomont) Anagn. and Komárek	0.2															
Merismopedia tenuissima Lemmerman		0.1														
Microcystis incerta Lemmerman										0.3	0.2					
Nostoc paludosum Kütz.						0.1										
Oscillatoria agardhii Gomont			0.3					4.8		1.8						
Oscillatoria limosa (Dylwin) C. Agardh							1.1							0.4	10.1	
Oscillatoria princeps Vaucher		3.6														
Oscillatoria sp.	0.2					1.5	0.1			4.2	0.2		1.0			
Oscillatoria subbrevis Schmidle					0.3	0.9		5.3	1.0	2.5	0.6			0.3	3.4	
Oscillatoria subtilissima Kütz. and De Toni	3.0			0.8		4.0	1.4								1.3	
Phormidium articulatum Gardner Anagn. and Komárek			1.1					0.2	0.7	2.2	0.4	1.5	3.4	0.4		1.4
Phormidium autumnale Gomont	3.0		16.9	0.8		0.4	0.8		3.8		0.5			2.3		
Phormidium diguetii (Gomont) Anagn. and Komárek		0.4	18.0	46.0	0.4	31.8	3.4	63.4	3.8	1.3	4.2	19.2	13.6	22.5		
Phormidium favosum Bory					0.6		0.2	0.1	0.4							
Phormidium formosum (Bory) Anagn. and Komárek			8.7						1.6				1.5			
Phormidium fragile Gomont			3.0	0.3			1.1	6.9				0.6	1.6			
Phormidium indunatum Kütz.				1.9												
Phormidium retzii (C. Agardh) Gomont	0.6		5.0		13.5	3.1	75.7	9.6		0.5	2.2					
Phormidium sp.		0.1		2.2	0.1	5.8	0.1		3.5			1.8	1.0	1.2		13.6
Phormidium tenue (C. Agardh and Gomont) Anagn. & Komárek			0.3	1.3	1.3	2.4	0.4		4.2		5.4	4.6	1.1	2.3		2.3
Plectonema gracillimum (Zopf) Hansg.						1.2										
Schizothrix lardacea (Ces.) Gomont				1.9												
Spirulina major Kütz.															8.1	
Spirulina temerrima Kütz.									2.2		3.7					
Synechococcus aeruginosus Nägeli						0.2		0.1				1.1	0.5			2.7
Synechococcus sp.				0.1			0.2						0.1			
Synechocystis sp.			0.1			0.1	0.8			0.8	0.4	0.2		0.1		
Xenococcus gracilus Lemmerman		0.1											0.1			
Xenococcus minimus Geitler		0.1														

(continued)

	Suggs 2015		Trace 2015		Flynn 2015		Hurricane 2015		Jones 2016		McAdoo 2016		M. Bone 2016		Will Hall 2016	
	May	August	May	August	May	August	May	August	May	August	May	August	May	August	May	August
Cryptophyta																
Cryptomonas erosa Ehrenb.				0.1							0.1					
Cryptomonas anomala F.E. Fritish					0.1								0.2			
Euglenophyta																
Euglena sp.		0.1														
Euglena tripteris (Duj.) Klebs		0.1														
Trachelomonas intermedia Dangeard		0.1														
Trachelomonas pulcherrima var. minor Playfair																0.5
Trachelomonas robusta Swirenko		0.1														
Trachelomonas sp.		0.1														
Ochrophyta																
Botrydiopsis sp.	0.1															
Ophiocytium desertum Printz													0.1			
Vaucheria sp.	7.3	10.8	0.3			0.6										
Rhodophyta																
Audouinella hermannii (Roth) Duby	0.2									15.0	6.7		1.0	5.4		2.3

Appendix 5. Abundance-weighted average (A-WA) of the concentration of benthic chlorophyll *a* for soft-algae taxa sampled August 2015 and August 2016. The standard deviation (SD) of the abundance-weighted average and ratio of SD to abundance-weighted average (SD/A-WA) are given for taxa in which more than one algal unit was recorded

	A-WA	SD	SD/A-WA
Chlorophyta			
Carteria globulosa Pascher	52.40		
Chaetopeltis orbicularis Berthold	23.04	7.40	0.32
Characium ambiguum H. Jaeger	136.10		
Chlamydomonas angulosa Dill	52.40		
Chlamydomonas cienkowskii Schmidle	136.10	0.00	0.00
Chlamydomonas globosa Snow.	95.38	47.06	0.49
Chlamydomonas gloeogama Korschikov	52.40		
Chlamydomonas patellaria Whitford	74.45	41.15	0.55
Cladophora glomerata (L.) Kütz.	135.50	24.99	0.18
Closterium acerosum (Schrank) Ehrenb.	134.80	116.53	0.86
Closterium moniliferum (Bory) Ehrenb.	133.70	84.73	0.63
Coelastrum microporum Nägeli	28.40		
Cosmarium galeritium Nordst.	136.10		
Eudorina elegans Ehrenb.	47.80		
Gloeocystis gigas (Kütz.) Langerh.	28.40		
Gloeocystis vesiculosa Nägeli	101.51	79.33	0.78
Hydrodictyon reticulatum (L.) Lagerh.	136.10	0.00	0.00
Oedogonium sp.	123.58	30.36	0.25
Pandorina morum (Müller) Bory	136.10		
Rhizoclonium hieroglyphicum (C. Agardh) Kütz.	52.40	0.00	0.00
Scenedesmus dimorphus (Turp.) Kütz.	217.20		
Scenedesmus sp.	217.20		
Selenastrum capricornutum Printz	136.10		
Spirogyra sp.	136.10	0.00	0.00
Stigeoclonium tenue (C. A. Ag.) Kütz.	52.40	0.00	0.00
Tetraselmis cordiformis (Carter) Stein	52.40		
Ulothrix cylindricum Prescott	217.20	0.00	0.00
Ulothrix variabilis Kütz.	136.10	0.00	0.00
Cyanobacteria			
Aphanocapsa elachista West and West	35.53	14.25	0.40
Aphanocapsa pulchra (Kütz.) Rabenhorst	136.10	0.00	0.00
Aphanothece castagnei (de Breb.) Rabenh.	39.80	15.61	0.39
Aphanothece nidulans Richter	111.97	91.86	0.82
Borzia periklei Anag.	132.50	119.78	0.90
Borzia trilocularis Cohn.	79.77	50.98	0.64
Calothrix stellaris Bornet and Flahault	28.40		
Chroococcus minimus (Keissler) Lemmerman	28.40		
Chroococcus minor (Kütz.) Nägeli	106.23	80.76	0.76
Chroococcus minutus Kütz.	66.16	84.43	1.28
Dactylococcopsis raphidioides Hansg.	176.65	57.35	0.32
Entophysalis rivularis	120.37	86.11	0.72
Gloeocapsa aeruginosa (Carm.) Kütz.	47.80		
Gloeocapsopsis cyanea (Krieg) Komárek and Anagn.	196.22	61.05	0.31
Gloeocapsopsis pleuroccapsoides (Novacek) Komárek and Anagn.	40.68	45.52	1.12
Heteroleibleinia kossinskajae (Elenkin) Anagn. and Komárek	48.04	22.18	0.46
Homeothrix crustaceae Woron.	28.40	0.00	0.00
Homeothrix juliana (Bornet and Flahault) Kirchner	56.86	7.73	0.14
Jaaginema pseudogeminatum	14.10	0.00	0.00
Komvophoron constrictum (Szafer) Anagn. and Komárek	106.82	72.41	0.68
Komvophoron munitum (Skuja) Anagn. and Komárek	57.14	25.45	0.45
Komvophoron schmidlei (Jaag) Anagn. and Komárek	60.94	9.29	0.15
Leptolyngbya angustissimum (West and West) Anagn. and Komárek	44.00	14.83	0.34
Leptolyngbya foveolarum (Mont.) Anagn. and Komárek	58.95	40.98	0.70
Leptolyngbya nostocrum (Bomont) Anagn. and Komárek	50.97	5.73	0.11
Lyngbya major Menegh.	64.08	9.79	0.15

(*continued*)

	A-WA	SD	SD/A-WA
Lyngbya martensiana Menegh.	56.54	5.55	0.10
Merismopedia tenuissima Lemmerman	136.10		
Microcystis incerta Lemmerman	217.20		
Nostoc paludosum Kütz.	28.40		
Oscillatoria agardhii Gomont	32.56	58.77	1.80
Oscillatoria limosa (Dylwin) C. Agardh	70.90	0.00	0.00
Oscillatoria princeps Vaucher	136.10	0.00	0.00
Oscillatoria subbrevis Schmidle	38.16	61.77	1.62
Oscillatoria subtilissima Kütz. and De Toni	36.17	12.84	0.35
Phormidium articulatum Gardner Anagn. and Komárek	104.08	80.70	0.78
Phormidium autumnale Gomont	64.33	11.51	0.18
Phormidium diguetii (Gomont) Anagn. and Komárek	39.64	25.69	0.65
Phormidium favosum Bory	14.10	0.00	0.00
Phormidium fragile Gomont	17.15	10.83	0.63
Phormidium indunatum Kütz.	56.61	1.63	0.03
Phormidium retzii (C. Agardh) Gomont	18.67	22.51	1.21
Phormidium tenue (C. Agardh & Gomont) Anagn. and Komárek	54.08	14.79	0.27
Plectonema gracillimum (Zopf) Hansgir	28.40	0.00	0.00
Schizothrix lardacea (Ces.) Gomont	56.90	0.00	0.00
Synechococcus aeruginosus Nägeli	50.51	25.42	0.50
Synechococcus sp.	56.90	0.00	0.00
Synechocystis sp.	133.88	92.26	0.69
Xenococcus gracilus Lemmerman	136.10		
Xenococcus minimus Geitler	136.10		
Cryptophyta			
Cryptomonas erosa Ehrenb.	56.90		
Cryptomonas anomala F.E. Fritish	49.40	8.49	0.17
Euglenophyta			
Euglena tripteris (Duj.) Klebs	136.10		
Trachelomonas intermedia Dangeard	136.10		
Trachelomonas pulcherrima var minor Playfair	47.80		
Trachelomonas robusta Swirenko	136.10		
Ochrophyta			
Vaucheria sp.	135.39	8.74	0.06
Rhodophyta			
Audouinella hermannii (Roth) Duby	135.07	73.86	0.55