Correlations of the Composition of Algae Assemblages to the Trophic State of Middle Tennessee Streams

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Molly Grimmett 05/05/2017

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Abstract

MOLLY GRIMMETT. Correlations of the Composition of Algae Assemblages to the Trophic State of Middle Tennessee Streams (Under the direction of DR JEFFERSON LEBKUECHER.)

The use of soft algae for bioassessment is less common relative to fish and macroinvertebrates because the response of soft-algae assemblages to changes in water quality is poorly understood. Algae assemblages at eight stream sites in seven watersheds in Middle Tennessee were studied to: 1) document the composition of algae assemblages, 2) assess the trophic state of the stream sites, 3) correlate the composition of soft-algae assemblages to trophic state, and 4) construct biotic indices using soft-algae taxa to help monitor trophic state. Two-hundred thirty-two soft-algae and diatom taxa were identified. The change of soft-algae composition between May and August was two-fold greater relative to the change of diatom composition. The trophic state of the sites was assessed by evaluating the nutrient concentration of water and benthic characteristics which included total phosphorus concentration of periphyton, chlorophyll-*a* concentration (mg/m^2 stream bottom), and ash-free dry mass of periphyton. Concentrations of total phosphorus of periphyton was a more accurate of an indicator of trophic state relative to nutrient concentrations of water as indicated by Pearson's correlation coefficients to benthic characteristics used to denote trophic state. Trophic state preferences of soft-algae taxa were evaluated by calculating the abundance-weighted average of the concentrations of chlorophyll *a* ($A\text{-}WA_{chl\ a}$) and the abundance-weighted average of pollution tolerance index ($A\text{-}WA_{PTI}$) for each soft-algae taxon. Trophic state preferences for of soft-algae taxa present were used to calculate algae trophic indices. The indices significantly correlate to the trophic state of streams. The algae trophic indices are the first indices to utilize periphyton characteristics as opposed to nutrient concentration of water to assign trophic-indicator values to soft-algae taxa to assess the trophic state of streams. The indices are easy to calculate, easy to interpret, and provide an additional method to monitor trophic state.

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Chapter I

Introduction

The term photoautotrophic periphyton refers to benthic algae, both diatoms and soft algae. Photoautotrophic periphyton is the trophic base of mid-order streams, providing autochthonous materials for higher trophic organisms (DeNicola 1996). Nutrient enrichment of an aquatic system provides growth-limiting nutrients to these photoautotrophic organisms possibly resulting in excessive growth that has detrimental effects on the system. The composition of photoautotrophic periphyton provides insight into the environmental condition of a stream. Knowledge of how nutrient concentration influences the composition of photoautotrophic periphyton is essential in understanding how eutrophication effects shallow lotic systems (Dodds 2006). Quantification of the impact of eutrophication on photoautotrophic periphyton is crucial in the assessment of stream water quality and is necessary to establish better water management practices.

Biomonitoring may determine the effect of water quality on the composition and structure of species assemblages and is often effective in the detection of impairment. Biomonitoring is used worldwide and with various types of organisms, most commonly fish, macroinvertebrates and diatoms (Carlisle 2008). Justus (2010), in a comparative study, evaluated the accuracy of using fish, macroinvertebrates, and diatoms in detecting low-level nutrient enrichment. They concluded there was a significant correlation of the three biotic indices to the nutrient-enrichment gradient. The diatom indices were the most sensitive to nutrient fluctuations ($p \leq 0.05$) relative to other organism indices applied. Due to the sensitivity and continuous presence of these organisms in their respective environment, biotic indices accurately reflect fluctuations in environmental conditions (Justus 2010, Dufrene 1997).

The trophic state of a lotic system can be inferred by the biomass of algae present (TDEC 2013). Quantification of trophic state is performed with a variety of methods however, each has certain limitations (Kurle & Cardinale 2011, Whitton 2012). Nutrient enrichment is commonly quantified through chemical analysis, though this practice may be inaccurate. Fluctuations of nutrients in a lotic system due to changes in flow regime make the evaluation the trophic state of a stream using water chemistry alone potentially inaccurate. Excessive algae biomass may result in greater uptake and sequestering of nutrients from the water causing diminished levels of nutrients.

Large quantities of algae biomass, as evaluated by chlorophyll-*a* concentrations, are indicative of eutrophication (Khan and Ansari 2005). Therefore, the use chlorophyll-*a* concentrations as a measurement of algae biomass, thus trophic state, is often employed. The biomass of algae present in a system can be easily calculated by determination of the chlorophyll-*a* concentration per meter squared because 1.5% of dry algae biomass is chlorophyll *a* (Eaton 2005). Algae growth is influenced by numerous abiotic and biotic characteristics of the stream reach making measurements of chlorophyll-*a* concentrations alone possibly inaccurate indicators of trophic state (Kurle & Cardinale 2011).

Diatoms are often the focus of bioassessment studies because the autecological information is well understood relative to soft algae (Porter et al. 2008, Stancheva et al. 2012). Diatom composition is commonly used in water quality assessment in many European countries, as well as some middle-eastern countries, and is becoming more common in the United States and Canada (Porter et al. 2008, Lebkuecher 2015, Stancheva et al. 2012, Lavoie et al. 2014). Diatoms are the preferable aquatic bioindicator due to their rapid lifecycles and are more accurate in the detection of small changes in nutrients and harmful chemicals or contaminants (Jüttner et al. 2003, Justus et al. 2010, Cantonati et al. 2014). Diatoms are beneficial in the identification of heavy metal contaminants, acidification, and nutrients, such as phosphorous and nitrogen, because exposure causes a decrease in abundance of specific taxa and does not result in fatality as it does in macroinvertebrates or fish (Cantonati et al. 2014). The pollution tolerance index (PTI) is the standard index utilizing diatoms to assess trophic state in many southeastern states (KDOW 2002). Diatom composition may not accurately portray trophic state. For example, *Cocconesis placentula* is associated with oligotrophic conditions, but is often epiphytic on filamentous algae, such as *Vaucheria,* which has a eutrophic association.

The composition of soft-algae assemblages in response to nutrient concentrations remains relatively unstudied compared to diatoms (Porter 2008, Smucker and Vis 2013). Few indices using soft algae currently exist and all utilize nutrient concentration of the water, typically soluble reactive phosphorus (Schneider and Lindstrøm 2011, Porter 2008). The periphyton index of trophic state (PIT) utilizied the composition of soft algae exclusively to evaluate the effects of nutrient concentration on lotic systems in northern Europe (Schneider and Lindstrøm 2011). The primary deficency of this index is the difficulty of demonstrating a correlation between water quality and percent composition of soft-algae taxa without accounting for geographic variation, as well as variation in stream quality and taxa present outside of northern Europe (Porter 2008).

Soft-algae taxa may be more affected by abiotic and biotic changes within the system relative to diatoms. For example, intermittent changes in water velocity may have a greater impact on soft-algae assemblages due to their greater surface area relative to diatoms (Whitton 2012). The algae trophic index (ATI_r) is an index that used the Pearson's correlation coefficient of soft-algae taxa abundance to soluble reactive phosphorus of the water as trophic-state indicates for the soft taxa present (Lebkuecher 2015). The ATI_r accurately portrayed the trophic state in Sulphur Fork due to the continuous state of enrichment from a wastewater treatment facility located upstream. The foundation of the ATI_r is the correlation of the soft-algae taxa to nutrient concentrations of the water, thus addition of taxa to this index outside of this stream is implausible due to the fact that of nutrient concentrations of water are often not an accurate indicator of trophic state.

For this study, soft-algae assemblages were analyzed using the abundance-weighted average of benthic characteristics, including chlorophyll-*a* concentrations, total phosphorus of benthic organics, ash-free dry mass of benthic organics, and the PTI, to assign trophic indicator values to soft-algae taxa present. The objective of this study was (1) to document algae composition and (2) utilize soft-algae composition in the assessment of trophic state of 8 stream sites in 7 watersheds. I hypothesize that biotic index values using abundance-weighted averages of benthic characteristics for soft-algae taxa present as the trophic indicator values will be an accurate predictor of trophic state for my study sites.

Chapter II

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). 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. In 2015 four sites were sampled on May 1, 2015 or May 2, 2015 and again on August 15, 2015 or August 16, 2015. In 2016 the four stream sites were sampled on May 8, 2015 and again on August 1, 2016 or August 11, 2016. Benthic characteristics including pigment concentrations of photoautotrophic periphyton, ash-free dry mass of benthic organics, and concentrations of total phosphorus of benthic organics, were determined from samples collected August 2015 and August 2016 on the same dates samples were collected to determine algae composition.

The choice of stream sites sampled reflects the attempt to pick sites ranging from hypereutrophic to oligotrophic and were based on visual assessments and listings by United States Environmental Protection Agency of nutrient-impaired and unimpaired stream reaches (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, both located in rural watersheds less affected by anthropogenic activity relative to most watersheds in Middle Tennessee, are listed as nutrient-unimpaired reference sites (TDEC 2009, 2016). 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):

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

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 Abundance of Soft-Algae and Diatom Taxa, Pigment Concentrations of Photoautotrophic Periphyton, Ash-Free Dry Mass of Benthic Organics, and Total Phosphorous Concentration of Periphyton

Cobble sampling occurred in the established five-meter reaches at depths between 0.07 m and 0.37 m and stream velocities between 0.15 $m \cdot s^{-1}$ 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-cm^2 and 18-cm^2 diameter with most of the surface area for periphyton growth parallel to flow were removed. One cobble from each plot was removed 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 plot sampled August 2015 and August 2016. One cobble was used to determine pigment concentrations of photoautotrophic periphyton and ash-free dry mass of benthic organics. These cobbles were placed in sealable plastic bags and transported to the lab on ice in darkness. One cobble was used to determine the concentration of total phosphorous of periphyton. The periphyton were removed in the field using a single-edge razor blade and scrub brush, placed on ice, and transported to the lab in darkness.

Pigment Concentrations of Photoautotrophic Periphyton and Ash-Free Dry Mass of benthic organics

The cobble collected in field 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-mL aliquots of periphyton suspended in acetone were placed in a mortar, ground with a pinch of sand and a pestle for 2 minutes, and filtered through Whatman no. 1 filter-paper circles. Optical density of the supernatant was determined at 664 nm to determine the concentration of chlorophyll *a*, then at 665 nm following acidification with 0.1 N HCl to determine the concentration of pheophytin *a*. Concentrations of chlorophyll *a* and pheophytin *a* were calculated as described by Eaton et al. (2005). Chlorophyll *a* is degraded to pheophytin *a* as plants and algae senesce, thus high concentrations of pheophytin *a* reveal poor physiological condition. Because healthy algae may have no detectable pheophytin *a* determined by optical density (OD) measurements, the chlorophyll *a* to pheophytin *a* ratio is indicated as the ratio of OD664 to OD665. OD664/OD665 values of 1.7 indicate no detectable pheophytin *a* was present. Pigment extract from algae with OD664/OD665 values near 1.7 indicate the algae were in excellent physiological condition. Extract from algae with OD664/OD665 values below 1.5 indicate the algae were in poor physiological condition (Eaton et al. 2005). Periphyton removed from cobble were dried by allowing the acetone to evaporate at 25^{o} 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 and 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 100X, 400X, and 1000X 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 1000X magnification were identified and tallied until a minimum of 200 valves from each stream site were identified, the minimum number of needed 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 (Whittaker and Fairbanks 1958).

Diatom Indices

The Pollution Tolerance Index of diatom assemblages (PTI; KDOW 2002) was calculated as:

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

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 $\leq$ 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 $\leq$ 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). In other words, the SI is the sum of *Navicula*, *Nitzschia*, *Surirella*, and the taxa formerly identified as *Navicula* and *Nitzschia* divided by the total no. of diatoms. The SI values range from 0 to 100. High SI values signify that sediments impact the structure of diatom assemblages. Given the highly erodible soils and substantial agriculture in Middle Tennessee, a SI values < 60 suggests an absence of an excessive impact of sediments on diatom assemblages (Lebkuecher et al. 2011).

Soft-Algae Assemblage Metrics and Indices

The relationship of the trophic state of the stream sites on 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 concentrations of chl *a* and the pollution tolerance index of diatoms. 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 is calculated as:

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

where: $A\text{-}WA_j$ 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 $A\text{-}WA_j$. Taxa more abundant at sites with greater values for a stream site characteristic will have a greater value for the A-WA.

Four 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:

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

where: n_j = number of taxon unit 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 four variations of the ATI differed by the stream site characteristic used to calculate the trophic-indicator values. The four trophic-indicator values utilized were abundance-weighted average (A-WA) of concentration of chlorophyll *a*, A-WA of the pollution tolerance index of diatom assemblages, A-WA of ash-free dry mass of benthic organics, and A-WA of total phosphorous concentration of benthic organics. Taxa not identified to species were excluded from index calculations.

Nutrient Concentrations of Water Samples and Benthic Organics

Concentration of nutrients in water were determined from water samples collected at midstream and 5 cm below the surface. Concentrations of total phosphorous of benthic organics were determined from samples scraped from cobble, desiccated for 24 h at 50°C, and ashed at 500° C for 2 hrs. Concentrations of soluble reactive phosphorus, NO_2 + NO_3 nitrogen, and total nitrogen of water samples and concentrations of total phosphorous of benthic organics 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-um 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 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).

Chapter III

Results

Benthic characteristics used to evaluate trophic state demonstrate that sites range from oligotrophic to eutrophic (Table 1). The chlorophyll-*a* concentrations determined from photoautotrophic periphyton at the sites sampled in August 2015 indicate oligotrophic conditions (0-60 chl-*a* $mg \cdot m^{-2}$, Biggs 2000) at the Hurricane Creek site, mesotrophic conditions (60-150 chl-*a* $mg \cdot m^{-2}$, Biggs 2000) at the Trace Creek and Flynn Creek sites, and near eutrophic conditions (>150 chl-*a* $mg \cdot m^{-2}$, Biggs 2000) at the Suggs Creek site. The concentrations of chlorophyll *a* of photoautotrophic periphyton at the Suggs Creek site were approaching nuisance levels and were significantly greater than the concentration of chlorophyll *a* relative to the other sites sampled in August 2015. The evaluation of chlorophyll-*a* concentrations, ash-free dry mass of benthic organics and total phosphorus concentration of benthic organics determined from photoautotrophic periphyton at the sites sampled in August 2016 indicate mesotrophic conditions at the McAdoo Creek, Marrow Bone Creek, and Will Hall Creek sites, and eutrophic conditions at the Jones Creek site. The concentrations of chlorophyll *a* of photoautotrophic periphyton at the Jones Creek site are above nuisance levels (>150 chl-*a* $mg \cdot m^{-2}$, Biggs 2000) and are significantly greater than the concentration of chlorophyll *a* at other sites in August 2016. The photoautotrophic periphyton were in excellent physiological condition at the sites sampled as indicated by the low concentrations of pheophytin relative to chlorophyll-*a* concentrations as indicated by high OD664 to OD665 ratios, except for the Suggs Creek site (Table 1).

Eutrophic conditions at the Suggs Creek and Jones Creek sites is supported by the high concentrations of total phosphorus of benthic organics relative to the other sites (Table 2). There is a significant correlation of total phosphorus of benthic organics to chlorophyll-*a* concentration in August 2015 and August 2016 demonstrating concentrations of total phosphorus of benthic organics is representative of trophic state (Table 3). Concentrations of soluble reactive phosphorus, $NO_2 + NO_3$, and total nitrogen were not correlated to trophic state as indicated by non-significant correlation coefficients, suggesting these metrics are poor indicators of trophic state of the sites we studied.

Two-hundred thirty-two algae taxa were identified, of which 114 were diatom taxa (Appendix 3) and 128 were soft-algae taxa (Appendix 4). Analysis of the composition of algae groups demonstrated diatoms are the most abundant algae group at every site for May 2015 and May 2016 and soft- algae are the most abundant algae group at every site for August 2015 and August 2016 (Table 4). Significant seasonal variations in percent composition between May 2015 and August 2015 and May 2016 and August 2016 were demonstrated by student's tests ($n = 8$, $p < 0.001$). Cyanobacteria composition decreased in response to increases in total phosphorus concentrations of benthic organics, ash-free dry mass of benthic organics, and concentrations of chlorophyll-*a*, demonstrating a clear effect of trophic state (Table 5). Diatom abundance was not significantly affected by the trophic state at these sites. Chlorophyta abundance is greater at sites with greater total phosphorus concentrations of benthic organics, ash-free dry mass of benthic organics, and concentrations of chlorophyll-*a*.

The diatom assemblages at each site has a high abundance of *Achnanthidium rivulare* Potapova and Poander (26.2% of all sites and dates) and *Achnanthidium minutissimum* (Kütz.) Czarn. (8.9% of all sites and dates) (Table 6). *Achnanthidium rivulare* is the most abundant diatom taxa across all sites and seasons for 2015. High percentages of the most abundant taxa contributed of the similarity observed in diatom assemblages between sites in 2015 and 2016 (Table 7).

The pollution tolerance index (PTI) values for all sites and seasons in 2015 and 2016 accurately reflect the trophic condition (Table 8) and support the previous assessment of trophic state utilizing concentrations of chlorophyll *a* (Table 1). The low PTI values for Jones Creek and Suggs Creek sites indicate eutrophic conditions. The higher PTI values for Hurricane Creek, Will Hall Creek, Flynn Creek, and Marrow Bone Creek indicate oligotrophic-mesotrophic conditions. The Jones Creek site is impacted by sedimentation and organic pollution as indicated by values for the siltation index and organic pollution index. While evenness, richness, and the Shannon Diversity Index aid in the description of the structure of diatom assemblages, they do not follow trophic state trends, thus making these inadequate indicators of the trophic state.

Of the soft-algae taxa sampled, *Cladophora glomerata* (16.9% of all sites and dates) and *Phormidium diguetii* (14.3% of all sites and dates) was in the greatest abundance at all sites in 2015 and 2016 (Table 9). Variability of the most abundant taxa between sites in 2015 and 2016 resulted in low similarity of soft-algae assemblages (Table 10). The mean percent similarity between the same sites sampled May and August illustrates low similarity of soft-algae and diatom assemblages between seasons (Table

11). Soft algae exhibited two-fold less similarity between seasons relative to diatoms. The Shannon Diversity Index, evenness, and richness of soft-algae assemblages reveal the structure of assemblages but fail to follow trophic state trends, thus making these metrics and indices poor indicators of trophic state of the sites studied.

Trophic indicator values for soft-algae taxa were derived from the abundance-weighted average of chlorophyll *a* (A-$WA_{chl\ a}$) and abundance-weighted average of the pollution tolerance index for diatom assemblages (A-WA_{PTI}) (Appendix 5). Taxa with a high A-$WA_{chl\ a}$ and A-WA_{PTI} values coupled with low standard deviations (SD) of the A-WA were interpreted as a more accurate indicator of trophic state. For the sites studied, *Cladophora glomerata* and *Vaucheria* sp. are indicators of nutrient-rich sites as indicated by both high A-$WA_{chl\ a}$ and A-WA_{PTI} values and low SD. *Chaetopeltis orbicularis* Berthold, *Aphanocapsa elachista,* West and West, and *Oscillatoria subtilissima* Kütz. and De Toni are indicators of nutrient-limited sites as indicated by low A-$WA_{chl\ a}$ and A-WA_{PTI} values and low SD.

Algae trophic index (ATI) values for each site in August of 2015 and August 2016 reveal the trophic state of the sites (Table 13). The ATI using A-$WA_{chl\ a}$ and A-WA_{PTI} trophic indicator values were the most accurate indicators of trophic state (Table 14). The A-$WA_{chl\ a}$ and A-WA_{PTI} are not significantly correlated to concentrations of soluble reactive phosphorus of the water (Table 14), $NO_2 + NO_3$ (data not shown), or total nitrogen (data not shown).

Chapter IV

Discussion

The determination of trophic state of water is often subjective in its utilization of concentrations of nutrients, biomass, and organism composition (Hilton et al. 2006). Estimates of photoautotrophic biomass is a common bioassessment method to measure the trophic state of water (Biggs 2000). Trace Creek and Hurricane Creek sites were the least impacted by nutrient enrichment as demonstrated by their low levels of chlorophyll *a*. Nuisance levels of chlorophyll *a* measured at the Jones Creek site, located directly downstream of a wastewater treatment facility, support conclusions from other studies that demonstrate nutrient-enrichment in the water results in greater photoautotrophic periphyton biomass (Khan & Ansari 2005). The low concentrations of chlorophyll-*a* relative to the high concentrations of pheophytin-*a* at the Suggs Creek indicate poor physiological health of the photoautotrophic periphyton. Poor physiological health was denoted by the low OD664 to OD665 ratio demonstrating high concentrations of pheophytin at the Suggs Creek site and may be the result of shading of the benthic photoautotrophic periphyton.

Worldwide periodic determinations of nutrient concentrations of water are required by law to monitor the trophic state of rivers and streams in many countries (Whitton 2013). The use of concentrations of soluble reactive phosphorus of the water to determine trophic state is common because soluble reactive phosphorus is the available form of phosphorus, which is often limited in these systems (Moss et al. 2013). Pulses of nutrient enrichment and uptake from autotrophic organisms may cause high or low nutrient measurements make chemical analyses of water samples inaccurate indicators of trophic state. The United States Geological Survey National Water-Quality Assessment (NAWQA) program ranked soft-algae taxa by eutrophication tolerance based on the abundance-weighted averages of $\log_{10}$-transformed concentrations of soluble reactive phosphorus of the water samples at sites across the United States (NAWQA 2005). NAWQA (2005) concluded that the values assigned to taxa did not accurately reflect trophic state Our results strengthen the conclusions of earlier studies that nutrient concentration may not be the most important factor affecting the percent composition of many algae taxa.

The two most abundant diatom taxa in all sites, *A. rivulare* and *A. minutissimum*, are common taxa for the southeastern United States (Ponader & Potapova 2007). The lower percent composition of *A. minutissimum* relative to *A. rivulare* is consistent with previous studies (Lebkuecher 2015, Ponader & Potapova 2007) and supports the conclusion that while both taxa are indicative of good water quality, *A. rivulare* is the more tolerant species of nutrient enrichment. Pollution tolerance index values accurately reflect trophic state at each site. The low PTI values of diatom assemblages found at Jones Creek and Suggs Creek sites are consistent with numerous other studies which demonstrated the impact of eutrophication on diatom composition (Rimet 2012).

The large number of motile diatom taxa and organic pollution tolerant diatom taxa at Jones Creek is indicative of sedimentation and organic pollution impacting the composition of diatom assemblages at this site. The Jones Creek site was the only site impacted by organic pollution and siltation as demonstrated by the high siltation and organic pollution index values relative to the other sites. Due to the highly erodible soils and substantial agriculture present in Middle Tennessee, a SI values > 60 suggests the presence of an excessive impact of sediments on diatom assemblages and OPI values > 40 suggest a significant impact of organic pollution (Lebkuecher et al. 2011).

The two most abundant soft-algae taxa in all sites, *C. glomerata* and *P.diguetii* are widespread and abundant taxa (Prescott 1982, Whitford and Schumaker 1984). Autecological information of almost all soft-algae taxa remains limited and soft-algae assemblages are therefore not commonly utilized in the assessment of trophic state, relative to diatoms (Passy and Larson 2011). Percent similarity of soft-algae and diatom taxa in 2015 and 2016 between the same sites revealed low mean similarity demonstrating the composition of algae taxa may vary drastically between seasons or even years. Soft-algae composition was two-fold variable between seasons at our sites relative to diatoms which is consistent with other studies (Vis et al. 1998, Lebkuecher 2015).

The weak correlation of nutrient concentrations of the water to benthic characteristics of the photoautotrophic periphyton at these sites is consistent with previous studies. Stancheva et al. (2012) identified 180 non-diatom benthic algae taxa to species from stream sites in southern California that varied from low total phosphorus (<10 μg/L) or high total phosphorus (≥10 μg/L). Of the 180 taxa, only 7 taxa were determined to be useful indicators of the concentration of total phosphorus using a model described by Dufrene and Legendre (1997). Stancheva (2012) also used bio-volume-weighted averages of total phosphorus concentrations to determine potential indicator species using total phosphorus concentrations optima and standard deviations for the taxa present in their sites. Twenty-one of the 180 taxa identified were determined to be indicative of high or low concentrations of total phosphorus. Stancheva (2012) concluded that nutrient concentrations of the water were not solely responsible for the structure of soft-algae assemblages at their sites.

Algae trophic indices developed using benthic characteristics to assign trophic indicator values to soft-algae taxa present allows for evaluation of lotic systems based on the response of soft-algae composition to trophic state. The results of this study demonstrate that $ATI_{chl\text{-}a}$ and ATI_{PTI} values are accurate indicators of trophic state as supported by significantly correlated coefficients to the other indicators of trophic state. These indices are superior to indices formulated using nutrient concentrations as indicator values for taxa present given that chlorophyll-*a* concentration and diatom composition is a more accurate indicator of trophic state than nutrient concentration of the water.

Chapter V

Conclusions

The effects of nutrient enrichment on soft-algae assemblages is quantifiable in terms of benthic characteristics such as total phosphorus concentration of benthic organics, chlorophyll-*a* concentration, and ash-free dry mass of benthic organics. Total phosphorus of the benthic organics was a more accurate indicator of trophic state relative to the nutrient concentrations of the water (i.e., soluble reactive phosphorus). The trophic state of the Suggs Creek and Jones Creek sites were verified as eutrophic, while Flynn Creek and Hurricane Creek sites were verified as oligotrophic. Seasonal variation of soft algae composition varied two-fold from spring to summer relative to diatoms. The trophic-state preferences of soft-algae taxa were denoted by the abundance-weighted average of chlorophyll-*a* concentrations (A-$WA_{chl\ a}$) and the PTI (A-WA_{PTI}). The algae trophic indices using the A-$WA_{chl\ a}$ and A-WA_{PTI} as trophic indicator values for soft-algae taxa accurately portray the trophic state.

Chapter VI

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Chapter VII
Tables

Table 1. Characteristics of photoautotrophic periphyton and ash-free dry mass of benthic organics 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}
OD664/0D665	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 organics ($g \cdot m^{-2}$)	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 organics, 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.

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

Table 3. Pearson's correlation coefficients for concentrations of chlorophyll *a* and ash-free dry weight of benthic organics to concentrations of total phosphorous of benthic organics 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 organics ($mg·m^{-2}$)	Soluble reactive phosphorous ($\mu g·L^{-1}$ water)	$NO_2 + NO_3$ ($\mu g·L^{-1}$ water)	Total nitrogen ($\mu g·L^{-1}$ water)
Aug. 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 wt. of benthic organics ($g·m^{-2}$)	1.0 (0.00)	- 0.52 (0.47)	- 0.64 (0.36)	- 0.12 (0.87)
Aug. 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 wt. of benthic organics ($g·m^{-2}$)	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.

2015	Suggs		Trace		Flynn		Hurricane	
	May	Aug	May	Aug	May	Aug	May	Aug
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							
2016	Jones		McAdoo		M. Bone		Will Hall	
	May	Aug	May	Aug	May	Aug	May	Aug
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

Table 5. Pearson's correlation coefficients for percent composition of diatoms, cyanobacteria, and Chlorophyta at sites sampled in August 2015 and August 2016 to periphyton characteristics used to assess trophic state determined from the same sites sampled August 2015 and August 2016. The significance of probability at the 95 % confidence level follows the Pearson's correlation coefficients in parentheses.

	Total phosphorous of benthic organics (mg m-2)	Chlorophyll *a* (mg m^{-2})	Ash-free dry wt. of benthic organics (g m^{-2})
Diatoms	- 0.25 (0.95)	0.26 (0.52)	0.02 (0.97)
Cyanobacteria	- 0.84 (0.009)	- 0.72 (0.04)	- 0.83 (0.01)
Chlorophyta	0.98 (0.0001)	0.64 (0.08)	0.97 (< 0.0001)

Table 6. 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)

Table 7. 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 8. *Metrics and indices of diatom assemblages at sites sampled in May 2015, August 2015, May 2016, and August 2016.*

2015	Suggs		Trace		Flynn		Hurricane	
	May	Aug	May	Aug	May	Aug	May	Aug
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
2016	Jones		McAdoo		M. Bone		Will Hall	
	May	Aug	May	Aug	May	Aug	May	Aug
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

Table 9. Most abundant soft-algae taxa at sites sampled May 2015, August 2015, May 2016, and August 2016. Numbers in parentheses represent percent composition.

May 2016			
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)
Aug 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)
Aug 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. diguetii* (22.5)	*Phormidium* sp. (13.6)
E. rivularis (10.2)	*K. constrictum* (9.1)	*L. foveolarum* (11.5)	*L. angustissimum* (9.1)

Table 10. 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
Aug 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
Aug 2016			
Big McAdoo	15		
Marrow Bone	22	40	
Will Hall	21	20	32

Table 11. 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

Table 12. Metrics and indices of soft-algae assemblages sampled May 2015, August 2015, May 2016, and August 2016.

2015	Suggs		Trace		Flynn		Hurricane	
	May	Aug	May	Aug	May	Aug	May	Aug
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

2016	Jones		McAdoo		M. Bone		Will Hall	
	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

Table 13. Algae trophic index (ATI) values of stream sites sampled August 2015 and August 2016. Sites are listed in order of most to least nutrient impaired as determined by ATI values. The ATI using abundance-weighted average (A-WA) of chlorophyll-a concentrations ($mg \cdot m^{-2}$) for soft-algae taxa of the assemblages as the trophic-indicator values is abbreviated $ATI_{chl\ a}$. The ATI using A-WA of the pollution tolerance index of diatom assemblages for soft-algae taxa of the assemblages as the trophic-indicator values is abbreviated ATI_{PTI}. The ATI using A-WA of ash-free dry weight of benthic organics ($g \cdot m^{-2}$) for soft-algae taxa of the assemblages as the trophic-indicator values is abbreviated ATI_{AFDW}. The ATI using A-WA of total phosphorous of benthic organics ($mg \cdot m^{-2}$) for soft-algae taxa of the assemblages as the trophic-indicator values is abbreviated ATI_{TP}.

	Suggs	Jones	Will Wall	McAdoo	M. Bone	Trace	Flynn	Hurricane
$ATI_{chl\ a}$	134.3	121.5	67.7	60.2	59.9	49.3	47.8	39.8
ATI_{PTI}	2.37	2.39	2.56	2.52	2.64	2.66	2.72	2.76
ATI_{AFDW}	49.8	20.6	10.5	11.6	9.5	8.3	6.8	6.8
ATI_{TP}	70.9	8.0	7.6	7.8	30.9	10.7	9.9	11.1

Table 14. Pearson's correlation coefficients of indices for algae assemblages sampled August 2015 and Augus 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 $ATI_{chl\ a}$. The ATI using abundance-weighted averages of the pollution tolerance index of diatoms as the trophic-indicator values is abbreviated ATI_{PTI}. The ATI using abundance-weighted averages of ash-free dry weight of benthic organics as the trophic-indicator values is abbreviated ATI_{AFDW}. The ATI using abundance-weighted averages of the concentration of total phosphorous of benthic organics as the trophic-indicator values is abbreviated ATI_{TP}.

Index	Total phosphorous of benthic organics (mg/m^2)	Ash-free dry weight of benthic organics (g·m^{-2})	Chl *a* (mg·m^{-2})	Pollution tolerance index of diatoms	Soluble reactive phosphorous of water (µg·L^{-1} water)
$ATI_{chl\ a}$	0.93 (0.001)	0.85 (0.01)	0.89 (0.003)	- 0.75 (0.03)	0.51 (0.20)
ATI_{PTI}	- 0.81 (0.02)	- 0.78 (0.02)	- 0.85 (0.01)	0.88 (0.004)	- 0.27 (0.52)
ATI_{AFDW}	0.98 (< 0.0001)	0.61 (0.11)	- 0.48 (0.23)	0.00 (0.99)	- 0.15 (0.71)
ATI_{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 diatoms	- 0.54 (0.17)	- 0.49 (0.22)	- 0.86 (0.006)		- 0.48 (0.23)

Appendices

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

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 S.W. 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	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug
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 prostratum (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									
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. & 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 acuminatum (Kütz.) Rabenh.																
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							
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. & Round					0.5											
Psammothidium subatomoides Hust.					0.9											1.2
Psammothidium sp.			0.5		0.9				1.4							
Pseudostaurosira trainorii Morales																
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 angusta Kütz.																
Surirella brebissonii Lange-Bert. & 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 & 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	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug	May	Aug
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 bijuga (Turp.) Lagerh.																
Scenedesmus dimorphus (Turp.) Kütz.			0.1							0.3	0.1					
Scenedesmus sp.										0.3					0.7	
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 & 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 & 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 & 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 & Anagn.						0.2			0.1	4.0	0.4				0.7	
Gloeocapsopsis pleuroccapsoides (Novacek) Komárek & 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. & 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 & Flahault) Kirchner				34.1		3.1							2.0	7.0		28.5
Jaaginema pseudogeminatum (G. Schmid) Anagn. & Komárek								0.1								
Komvophoron constrictum (Szafer) Anagn. & Komárek	0.1	0.1								5.5		9.1				
Komvophoron munitum (Skuja) Anagn. & Komarek		0.1		0.1	0.3	0.4					1.7	3.3	0.9	0.1	1.3	
Komvophoron schmidlei (Jaag.) Anagn. & Komárek											0.6	6.4		2.3		
Leibleinia nordgaardii (Wille) Anagn. & Komárek	1.2				0.8											

Leptolyngbya angustissimum (West and West) Anagn. & 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. & 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. & 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. & Gomont) Anagn. & 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. & De Toni	3.0			0.8		4.0	1.4								1.3	
Phormidium articulatum Gardner Anagn. & 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. & 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. & 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 & 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														
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 chlorophyll *a* of photoautotrophic periphyton for soft-algae taxa sampled August 2015 and August 2016 and A-WA of the pollution tolerance index of diatom assemblages (PTI) 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 1 algal unit was recorded.

	Chlorophyll *a*			PTI		
	A-WA	SD	SD/A-WA	A-WA	SD	SD/A-WA
Chlorophyta						
Carteria globulosa Pascher	52.40			2.31		
Chaetopeltis orbicularis Berthold	23.04	7.40	0.32	2.90	0.04	0.001
Characium ambiguum H. Jaeger	136.10			2.37		
Chlamydomonas angulosa Dill	52.40			2.31		
Chlamydomonas cienkowskii Schmidle	136.10	0.00	0.00	2.37	0.00	0.00
Chlamydomonas globosa Snow.	95.38	47.06	0.49	2.40	0.10	0.04
Chlamydomonas gloeogama Korschikov	52.40			2.31		
Chlamydomonas patellaria Whitford	74.45	41.15	0.55	2.38	0.11	0.05
Cladophora glomerata (L.) Kütz.	135.50	24.99	0.18	2.37	0.12	0.05
Closterium acerosum (Schrank) Ehrenb.	134.80	116.53	0.86	2.13	0.25	0.12
Closterium moniliferum (Bory) Ehrenb.	133.70	84.73	0.63	2.31	0.34	0.15
Coelastrum microporum Nägeli	28.40			2.87		
Cosmarium galeritium Nordst.	136.10			2.37		
Eudorina elegans Ehrenb.	47.80			2.62		
Gloeocystis gigas (Kütz.) Langerh.	28.40			2.87		
Gloeocystis vesiculosa Nägeli	101.51	79.33	0.78	2.44	0.37	0.15
Hydrodictyon reticulatum (L.) Lagerh.	136.10	0.00	0.00	2.37	0.00	0.00
Oedogonium sp.	123.58	30.36	0.25	2.44	0.16	0.07
Pandorina morum (Müller) Bory	136.10			2.37		
Rhizoclonium hieroglyphicum (C. Agardh) Kutz.	52.40	0.00	0.00	2.31	0.00	0.00

Scenedesmus dimorphus (Turp.) Kütz.	217.20			1.95		
Scenedesmus sp.	217.20			1.95		
Selenastrum capricornutum Printz	136.10			2.37		
Spirogyra sp.	136.10	0.00	0.00	2.37	0.00	0.00
Stigeoclonium tenue (C. A. Ag.) Kütz.	52.40	0.00	0.00	2.31	0.00	0.00
Tetraselmis cordiformis (Carter) Stein	52.40			2.31		
Ulothrix cylindricum Prescott	217.20	0.00	0.00	1.95	0.00	0.00
Ulothrix variabilis Kütz.	136.10	0.00	0.00	2.37	0.00	0.00
Cyanobacteria						
Aphanocapsa elachista West & West	35.53	14.25	0.40	2.79	0.17	0.06
Aphanocapsa pulchra (Kütz.) Rabenhorst	136.10	0.00	0.00	2.37	0.00	0.00
Aphanothece castagnei (de Breb.) Rabenh.	39.80	15.61	0.39	2.74	0.18	0.07
Aphanothece nidulans Richter	111.97	91.86	0.82	2.43	0.42	0.17
Borzia periklei Anag.	132.50	119.78	0.90	2.29	0.47	0.21
Borzia trilocularis Cohn.	79.77	50.98	0.64	2.58	0.24	0.09
Calothrix stellaris Bornet and Flahault	28.40			2.87		
Chroococcus minimus (Keissler) Lemmerman	28.40			2.87		
Chroococcus minor (Kütz.) Nägeli	106.23	80.76	0.76	2.47	0.38	0.15
Chroococcus minutus Kütz.	66.16	84.43	1.28	2.69	0.41	0.15
Dactylococcopsis raphidioides Hansg.	176.65	57.35	0.32	2.16	0.30	0.14
Entophysalis rivularis	120.37	86.11	0.72	2.35	0.39	0.17
Gloeocapsa aeruginosa (Carm.) Kütz.	47.80			2.62		
Gloeocapsopsis cyanea (Krieg) Komárek & Anagn.	196.22	61.05	0.31	2.05	0.30	0.15
Gloeocapsopsis pleuroccapsoides (Novacek) Komárek & Anagn.	40.68	45.52	1.12	2.81	0.22	0.08
Heteroleibleinia kossinskajae (Elenkin) Anagn. & Komárek	48.04	22.18	0.46	2.63	0.25	0.10

Homeothrix crustaceae Woron.	28.40	0.00	0.00	2.87	0.00	0.00
Homeothrix juliana (Bornet & Flahault) Kirchner	56.86	7.73	0.14	2.58	0.08	0.03
Jaaginema pseudogeminatum	14.10	0.00	0.00	2.94	0.00	0.00
Komvophoron constrictum (Szafer) Anagn. & Komárek	106.82	72.41	0.68	2.22	0.17	0.08
Komvophoron munitum (Skuja) Anagn. & Komárek	57.14	25.45	0.45	2.41	0.20	0.08
Komvophoron schmidlei (Jaag) Anagn. & Komárek	60.94	9.29	0.15	2.50	0.21	0.08
Leptolyngbya angustissimum (West and West) Anagn. & Komárek	44.00	14.83	0.34		0.23	0.09
Leptolyngbya foveolarum (Mont.) Anagn. & Komárek	58.95	40.98	0.70	2.69	0.24	0.09
Leptolyngbya nostocrum (Bomont) Anagn. & Komárek	50.97	5.73	0.11	2.34	0.13	0.06
Lyngbya major Menegh.	64.08	9.79	0.15	2.63	0.16	0.06
Lyngbya martensiana Menegh.	56.54	5.55	0.10	2.57	0.06	0.02
Merismopedia tenuissima Lemmerman	136.10			2.37		
Microcystis incerta Lemmerman	217.20			1.95		
Nostoc paludosum Kütz.	28.40			2.87		
Oscillatoria agardhii Gomont	32.56	58.77	1.80	2.85	0.29	0.10
Oscillatoria limosa (Dylwin) C. Agardh	70.90	0.00	0.00	2.72	0.00	0.00
Oscillatoria princeps Vaucher	136.10	0.00	0.00	2.37	0.00	0.00
Oscillatoria subbrevis Schmidle	38.16	61.77	1.62	2.83	0.30	0.11
Oscillatoria subtilissima Kütz. & De Toni	36.17	12.84	0.35	2.78	0.15	0.05
Phormidium articulatum Gardner Anagn. & Komárek	104.08	80.70	0.78	2.37	0.35	0.15
Phormidium autumnale Gomont	64.33	11.51	0.18	2.68	0.09	0.03
Phormidium diguetii (Gomont) Anagn. & Komárek	39.64	25.69	0.65	2.74	0.20	0.07

Phormidium favosum Bory	14.10	0.00	0.00	2.94	0.00	0.00
Phormidium fragile Gomont	17.15	10.83	0.63	2.90	0.13	0.04
Phormidium indunatum Kütz.	56.61	1.63	0.03	2.54	0.01	0.004
Phormidium retzii (C. Agardh) Gomont	18.67	22.51	1.21	2.92	0.11	0.04
Phormidium tenue (C. Agardh & Gomont) Anagn. & Komárek	54.08	14.79	0.27	2.60	0.20	0.08
Plectonema gracillimum (Zopf) Hansgir	28.40	0.00	0.00	2.87	0.00	0.00
Schizothrix lardacea (Ces.) Gomont	56.90	0.00	0.00	2.54	0.00	0.00
Synechococcus aeruginosus Nägeli	50.51	25.42	0.50	2.54	0.23	0.09
Synechococcus sp.	56.90	0.00	0.00	2.54	0.00	0.00
Synechocystis sp.	133.88	92.26	0.69	2.29	0.42	0.18
Xenococcus gracilus Lemmerman	136.10			2.37		
Xenococcus minimus Geitler	136.10			2.37		
Cryptophyta						
Cryptomonas erosa Ehrenb.	56.90			2.54		
Cryptomonas anomala F. E. Fritish	49.40	8.49	0.17	2.38	0.20	0.08
Euglenophyta						
Euglena tripteris (Duj.) Klebs	136.10			2.37		
Trachelomonas intermedia Dangeard	136.10			2.37		
Trachelomonas pulcherrima var minor Playfair	47.80			2.62		
Trachelomonas robusta Swirenko	136.10			2.37		
Ochrophyta						
Vaucheria sp.	135.39	8.74	0.06	2.37	0.04	0.02
Rhodophyta						
Audouinella hermannii (Roth) Duby	135.07	73.86	0.55	2.37	0.38	0.16