Final Report

IMPACTS OF TROPHIC STATE ON THE COMPOSITION OF ALGAE ASSEMBLAGES OF THE HARPETH RIVER IN MIDDLE TENNESSEE

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Submitted to the Harpeth Conservancy

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ABSTRACT

The concentration of total phosphorus and total nitrogen of water samples, biomass of periphyton, and composition of soft-algae and diatom assemblages in the Harpeth River at two sites upstream and two sites downstream of Franklin, Tennessee were evaluated to assess the impact of nutrient concentrations on the integrity of photoautotrophic periphyton. Nutrient impairment of all four sites was indicated by eutrophic concentrations of total phosphorus and eutrophic concentrations of periphyton biomass. Percent composition of 186 taxa of algae were documented: 92 taxa of soft algae and 94 taxa of diatoms. Analyses of algae composition by indices including the algae trophic index for soft-algae assemblages and the pollution tolerance index for diatom assemblages indicate impairment by nutrient enrichment was greatest at the river site located immediately downstream of the Franklin Wastewater Treatment Plant in Franklin. Impairment by excessive concentrations of organic matter and inorganic sediments was indicated at the two river sites downstream of Franklin by high values for the organic pollution index for diatom assemblages and the siltation index for diatom assemblages, respectively. The results indicate that degradation of water quality as the Harpeth River flows through Franklin alters the composition of photoautotrophic periphyton and are consistent with an earlier study by Burkholder (2016) which indicates eutrophication by the Franklin Wastewater Treatment Plant negatively impacts the biotic integrity of the Harpeth River.

INTRODUCTION

Bioassessments using algae

Nutrient enrichment often results in unhealthy changes of nutrient stoichiometry and major shifts in the structure of aquatic communities (Burkholder and Glibert 2013). Bioassessments that characterize and quantify the impacts of eutrophication are prerequisites to monitoring the efficacy of management practices designed to improve the integrity of nutrient-impaired waters (Smucker and Vis 2009). Algae are a major component of the trophic base of most shallow lotic systems and assemblage composition may reflect habitat quality (Stancheva and Sheath 2016). The composition of algae assemblages of the majority of streams in the Interior Plateau Level III Ecoregion is unknown. This lack of basic knowledge limits the ability of watershed managers to monitor changes of habitat quality. This work documents the composition of algae assemblages essential to monitor the effects of water quality in the upper and middle reaches of the Harpeth River in Middle Tennessee.

Methods to evaluate the impact of nutrient enrichment include measurements of chlorophyll (chl) *a*, ash-free dry mass of benthic organics (AFDM), and nutrient concentrations of water, all of which may not accurately denote trophic state. Estimates of the biomass of photoautotrophic periphyton by measurements of the concentration of benthic 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 concentrations of benthic chl *a* $\leq$ 20 $mg \cdot m^{-2}$ as oligotrophic and concentrations > 70 $mg \cdot m^{-2}$ as eutrophic. The use of concentration of chl *a* as an indicator of trophic state is complicated by the influence of many abiotic and biotic characters including irradiance,

temperature, water velocity, herbivory, and time period between spates of high discharge (Anderson et al. 1999, Kurle and Cardinale 2011). Ash-free dry mass of benthic organics (AFDM) may be influenced by the same factors that influence the concentration of chl *a*. In addition, AFDM is affected by organic inputs which differ by season and stream bank characteristics. Chemical analyses do not indicate ecological condition and may not accurately reflect water quality (Andrus et al. 2013). Pulses of pollution may be missed during sampling and water with a high concentration of biomass may have low nutrient concentrations due to high nutrient demand (Dodds 2006). Organism composition is often the most accurate indicator of trophic state relative to biomass and nutrient concentrations of water (Stancheva et al. 2012). The advantages for the use of algae composition as indicators of habitat quality result from the fact that algae composition reflects the history of pollution levels and is less influenced by changes in discharge which affect biomass (Kelly and Whitton 1995).

Bioassessments using diatoms

Diatom composition is the constituent of photoautotrophic periphyton most widely used to assess trophic state relative to soft (non-diatom) algae state because more autecological information exists for diatoms (Rimet 2012). The composition of diatom assemblages often reflects the impacts of trophic state, organic pollution, and siltation and thus can be used to support proposed best management practices (Smucker and Vis 2009). Evaluations of the composition of diatom assemblages are used by most European countries to satisfy the requirement by the Water Framework Directive of the European Union to regularly assess the phytobenthos of rivers (Schneider et al. 2013). Several US states including Oklahoma, Montana, Kentucky, and Texas use evaluations

of diatom composition as a standard protocol to monitor changes of water quality (Stevenson et al. 2008, Szczepocka and Szule 2009). The pollution tolerance index for diatom assemblages (PTI) reveals the impact of nutrient concentration on the diatom assemblage and the trophic state of water (KDOW 2002). The PTI is similar to the trophic diatom index (Kelly 1998) and other diatom indices which use relative-abundance and eutrophication-tolerance values assigned to taxa (Lange-Bertalot 1979, Leclercq and Maquet 1987, Watanbe *et* al. 1988). The eutrophication-tolerance value of a taxon is determined from autecological information, and for the PTI, ranges from 1 to 4 (KDOW 2002, Barbour et al. 1999). Taxa very tolerant to eutrophic conditions are assigned a eutrophication-tolerance value of 1. Taxa very intolerant of eutrophic conditions are assigned a eutrophication-tolerance value of 4.

Bioassessments using soft algae

Soft algae are a major component of the trophic base of mid-order lotic systems (Stevenson 1996) and encompass an unknown number of taxa from several phyla (Graham et al. 2016). Only a few indices have been developed which utilize soft-algae taxa to evaluate the trophic state of lotic systems (Gutowski et al. 2004, Schaumburg et al. 2004, Schneider and Lindstrøm 2011, Fetscher et al. 2014, Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017). 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 and Larson 2011, Whitten 2012). Several characteristics of soft algae contribute to the scarcity of data correlating environmental conditions to abundance. Soft-algae taxa may be more affected by intermittent changes of water velocity relative to diatoms due to their greater diversity of

surface area (Porter 2008, Whitton 2012). The greater phylogenetic diversity for soft algae relative to diatoms most likely contributes to greater differences of ecological interactions complicating the relationships of composition to an environmental condition (NAWQA 2005). Despite the challenges associated with using soft-algae taxa as indicators of trophic state, the few indices developed do accurately denote the trophic state of aquatic habitats in the ecoregions they were designed to test. The algae trophic index (ATI) uses trophic-indicator values based on benthic concentrations of chl *a* at sites across Middle Tennessee and accurately depicts the trophic state of lotic systems in Middle Tennessee (Grimmett and Lebkuecher 2017).

METHODS AND MATERIALS

The study area: Harpeth River Watershed

The Harpeth River Watershed drains 223,516 ha of the central region of the Interior Plateau Level III Ecoregion of the United States. The geologic base of the ecoregion is limestone which includes some chert, shale, siltstone, sandstone, and dolomite (Griffith et al. 1997). The forests are Western Mesophytic and consist largely of *Quercus* and *Carya* species (Baskin et al. 1997). Much of the watershed is used to produce agriculture products including corn, soybean, and livestock (TDEC 2017a). Most of the watershed is within the Outer and Inner Nashville Basin Level IV Ecoregions which consist of surface waters with naturally high concentrations of phosphorus due partially to the high phosphorus concentrations of the carbonate (USGS 1999).

The Harpeth River flows northwest 185 km from its source near Eagleville, Tennessee in rural Middle Tennessee to where it enters the Cumberland River

approximately 25 km northwest of Nashville, Tennessee. The middle reaches flow through Franklin, Tennessee, a large suburb of Nashville, with a population of 75,000 as of 2015 (USCB, 2015). The river serves as the region's water supply and sewage disposal. The lower portion is designated as a scenic river under the Tennessee Scenic Rivers Act and is popular for swimming, canoeing, and fishing (TDEC 2017b).

Sampling site locations and dates

Four sites were sampled in the Harpeth River on September 30, 2017 (Appendix 1) from river mile 106 (site 1; uppermost river site sampled) to river mile 62.4 (site 4; lowermost river site sampled). The uppermost site is located 12 km east-southeast of Franklin, Tennessee in a rural, agricultural region. Site two (river mile 90.5) and site three (river mile 80) are located in densely populated, urban areas of Franklin, 3 km east-southeast of downtown and 5 km northwest of downtown, respectively. Effluent from the Franklin Water Treatment Facility enters the river at river mile 85.2, 5 km upstream of site 3. The facility treats approximately 12-million gallons of wastewater per day from a 114-million gallon raw-water reservoir (CFWD 2017). Site 4 is located 15 km north-northwest of Franklin and 20 km southeast of downtown Nashville in an area with a mix of neighborhoods and agriculture.

Sampling cobbles to determine periphyton characteristics

Cobble sampling occurred in runs with velocities between 0.1 $m \cdot s^{-1}$ and 0.3 $m \cdot s^{-1}$ at depths between 0.1 m and 0.25 m. Four plots in each run were established with 0.25 m^2 wire frames placed approximately 1.25 m apart. Two 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. If a plot did not contain 2 cobbles appropriate for

sampling, cobbles closest to the plot were removed. One cobble from each plot was 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. One cobble from each plot was used to determine pigment concentrations of photoautotrophic periphyton and ash-free dry mass of benthic organic matter. These cobbles were placed in self-sealable plastic bags and transported to the lab on ice in darkness.

Periphyton pigment concentrations and ash-free dry mass

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-mL 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* corrected for pheophytin *a* were calculated as described by APHA (2017). The chl *a* to pheophytin *a* ratio was indicated as the ratio of OD_{664} to OD_{665} (APHA 2017).

Periphyton removed from cobble was dried by allowing the acetone to evaporate at 25 ^{o}C. Ash-free dry mass and inorganic sediment weights were determined as described by APHA (2017). Ash-free dry weights of benthic organic matter 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 were compared using Tukey-Kramer Honestly Significant Difference Tests preceded by Analysis of Variance Tests. Assay means were considered significantly different if they differed at the experimentwise-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 800 units were counted.

A multi-habitat sampling technique was employed to identify additional soft-algae taxa associated with substrates other than the cobble sampled to determine percent

composition. Samples were collected from the water column, cobbles, sediment, wood, detritus, aquatic flora, and snail shells in riffles, runs, and pools. Algae associated with cobbles were removed using a single-edged razor blade and a test tube brush. Algae associated with sand, silt, and clay were sampled using a plastic pipet with the tip cut off to increase tip diameter and removing approximately 5 mm of surface sediment. Algae associated with wood were sampled by scraping the wood surface with a single-edged razor blade. Algae associated with small substrates such as organic debris were sampled by collecting sections of the substrate. Epiphytic algae on aquatic mosses and macrophytes were sampled by collecting sections of the shoots. Samples were preserved in 1 % glutaraldehyde adjusted to pH 7.0 with NaOH, and concentrated by settling in darkness. Wet mounts from each habitat were searched using 100 X, 400 X, and 1000 X magnification until no new taxa were observed in at least 5 consecutive wet mounts. Soft-algae taxa identified were recorded as present.

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^{O} 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.

Metrics and indices

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).

The pollution tolerance index for 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).

The organic pollution index (OPI) is the percentage of diatoms tolerant of organic pollution listed in Kelly (1998). OPI values of $\geq$ 20 infer organic pollution impacts the composition of diatom assemblages and values > 40 infer the habitat is severely impaired by excessive concentration of organic matter (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 number of diatoms. The SI values range from 0 to 100. High SI values signify that sediments impact the structure of diatom assemblages. Belton et al. (2005) suggested that SI values near 40 indicate an impact of sediments on diatom assemblages.

The algae trophic index of soft algae assemblages (ATI) was calculated as:

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

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 trophic-indicator values are the abundance-weighted average (A-WA) of concentration of chl *a*, listed in Grimmett and Lebkuecher (2017). Taxa not identified to species were excluded from index calculations.

Concentrations of total phosphorus and total nitrogen of water samples

Nutrient concentrations of water samples collected approximately 5 cm below the surface were determined by Hancock Biological Station at Murray State University in Murray, Kentucky using a Lachat QuickChem 8500 Flow Injection Analyzer (Lachat Instruments, 5600 Lindbergh Dr., Loveland, Colorado 80538). Concentrations of total phosphorus were determined using the persulfate-digestion and the ascorbic-acid method (APHA 2017). Concentrations of total nitrogen were determined by the persulfate-digestion and cadmium-reduction method (APHA 2017).

Reach morphological characteristics

Two transects from the opposing banks and 5 m apart were established in reaches near each sampling site. Transect widths and depths at 1/3 intervals between the banks of each transect were measured. Velocity was determined as the time required for a density-neutral object to travel 5 m downstream. Discharge was calculated using the equation from Robins and Crawford (1954): Discharge = Width · Depth · Velocity · 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. Canopy angle was estimated visually as the angle between the tops of the vegetation on each bank at midstream. Reach morphological characteristics were determined to provide a depiction of the abiotic characteristics of the reaches sampled (Appendix 2).

Results and Discussion

Concentrations of total phosphorus and total nitrogen

Concentrations of total phosphorus (TP) of water from the four sites sampled (Table 1) were well above 75 µg $TP \cdot L^{-1}$, the value suggested by Dodds et al. (1998) to designate lotic systems as eutrophic. The high concentrations of TP at all four sites likely reflect the heavy anthropogenic activities in the watershed and naturally high concentrations of phosphorus in the limestone bedrock (USGS 1999). Concentrations of approximately 180 µg $TP \cdot L^{-1}$ are suggested to be a more realistic expectation of moderate levels of P in surface waters in the Nashville Basin (TDEC 2005). Concentrations of TP were substantially greater at site 3 (river mile 80), 5 km

downstream of the Franklin Wastewater Treatment Facility. Concentrations of total nitrogen (TN) at the sites were in or near the range considered mesotrophic for streams ($\geq$ 700 $\mu g \cdot L^{-1}$ to 1500 $\mu g \cdot L^{-1}$) by Dodds et al. (1998). Concentrations of TN were lowest at the uppermost site and greatest at the site immediately downstream of the wastewater treatment plant. The TN:TP ratios were lowest at the two sites downstream of Franklin as a result of the very high concentrations of TP at these sites. The impacts of changes in nutrient stoichiometry on ecological integrity are often difficult to document (Burkholder et al. 2010) but are known to promote unnatural growths of harmful algae (Gobler et al. 2016). Numerous studies demonstrate the need for management of both phosphorous and nitrogen concentrations to maintain algal assemblages with abundances of taxa typical of healthy communities (Burkholder and Glibert 2013).

Concentrations of chlorophyll a and ash-free dry mass

The concentrations of chlorophyll (chl) *a* corrected for pheophytin *a* (Table 1) were > 70 $mg \cdot m^{-2}$, the value suggested by Dodds et al. (1998) to designate lotic systems as eutrophic. None of the OD_{664}/OD_{665} values of the pigment extracts were below 1.5, the threshold value used to indicate the algae were in poor physiological condition (APHA 2017). The concentrations of ash-free dry mass of benthic organic matter (AFDM) at the sites sampled were all > 10 $g \cdot m^{-2}$, a value considered indicative of eutrophic environments based on earlier studies (O'Brian and Wehr 2010, Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017).

Composition of soft-algae assemblages

We identified 186 taxa of algae: 92 taxa of soft algae (Appendix 3) and 94 taxa of diatoms (Appendix 4). Over 20 taxa were identified which were not known to occur in Middle Tennessee. Especially noteworthy taxa identified include *Chilomonas* sp., a nonphotosynthetic cryptomonad, and *Paulinella chromatophora* Lauterborn, a filose thecamoeba with primitive, cyanobacteria-like plastids. Genetic uniqueness of *P. chromatophora* plastids suggests that all plastids were not acquired from a single primary endosymbiotic event and thus implies that the Archaeplastida supergroup may not be monophyletic (Nowack et al. 2008).

The most abundant soft taxon sampled was the filamentous Rhodophyta *Audouinella hermannii* (Roth) Duby (16.0 %) due to its high abundance at the three lowermost sites (Table 2). The second most abundant soft-algae taxon was the filamentous cyanobacterium *Leptolyngbya foveolarum* (Mont.) Anagn. & Komárek (11.4 %) and was present at all four sites. The third and fourth most abundant soft-algae taxa were the filamentous cyanobacterium *Phormidium diguetii* (Gomont) Anagn. & Komárek (10.4 %) due to its high abundance at the uppermost site, and the filamentous cyanobacterium *Leptolyngbya angustissimum* (West & West) Anagn. & Komárek (6.7 %) due to its high abundance at the lowermost site.

Differences of composition of algae groups

The percent composition of algae groups differed dramatically between sites (Table 3). The uppermost site was dominated by cyanobacteria and the lowermost site was dominated by diatoms. The dominance of the uppermost site by cyanobacteria was a result of the high abundance of *Phormidium* taxa (69 %). We do not know the reason for

the differences in abundance of diatoms and soft algae at the sites. Lebkuecher et al. (2015) and Grimmett and Lebkuecher (2017) found no correlation between trophic state and abundances of diatoms verses soft algae in Middle Tennessee streams. The abundance cyanobacteria was substantially lower while the abundance of Chlorophyta was substantially greater at sites 3 and 4 (lowermost sites) relative to sites 1 and 2 (uppermost sites). These results are consistent with earlier studies that demonstrated cyanobacteria biomass (Perona et al. 1998) and diversity (Douterelo et al. 2004) were lower at river sites with higher concentrations of soluble reactive phosphorous of water samples in central Spain. Similar significantly lower abundances of cyanobacteria relative to Chlorophyta at sites with higher concentrations of nutrients occur in other Middle Tennessee streams (Grimmett and Lebkuecher 2017). Interpretation of the effects of trophic state on the abundance of algal groups is complicated by the fact that temperature and thus season may be the dominant factor controlling abundance (Allan and Castillo 2009). In addition, interactions between temperature and nutrient-concentrations also affects the abundance of algal groups (Burkholder and Glibert 2013). For example, 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, yet growth may be limited by silica limitations following spring diatom blooms. In general, 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 higher temperatures (DeNicola 1996).

The soft-algae and diatom assemblages were distinct from each other (Table 4). The greater similarity of the soft-algae assemblages between sites 1 and 2 is due to the abundance of *Phormidium diguetii* and *Leptolyngbya foveolarum* at both sites (Table 2). The larger dissimilarity of soft-algae assemblages relative to diatom assemblages between sites of the Harpeth River is consistent with earlier studies of several Middle Tennessee streams. A study by Lebkuecher et al. (2015) of three mesotrophic sites and one hypereutrophic site in Sulphur Fork Creek in Middle Tennessee demonstrated that 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 %. Soft algae assemblages at two oligotrophic-mesotrophic sites in the upper reach of Sulphur Fork Creek 11 river km apart sampled in August were only 16 % similar. In a different study of eight sites in eight streams in Middle Tennessee, the mean percent similarity between May and August was 24 $\pm$ 3 SE for soft-algae assemblages and 42 $\pm$ 4 for diatom assemblages (Grimmett and Lebkuecher 2017).

Metrics and indices for soft-algae assemblages

The 92 taxa of soft algae identified and richness of the sites (Table 5) demonstrate that the soft-algae assemblages studied are diverse. For example, Henderson and Luttenton (2007) identified 67 taxa of soft algae at 16 sites in 5 streams in the Little River basin of western Kentucky. Zalack et al. (2006) sampled a stream in southeastern Ohio each season for two consecutive years and identified 70 soft-algae taxa from samples collected in fall, 48 in winter, 49 in spring, and 58 in summer. Lebkuecher et al. (2015) identified 63 soft-algae taxa associated with cobble at four sites in Sulphur Fork

Creek in the Red River Watershed of in northern Middle Tennessee with richness of the sites ranging from 15 to 27. Grimmett and Lebkuecher (2017) identified 128 soft-algae taxa during the spring and summer at eight stream sites in Middle Tennessee with richness of the sites ranging from 18 to 39. In an effort to identify all species of organisms in the Great Smoky Mountains National Park, 512 soft-algae taxa were documented as of 2007 (Johansen et al. 2007).

The diversity of soft-algae taxa increased downstream (Table 5). The similar values for the Shannon diversity index among sites is due partially to similar evenness. The lack of substantial differences of the Shannon diversity index between sites support the conclusions of several earlier studies that values for the Shannon diversity index for algae assemblages may not correlate to habitat quality (Carlisle et al. 2008). For example, lotic systems with poor quality water may have few taxa with the individuals evenly distributed resulting in a high evenness value (Pontasch et al. 1989).

Values for the algae trophic index (ATI) indicate that the composition of soft algae at site 3 is most impacted by eutrophication (Table 5). The low ATI value for the uppermost site results largely from the high abundance of *Phormidium diguetii* which is assigned a low trophic-indicator value for the ATI which indicates this taxon is most abundant at sites in Middle Tennessee which are not eutrophic (Grimmett and Lebkuecher 2017). The higher values for the ATI at the lower 3 sites is due largely to the high abundances of *Audouinella hermannii.* The highest value for the ATI at site 3 is due largely to the high abundance *Cladophora glomerata* (L.) Kütz. *Audouinella hermannii* and *Cladophora glomerata* are assigned trophic-indicator values for the ATI

which indicate these taxa are abundant at eutrophic sites in Middle Tennessee (Grimmett and Lebkuecher 2017).

Composition of diatom assemblages

The most abundant diatom taxa sampled (Table 6) was *Achnanthidium rivulare* Potapova & Ponander (10.4 %) due largely to its high abundance at sites other than site 3. The second most abundant diatom taxon was *Navicula minima* Grun. (7.6 %) common at all four sites. The third and fourth most abundant diatom taxa are *Cymbella affinis* Kütz. (6.8 %) due to its high abundance at the uppermost site, and *Achnanthidium minutissimum* (Kütz.) Czarn. common at all four sites. *Achnanthidium* are common in the southeastern United States (Ponader and Potapova 2007). The lower abundances of *Achnanthidium* at sites 3 and 4 are consistent with lower abundances in Middle Tennessee streams most impacted by nutrient enrichment (Grimmett and Lebkuecher 2007) and the characterization of this genus as less common in streams with poor quality water (KDOW 2002). The high abundance of *Achnanthidium deflexa* Reimer at site 1 relative to the other sites is consistent with the characterization of this taxon as an indicator of good quality water (KDOW 2002).

Metrics and indices for diatom assemblages

The 94 diatom taxa identified and the diatom taxa richness of the sites (Table 7) demonstrate the diatom assemblages studied are diverse. For example, Lebkuecher et al. (2015) identified 91 diatom taxa associated with cobble at four sites in Sulphur Fork Creek in the Red River Watershed in northern Middle Tennessee with richness ranging from 31 to 49. Grimmett and Lebkuecher (2017) identified 114 diatom taxa during the spring and summer at eight stream sites in Middle Tennessee with richness of the sites

ranging from 17 to 48. The values for the Shannon diversity index for diatom assemblages (Table 7) support the conclusions made from examining the Shannon diversity index values for the soft-algae assemblages at the same sites that diversity and evenness may not decrease with eutrophication. Values for the pollution tolerance index for diatom assemblages (PTI) at the sites studied are $\leq$ 2.6 (Table 7), which indicate eutrophic conditions (Lebkuecher et al. 2011). The greatest PTI value for the assemblage at site 1 is due largely from the abundance of *Achnanthidium* taxa (52 %) and *Cymbella affinis* (16.5 %) which are assigned pollution tolerance values of 3 or 4 (KDOW 2002). The lowest PTI value for the assemblage at site 3 is due largely from the low abundance of *Achnanthidium* taxa (8.3 %, Appendix 4) and the greatest abundance of *Navicula minima* (11.0 %), designated as an indicator of poor water quality (KDOW 2008). The low PTI values of the Harpeth River sites are similar to those of other stream sites in predominately agricultural and urban regions impaired by nutrient enrichment in Middle Tennessee. PTI values for stream sites in Middle Tennessee considered the most nutrient impaired such as a Jones Creek site located 5 km downstream of the Jones Creek Wastewater Treatment Plant near Dickson, Tennessee, a Sulphur Fork Creek site located 0.5 km downstream of the Springfield Waste Water Treatment Plant near Springfield, Tennessee, and a Suggs Creek site in Nashville, Tennessee range from 2.3 to 2.0 (Lebkuecher et al 2015, Grimmett and Lebkuecher 2017). PTI values for stream sites in Middle Tennessee considered reference sites with good water quality, such as those located in Buzzard Creek in the Red River Watershed, Hurricane Creek in the Lower Duck Watershed, and Flynn Creek in the Cordell Hull Watershed range from 2.8 to 3.0 (Lebkuecher et al. 2011, Grimmett and Lebkuecher 2017).

The values for the organic pollution index (OPI) > 20 for the diatom assemblages at sites 3 and 4 suggest these assemblages may be impacted by organic pollution. The higher OPI values for sites 3 and 4 are due largely to the greater abundance of *Nitzschia* and small *Navicula* taxa < 12 µm long (Appendix 4), many of which are tolerant of organic pollution (Kelly 1998). The OPI values for site 3 and site 4 are well below the threshold value of 40 which indicates severe impairment. Values well above 40 are common in reaches known to have very high concentrations of organics such as Elk Fork Creek in the Red River Watershed (Lebkuecher et al. 2011) and Jones Creek downstream of the Jones Creek Wastewater Treatment Plant near Dickson, Tennessee (Grimmett and Lebkuecher 2017).

Values for the siltation index (SI) for diatom assemblages at sites 3 and 4 and suggest these sites are impacted by siltation. For example, diatom assemblages in two morphologically similar watersheds in New Jersey with 1 % and 28 % agriculture had mean SI values of 18 $\pm$ 7 and 43 $\pm$ 4, respectively (Belton et al. 2005). SI values are most informative when comparing values from stream sites within the same ecoregion given the effects of soil erodibility and land use on the composition of diatom assemblages. SI values of six stream sites in the Red River Watershed in northern Middle Tennessee which has highly erodible soils and where > 60 % of the land is used for agriculture was 54 at the watershed's reference site and 78 at the site most impacted by siltation (Lebkuecher et al 2011).

CONCLUSIONS

This study documents the composition of soft-algae and diatom assemblages necessary to monitor the integrity of photoautotrophic periphyton in the upper and middle reaches of the Harpeth River. Superfluous biotic impairment by eutrophication of the river sites downstream of Franklin, Tennessee is demonstrated by the very high concentrations of total phosphorous of water samples and values for the algae trophic index for soft-algae assemblages and the pollution tolerance index for diatom assemblages. The results indicate that degradation of water quality as the Harpeth River flows through Franklin alters the composition of photoautotrophic periphyton and are consistent with an earlier study by Burkholder (2016) which indicates eutrophication by the Franklin Wastewater Treatment Plant negatively impacts the biotic integrity of the Harpeth River.

ACKNOWLEDGMENTS

The research was funded by the Harpeth Conservancy and the Department of Biology at Austin Peay State University.

Table 1. Concentrations of total phosphorus and total nitrogen of water samples and periphyton characteristics at river sites sampled. Means ± standard error for concentrations of chlorophyll *a*, ratios of optical density $(OD)_{664}$ to OD_{665} of pigment extracts, and ash-free dry mass of benthic organic matter represent four replicates and are not significantly different at the experiment-wise error rate of alpha = 0.05.

Characteristic/Site	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Total phosphorus ($\mu g \cdot L^{-1}$)	310	360	1035	515
Total N ($\mu g \cdot L^{-1}$)	687	1010	1434	868
TN:TP ratio	2.2	2.8	1.4	1.7
Chlorophyll *a* ($mg \cdot m^{-2}$)	135 ± 33	103 ± 11	151 ± 13	134 ± 47
Ratio of OD_{664} to OD_{665}	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.5 ± 0.0
Ash-free dry mass of benthic organic matter ($g \cdot m^{-2}$)	20.6 ± 5.7	15.1 ± 1.4	12.9 ± 1.6	20.3 ± 6.3

Table 2. Most abundant soft-algae taxa sampled. Numbers in parentheses represent percent composition.

Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
P. diguetii (29)	*A. hermannii* (22)	*A. hermannii* (19) *C. glomerata* (19)	*L. angustissimum* (24)
P. fragile Gomont (16)	*L. foveolarum* (25)	*Oedogonium* sp. (10)	*A. hermannii* (23)
L. foveolarum (11)	*P. diguetii* (13)	*G. cyanea* (9)	*L. foveolarum* (8)

Table 3. Percent composition of algae groups.

	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Bacillariophyceae (diatoms)	6.1	28.9	63.8	78.8
Soft algae	93.9	71.1	36.2	21.2
Cyanobacteria	91.2	53.3	16.4	2.7
Chlorophyta	2.5	2.1	11.8	12.2
Ochrophyta (other than diatoms)			0.9	1.5
Rhodophyta		15.6	7.0	4.8
Cryptophyta		0.1		

Euglenophyta	0.1		0.1	

Table 4. Percent similarity of soft-algae and diatom assemblages between sites.

Soft algae	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Site 1. River mile 106	47	15	17
Site 2. River mile 90.5		34	41
Site 3. River mile 80.0			39
Diatoms			
Site 1. River mile 106	58	32	45
Site 2. River mile 90.5		45	49
Site 3. River mile 80.0			56

Table 5. Metrics and indices for soft-algae assemblages.

	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Taxon richness	28	36	38	46
Genus richness	16	19	24	26
Shannon diversity index	2.4	2.5	2.7	2.6
Evenness	0.72	0.70	0.73	0.68
Algae trophic index	37	71	107	91

Table 6. Most abundant diatom taxa sampled. Numbers in parentheses represent percent composition.

Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
A. rivulare (16.5) *C. affinis* (16.5)	*A. rivulare* (15.0)	*N. cryptotenella* (8.6)	*N. minima* (11.4)
A. minutissimum (11.0)	*Psammothidum* sp. (7.4)	*M. varians* (5.9)	*A. rivulare* (7.6)
A. deflexa (8.0) *Navicula minima* (8.0)	*A. minutissimum* (7.3)	*N. minima* (5.4)	*N. cryptotenella* (5.2)

Table 7. Metrics and indices for diatom assemblages.

	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Taxon richness	36	48	52	52
Genus richness	18	22	22	19
Shannon diversity index	2.9	2.6	3.4	3.5
Evenness	0.80	0.83	0.87	0.89
Pollution tolerance index	2.64	2.55	2.20	2.41
Organic pollution index	14.0	18.6	23.4	24.8
Siltation index	21.5	33.3	48.2	48.1

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Appendix 1. Locations of sites sampled in the Harpeth River.

Site number	River mile	Location
Site 1 (uppermost site)	106	100 m up stream of McDaniel Road Bridge, McDaniel, TN.
Site 2	90.5	20 m downstream of Forest River Golf Course Bridge, Franklin, TN.
Site 3	80.0	100 m upstream of Cotton Lane Bridge, Franklin, TN.
Site 4 (lowermost site)	62.4	50 m upstream of Hwy 100 Bridge, Bellevue, TN.

Appendix 2. Morphological characteristics (mean ± SE) of reaches sampled in the Harpeth River.

Characteristic	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Discharge ($m^3 \cdot s^{-1}$)	0.2 ± 0.0	1.5 ± 0.2	3.3 ± 0.2	3.9 ± 0.3
Width (m)	9.8 ± 0.4	11.3 ± 0.2	15.7 ± 0.5	24.7 ± 0.7
Depth (m)	0.1 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
Velocity ($m \cdot s^{-1}$)	0.2 ± 0.0	0.4 ± 0.1	0.7 ± 0.0	0.5 ± 0.0
Benthic substrate < 64 mm (%)	7 ± 1	46 ± 5	80 ± 3	18 ± 3
Canopy angle (degrees)	135	90	70	50

Appendix 3. Percent composition of soft-algae taxa associated with cobbles listed in alphabetical order. Additional soft-algae taxa identified by multi-habitat sampling are listed as present (P).

	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Chlorophyta				
Characium ambiguum H. Jaeger				0.1
Chlamydomonas angulosa Dill				0.1
Chlamydomonas globosa Snow			0.3	0.1
Chlamydomonas gloeogama Korschikov				0.1
Chlamydomonas patellaria Whitford	0.1			0.5

Chlamydomonas sp.	0.1		0.3	0.4
Cladophora glomerata (L.) Kütz.	P	P	19.7	P
Closterium acerosum (Schrank) Ehrenb.			0.9	
Closterium ehrenbergii Menegh				0.1
Closterium leibleinni Kütz.		0.1		
Closterium moniliferum (Bory) Ehrenb.		0.2	0.1	
Closterium sp.			P	0.1
Coleochaete obicularis Pringsh				P
Cosmarium botrytis Menegh.			0.1	1.2
Entransia sp.				1.2
Gloeocystis vesiculosa Nägeli		0.5	1.3	1.0
Oedogonium sp.	2.4	P	9.5	2.4
Pandorina morum (Müller) Bory				0.1
Pediastrum simplex Meyen			0.1	
Rhizoclonium hieroglyphicum (C. Agardh) Kütz.	P			
Scenedesmus abundans (G. Kirchn.) Chodat	0.1			
Scenedesmus acuminatus (Lagerh.) Chodat		0.1		
Scenedesmus quadricauda (Turp.) Bréb.			0.4	
Selenastrum capricornutum Printz		0.1		
Spirogyra sp.		2.0		
Stigeoclonium tenue (C. A. Ag.) Kütz.				3.7
Tetraedron sp.				0.5
Ulothrix sp.				0.7
Ulothrix zonata (Weber & Mohr) Kütz.	P			
Cyanobacteria				
Aphanothece nidulans Richter			0.1	0.6
Aphanothece sp.				0.1
Arthrospira jenneri (Kütz.) Stitz.		0.2		
Borzia trilocularis Cohn.			0.6	
Calothrix sp.		0.5		0.4
Callothrix stellaris Bornet & Flahault				0.2
Chamaesiphon incrustans Grunrow		P	1.3	
Chroococcus minimus (Keissler) Lemmerm.				0.1
Chroococcus minor (Kütz.) Nägeli		0.3		0.1
Chroococcus minutus Kütz.		0.1		
Chroococcus pallidus Nägeli				0.1
Chroococcus turgidus (Kütz.) Nägeli	0.1			
Dactylococcopsis raphidioides Hansg.				0.1
Entophysalis rivularis Kuetz.			0.1	
Gloeocapsopsis cyanea (Krieg) Komárek & Anagn.	1.8	1.7	8.9	2.0

Gloeocapsopsis pleuroccapsoides (Novacek) Komárek & Anagn.	0.2			0.6
*Heteroleibleinia kossinskajae (*Elenkin*)* Anagn*.* & Komárek	0.1			
*Homeothrix juliana (*Bornet & Flahault) Kirchner	4.3	4.0		
Komvophoron constrictum (Szafer) Anagn. *&* Komarek		0.6	0.6	
Komvophoron munitum (Skuja) Anagn. & Komarek		0.3		1.6
Komvophoron schmidlei (Jaag.) Anagn. & Komárek		3.3		3.0
Leibeinia sp.		P		
*Leptolyngbya angustissimum (*West and West) Anagn. & Komárek			2.5	24.4
Leptolyngbya foveolarum (Mont.) Anagn. & Komárek	10.5	25.4	1.3	8.2
Leptolyngbya sp.		0.6		
Lyngbya major Menegh.	3.0	2.4		
Lyngbya martensiana Menegh.	4.4	2.5	3.2	
Merismopedia punctata Meyen	0.1			
Microcystis incerta Lemmerm.	0.1	0.1	1.5	1.1
Microcystis sp.				0.4
Oscillatoria agardhii Gomont		1.9	2.5	
Oscillatoria rubescens DeCandoll		3.0		
Oscillatoria sp.	0.6	1.3	1.4	4.8
Oscillatoria subbrevis Schmidle	1.3	0.8	1.5	0.5
Oscillatoria subtilissima Kütz. & De Toni		1.2	0.6	
Phormidium articulatum (Gardner) Anagn. & Komárek	3.2	2.1	0.8	1.7
Phormidium autumnale Gomont	3.0			
Phormidium diguetii (Gomont) Anagn. & Komárek	28.8	12.9		0.2
*Phormidium formosum (*Bory) Anagn. & Komárek	2.4	2.0		
Phormidium fragile Gomont	16.1	5.1	1.1	
Phormidium indundatum Kütz			0.9	
Phormidium retzii (C. Agardh) Gomont	10.8	1.0		
Phormidium sp.	0.6	1.0	5.7	2.7
Phormidium tenue (C. Agardh & Gomont) Anagn. & Komárek	2.5		1.6	
Phormidium terebriforme (C. Agardh & Gomont) Anagn. & Komárek	1.7	0.4	8.2	0.2
Spirulina major Kütz.				1.8
Spirulina nordstedtii Gomont				1.8

Spirulina princeps (W. West and G.S. West) G. S. West	1.2			
Spirulina sp.		0.3		
Spirulina temerrima Kutz.		0.2		
Synechococcus aeruginosus Nägeli	0.2		0.3	0.4
Synechococcus sp.			0.1	
Synechocystis sp.			0.1	0.2
Cryptophyta				
Chilomonas sp.		0.1		
Chroomonas sp.				0.1
Dinophyta				
Ceratium hirundinella (O.F.M.) Schrank				P
Euglenophyta				
Euglena minuta Prescott			0.1	
Euglena proxima P.J.L. Dang.	0.1			
Euglena sp.			0.1	
Phacus sp.			0.1	
Ochrophyta				
Vaucheria sp.			2.5	7.2
Rhodophyta				
Audouinella hermannii (Roth) Duby		22.0	19.3	22.7
Compsopogon coeruleus (Balbis) Montagne			P	
Cercozoa				
Paulinella chromatophora Lauterborn		P		

Appendix 4. Percent composition of diatom taxa associated with cobbles listed in alphabetical order.

	Site 1. River mile 106	Site 2. River mile 90.5	Site 3. River mile 80.0	Site 4. River mile 62.4
Achnanthes pinnata Hust.	2.0	0.5	0.5	
Achnanthidium deflexa Reimer	8.0		0.5	
Achnanthidium eutrophilum Lange-Bert.				0.5
Achnanthidium exiguum var. *constrictum* (Grun.) Anderson				0.5
Achnanthidium latecephalum Kobayasi			0.5	0.5
Achnanthidium minutissimum (Kütz.) Czarn.	11.0	7.3	1.8	5.7

Achnanthidium rivulare Potapova & Ponander	16.5	15.0	2.3	7.6
Achnanthidium sp.	4.0	1.9	2.7	3.3
Amphora minutissima W. Sm.	0.5		0.5	
Amphora perpusilla Grun.	2.0	4.4	3.2	3.8
Amphora sp.		1.0		
Amphora veneta Kütz.		1.0		
Bacillaria paradoxa Gmelin	1.5	1.5	2.3	4.8
Cocconeis pediculus Ehrenb.			0.5	1.4
Cocconeis placentula Ehrenb.	3.0	4.8	4.5	4.8
Cocconeis placentula var. *euglypta* Ehrenb.				1.0
Craticula halophila (Grun.) G. D. Mann		0.5		
Cyclotella meneghiniana Kütz.			0.5	1.0
Cymatopleura elliptica (Bréb.)W. Sm.	0.5			
Cymatopleura solea (Bréb. & Godey) W. Sm.				0.5
Cymbella affinis Kütz.	16.5	5.8	3.6	1.4
Cymbella sp.			0.5	
Cymbella tumida (Bréb.) Van Heurck			0.9	
Diatoma vulgaris Bory				0.5
Encyonema appalachianum Potapova	4.5	1.9	5.0	2.4
Encyonema prostratum (Berk.) Kütz.			0.5	
Gomphoneis olivacea (Horn.) Daws.			0.9	0.5
Gomphonema brasiliense Grun.	0.5			
Gomphonema minutum Ag.		0.5		1.0
Gomphonema parvulum (Kütz.) Kütz.	0.5		0.5	1.4
Gomphonema pumilum (Grun.) Reich. & Lange-Bert.				0.5
Gomphonema sp.			0.5	0.5
Gyrosigma acuminatum (Kütz.) Rabenh.	0.5	1.5	1.8	2.4
Gyrosigma obtusatum (Sull. & Wormley) Boyer				0.5
Gyrosigma scalproides (Rabenh.) Cleve		0.5	1.8	1.0
Karayeva clevei (Grun.)				
Karayeva clevei var. *rostrata* Hust.		0.5		
Luticola goeppertiana (Bleish) D.G. Mann		0.5	0.5	
Melosira varians Ag.			5.9	
Navicula atomus (Kütz.) Grun.	1.0			
Navicula capitatoradiata Germ.	2.0	1.9	0.9	1.9
Navicula cari Ehrenb.			0.5	
Navicula cryptocephala Kutz.			0.5	
Navicula cryptotenella Lange-Bert.			8.6	5.2
Navicula decussis Østrup	0.5	0.5		
Navicula gregaria Donk.		1.0	0.5	

Navicula lanceolata (Ag.) Ehrenb.		0.5		0.5
Navicula menisculus Schum.	0.5		2.7	1.4
Navicula menisculus var*. upsaliensis* (Grun.) Grun.		0.5		
Navicula minima Grun.	8.0	5.4	5.4	11.4
Navicula reichardtiana Lange-Bert.	0.5	1.9	0.9	
Navicula reinhardii Grun.	0.5		0.5	
Navicula rhynchocephala Kütz.	0.5	1.9	0.9	
Navicula sp. (< 12 µm length)		1.0	4.5	2.9
Navicula sp. (> 12 µm length)		1.5	5.0	2.9
Navicula subminuscula Mang.		1.0		
Navicula subrotundata Hust.				0.5
Navicula symmetrica Patr.		0.5	0.5	
Navicula tenelloides Hust.				0.5
Navicula veneta Kütz.			0.9	1.0
Navicula viridula (Kütz.) Ehrenb.	1.0	1.0	3.6	0.5
Navicula viridula var. *linearis* Hust.				1.4
Neidium alpinum Hust.				0.5
Nitzschia acicularis (Kütz.) W. Sm.		3.4		
Nitzschia amphibia Grun.				2.4
Nitzschia capitellata Hust.	1.0	1.0		
Nitzschia constricta (Kütz.)			0.9	1.0
Nitzschia disputata (Kütz.)		0.5		
Nitzschia dissipata (Kütz.) Grun.		1.5	0.5	1.9
Nitzschia dissipata var. *media* (Hantz.) Grun.				0.5
Nitzschia flexa Schum.	0.5		0.9	1.0
Nitzschia frustulum (Kütz.) Grun.	0.5		0.5	
Nitzschia inconspicua Grun.			0.5	1.4
Nitzschia linearis (Ag.) W. Sm.		0.5	0.9	
Nitzschia microcephala Grun.				0.5
Nitzschia minuta Bleisch		0.5		
Nitzschia palea (Kütz.) W. Sm.	2.0	1.0		2.0
Nitzschia sociabilis Hust.			5.0	
Nitzschia sp.	1.0	2.4	1.8	2.0
Nitzschia sublinearis Hust		0.5		0.5
Pinnularia sp.	1.0			
Planothidium lanceolatum var*. dubia* Grun.		0.5	0.5	1.0
Psammothidium curtissimum (Carter) Aboal	4.0	6.9		
Psammothidium sp*.*		7.4	0.5	
Reimeria sinuata (Greg.) Kociolek & Stoermer		1.0	0.9	
Rhoicosphenia curvata (Kütz.) Grun.		1.5	3.6	2.9

Sellaphora seminulum (Grun.) D. G. Mann.	2.5	1.5	1.8	5.0
Stephanodiscus parvus Stoermer & Hakansson	0.5			
Stephanodiscus sp.		0.5		
Surirella brebissonii Lange-Bert. & Krammer		0.5		0.5
Surirella linearis W. Sm.	0.5			
Surirella ovalis Breb.	0.5			
Surirella ovata var. *pinnata* (W. Sm.) Brun.	0.5	1.9	0.5	
Synedra rumpens Kütz.				0.5
Synedra ulna (Nitz.) Ehrenb.			0.5	