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Photoautotrophic-Periphyton Composition in Reaches with Differing Nutrient Concentrations in the Harpeth River of Middle Tennessee

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ABSTRACT Four sites of the Harpeth River, two upstream and two downstream of the Franklin Wastewater Treatment Facility in Franklin, Tennessee, were sampled to assess the impact of nutrient enrichment on the integrity of photoautotrophic periphyton. Concentrations of total phosphorus of water samples ranged from 310 $\mu g \cdot L^{-1}$ at the uppermost site to 1035 $\mu g \cdot L^{-1}$ at the site immediately downstream of the wastewater treatment facility. Concentrations of total nitrogen of water samples ranged from 687 $\mu g \cdot L^{-1}$ at the uppermost site to 1,434 $\mu g \cdot L^{-1}$ at the site immediately downstream of the wastewater treatment facility. Concentrations of benthic chlorophyll *a* did not differ significantly among the sites and ranged from 103 $\pm$ 11 $mg \cdot m^{-2}$ at the site immediately upstream of the wastewater treatment facility to 151 $\pm$ 13 $mg \cdot m^{-2}$ at the site immediately downstream. Percent composition of 186 algae taxa were documented: 92 taxa of soft-bodied algae and 94 taxa of diatoms. Values for the algae trophic index for soft-bodied algal assemblages increased from 71 at the site immediately upstream of the wastewater treatment facility to 107 at the site immediately downstream. Values for the pollution tolerance index for diatom assemblages decreased from 2.55 at the site immediately upstream of the wastewater treatment facility to 2.20 at the site immediately downstream. These index values demonstrate that the assemblages of soft-bodied algae and diatoms immediately downstream the wastewater treatment facility had a greater relative abundance of taxa tolerant of eutrophic conditions compared to the assemblages at the site immediately upstream.

Key words: Algae, chlorophyll *a*, diatoms, Harpeth River, soft-bodied algae, trophic state.

INTRODUCTION 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 periphyton habitat quality (Stancheva and Sheath 2016). The composition of algae assemblages of the majority of streams in the Interior Plateau Level III Ecoregion that extends from southern Indiana and Ohio to northern Alabama is unknown (Grimmett and Lebkuecher 2017). This lack of knowledge limits the ability of watershed managers to monitor changes of habitat quality. This work documents the composition of algal assemblages as they are used to assess the effects of water quality in the upper and middle reaches of the Harpeth River in Middle Tennessee.

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Common methods to evaluate the impact of nutrient enrichment of shallow lotic systems include measurements of the concentrations of benthic chlorophyll *a* (chl *a*), ash-free dry mass of benthic organics (AFDM), nutrient concentrations in water, and organism composition. 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, volume of submerged wood, and time period

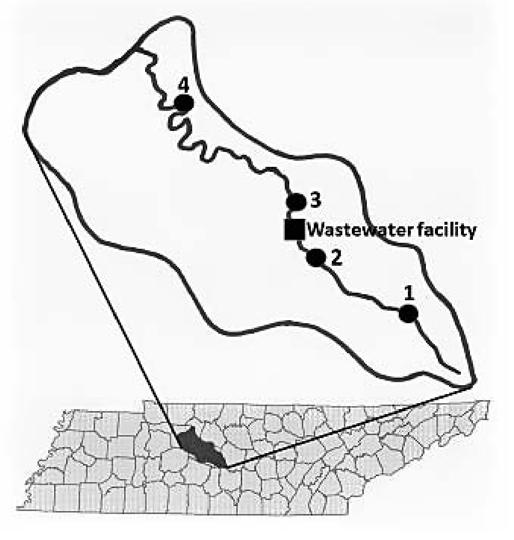

Figure 1. Location of Harpeth River Watershed (black area) in Tennessee, location of the Franklin Wastewater Treatment Facility (square), and locations of the four sampling sites (circles). Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

between spates of high discharge (Lebkuecher et al. 1998, Kurle and Cardinale 2011). Ash-free dry mass of benthic organics may be influenced by the same factors that influence the concentration of chl *a*. In addition, AFDM is affected by organic inputs that differ by season, streambank characteristics, and allochthonous inputs (Lebkuecher et al. 2015). 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). Periphyton composition is often a more accurate indicator of trophic state relative to biomass and nutrient concentrations partially because of rapid, yet sustained changes of composition as a result of changes of habitat quality.

Bioassessments using algal assemblages are most useful for management practices because they reflect a wide range of anthropogenic impacts (Rimet 2012). Several states including Kentucky, Montana, Oklahoma, 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 objectives of this study were to document the richness, composition, and biomass of algae in the Harpeth River at two sites upstream and at two sites downstream of the Franklin Wastewater Treatment Facility to assess the impact of nutrient enrichment on the algal assemblages.

METHODS

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 and includes some chert, shale, siltstone, sandstone, and dolomite (Griffith et al. 1997). Mesophytic forests (including *Quercus* and *Carya* species) surround the watershed, much of which has been converted to row crops and cleared for livestock (Tennessee Department of Environment and Conservation [TDEC] 2017). Most of the watershed is within the Outer and Inner Nashville Basin Level IV Ecoregions and consists of surface waters with naturally high concentrations of phosphorus (P) due to the high P concentrations of the carbonate (TDEC 2005). The Harpeth River flows northwest 185 km from its source near Eagleville, Tennessee in rural Middle Tennessee and 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.

Sampling Site Locations and Dates

Four sites were sampled in the Harpeth River on September 30, 2017 (Figure 1): two sites upstream and two sites downstream of the Franklin Wastewater Treatment Facility in downtown Franklin (Appendix 1). The uppermost site (Site 1) is located approximately 17 km upstream of Franklin in a rural, agricultural region. Site 2 is located approximately 4 km upstream of downtown Franklin in a densely populated neighborhood. Effluent from the Franklin Wastewater Treatment Facility enters the river at river near downtown Franklin approximately 5 km downstream of Site 2 and approximately 5 km upstream of Site 3. Site 3 is located approximately 6 km downstream of downtown Franklin in a densely populated neighborhood. Site 4 is located approximately 16 km downstream of Franklin in an area with a mix of neighborhoods and agriculture.

Field Methods

Reach morphological characteristics. Two transects that spanned the stream perpendicular to the direction of flow 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 and discharge were determined as described by Lebkuecher et al. (2015) using the equations of Robins and Crawford (1954). The percent of benthic substrates smaller than very coarse gravel (32 mm diameter) 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 as an indicator of canopy cover. Reach morphological characteristics were determined to provide a depiction of the abiotic characteristics of the reaches sampled (Appendix 2).

Sampling cobbles to determine periphyton characteristics. Cobble sampling occurred in runs with velocities between 0.1 m s^{-1} and 0.3 $m \cdot s^{-1}$ at depths between 0.1 m and 0.25 m. Four plots in each of the four river sites were established with 0.25 m^2 wire frames placed approximately 1.25 m apart at midstream. Two cobbles nearest to the plot center between 12-cm and 18-cm diameter were removed. If a plot did not contain two cobbles appropriate for sampling, cobbles closest to the plot were removed. One cobble from each plot was used to determine the percent composition of soft-algae and diatom taxa. Algae were removed from cobbles in the field using a single-edge razor blade and scrub brush. Algae removed from the four cobbles per site were combined, preserved in 1% glutaraldehyde adjusted to pH 7.0 with NaOH. One cobble from each plot was used to determine pigment concentrations of algae and AFDM. These four cobbles were placed in self-sealable plastic bags and transported to the lab on ice in darkness. There was no effort to control for site environmental cofactors that can modulate algal growth.

Multi-habitat sampling of soft-bodied algae. A multi-habitat sampling technique was employed to identify additional soft-bodied algal taxa not found associated with the cobbles sampled to determine percent composition. Diatoms were not identified from multi-habitat samples due to time constraints and because much more is known about the richness of diatoms relative to soft-bodied algae in Tennessee streams (Lebkuecher et al. 2015). Multi-habitat samples were not collected using a plot system nor were the additional soft-bodied taxa found enumerated. Multi-habitat 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 pipette 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.

Laboratory Methods

Periphyton pigment concentrations and ash-free dry mass. Methods to determine periphyton pigment concentrations and AFDM followed those described in Lebkuecher et al. (2015) and Grimmett and Lebkuecher (2017). One cobble was placed in a glass pan containing 0.1 L of 90% acetone and the periphyton was 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 chl *a*, then at 665 nm following acidification with 0.1 N HCl to determine the concentration of pheophytin *a*. Optical density determinations occurred within minutes of acetone extraction and in very low light to ensure pheophytin was not degraded. Concentrations of chl *a* corrected for pheophytin *a* were calculated using the equations of Lorenzen (1967). The chl *a* to pheophytin *a* ratio was indicated as the ratio of OD_{664} to OD_{665} (Baird et al. 2017).

Ash-free dry mass of organic benthic matter was determined following evaporation of the acetone at 25°C. During the drying process, periphyton was transferred to beakers, then to the same crucibles in which weights were measured to ensure no loss of organic matter. Ash-free dry mass of organic benthic matter and inorganic sediment weights were determined as described by Baird et al. (2017). Values for AFDM 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).

Statistical methods. Means for concentrations of chl *a*, optical density (OD) ratios of OD_{664} to OD_{665} of pigment extracts, and AFDM were compared using Tukey-Kramer honestly significant difference tests preceded by analysis of variance tests. The data were homoscedastic and normal. Assay means were considered significantly different if they differed at the experiment-wise error rate of $\alpha = 0.05$.

Identification of soft-bodied algal taxa. Large filamentous algae were cut with scissors such that well-mixed aliquots of filamentous-algae sections and microalgae in 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). Macroalgae were not separated from microalgae. Soft algae within a 2-mm^2 square were observed at 100×, 400×, and 1000× 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 as recommended by Stevenson and Bahls (2006). Taxa were enumerated until at least 800 units counted, or for samples with very little soft-bodied algae relative to diatoms, until at least 20 wet mounts were observed. Wet mounts from samples collected by multi-habitat sampling were searched using 100×, 400×, and 1000× magnification until no new taxa were observed in at least five consecutive wet mounts. Taxa identified from multi-habitat samples were recorded as present and thus were not compared to taxa sampled from cobbles to determine relative abundance. 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 overall soft-bodied algal units and overall diatom units at each site was estimated by counting the number of soft-bodied algae units and diatom units in 2-mm squares of the ruled microscope slide until at least 800 units were counted.

Identification of diatom taxa. Frustule preparation for permanent mounts followed the methods of Carr et al. (1986). Organic debris and intracellular material were completely 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. Valve ornamentations were clearly visible using 1000× magnification. All valves in the field of view at 1000× 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 2008). Primary taxonomic references used to identify diatom taxa included Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b), and Ponader and Potapova (2007). The permanent mounts are maintained in the Austin Peay State University Herbarium in Clarksville, Tennessee.

Calculation of metrics and indices. Shannon diversity index (H′) and evenness (J) of soft-algae and diatom assemblages were calculated to provide information about assemblage structures by the equations of Shannon and Weaver (1949):

$$H' = -\Sigma(P_i \ln P_i); \quad (1)$$

$$J = H'/\ln S, \quad (2)$$

where P_i = abundance of species i, and S = richness (number of taxa). Pairwise percent similarities of diatom and soft-algae assemblages associated with cobble were calculated as the sum of the smaller of the two percent-composition values for all taxa common to two sites (Whittaker and Fairbanks 1958). A percent-similarity value of 0 indicates that the two assemblages compared do not have a single taxon in common. A percent-similarity value of 100 indicates that the two assemblages compared have the exact same percent composition of taxa.

The pollution tolerance index (PTI) for diatom assemblages (KDOW 2008) was calculated as:

$$\mathrm{PTI} = \left[\Sigma_{j=1\mathrm{sp.}} n_j \; t_j\right]/N, \tag{3}$$

where n_j = number of individuals of taxon j, t_j = eutrophication-tolerance value (one to four; KDOW 2008) of taxon j, and N = total number of individuals assigned a eutrophication-tolerance value and tallied to calculate the index. The PTI ranges from one (all taxa very tolerant to eutrophic conditions) to four (all taxa very intolerant of eutrophic conditions). A value for the PTI < 2.6 indicates an assemblage is impaired by eutrophic conditions as determined by comparisons of PTI values of stream sites with different trophic states in Middle Tennessee (Lebkuecher et al. 2011).

The organic pollution index (OPI) is the percentage of diatoms tolerant of organic pollution listed in Kelly (1998). Organic pollution index values of >20 imply organic pollution impacts the composition of diatom assemblages and values >40 imply the habitat is severely impaired by excessive concentration of organic matter (Kelly 1998). The siltation index (SI) is the sum of *Navicula*, *Nitzschia*, and *Surirella*, and the taxa formerly identified as *Navicula*, *Nitzschia*, and *Surirella* divided by the total number of diatoms and multiplied by 100 (Bahls 1993). Motile diatoms other than *Navicula sensu lato*, *Nitzschia senu lato*, and *Surirella* are not used to calculate the SI (Bahls 1993). 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:

$$\mathrm{ATI} = \left[\Sigma_{j=1\ \mathrm{taxon}} n_j \; ti_j\right]/N, \tag{4}$$

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 (Grimmett and Lebkuecher 2017). The trophic-indicator values are the abundance-weighted average of the concentration of benthic chl *a*, listed in Grimmett and Lebkuecher (2017). Taxa not identified to species were excluded from index calculations and included less than 10% of the algae observed. The algae trophic index of soft-algae assemblages values for stream reaches studied by Grimmett and Lebkuecher (2017) were very similar to and significantly correlated with concentrations of benthic chl *a* ($\mathrm{mg{\cdot}m^{-2}}$). Therefore, ATI values correspond with Dodds et al. (1998) suggested classification of temperate streams with chl *a* concentrations $<20\ \mathrm{mg{\cdot}m^{-2}}$ as oligotrophic and concentrations $>70\ \mathrm{mg{\cdot}m^{-2}}$ as eutrophic.

Concentrations of total phosphorus and total nitrogen of water samples. Nutrient concentrations of water samples collected approximately 5 cm below the surface were determined using a Lachat QuickChem 8500 Flow Injection Analyzer (Lachat Instruments, Loveland, Colorado). Concentrations of total phosphorus (TP) were determined using the persulfate-digestion and the ascorbic-acid method (Baird et al. 2017). Concentrations of total nitrogen (TN) were determined by the persulfate-digestion and cadmium-reduction method (Baird et al. 2017). Concentrations of TP and TN were determined because Dodds (2003) suggested concentrations of TP and TN are stronger indicators of trophic state relative to the concentrations of dissolved forms of N and P. In addition, concentrations of TP and TN are used to designate the trophic state of streams by Dodds et al. (1998).

RESULTS

Nutrient Concentrations and Periphyton Biomass

Water samples from all four sites contained eutrophic concentrations of TP and ranged from $310\ \mu\mathrm{g{\cdot}L^{-1}}$ at the uppermost site to $1035\ \mu\mathrm{g{\cdot}L^{-1}}$ at the site immediately downstream of the wastewater treatment facility (Table 1). Concentrations of total nitrogen (TN) were in or near the range considered mesotrophic for streams and ranged from $687\ \mu\mathrm{g{\cdot}L^{-1}}$ at the uppermost site to $1434\ \mu\mathrm{g{\cdot}L^{-1}}$ at site three immediately downstream of the wastewater treatment facility. The TN:TP ratios were lowest at the two sites downstream of the Franklin Wastewater Treat-

Table 1. Concentrations of total phosphorus (TP) and total nitrogen (TN) of water samples and periphyton characteristics at sites sampled in the Harpeth River. Means ± standard error for concentrations of chlorophyll *a*, optical density (OD) ratios of 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 $\alpha = 0.05$. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

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 Nitrogen ($\mu g \cdot L^{-1}$)	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

ment Facility as a result of the very high concentrations of TP at these sites.

Concentrations of benthic chlorophyll chl *a* (Table 1) were all >103 $mg \cdot m^{-2}$ and did not differ significantly. None of the optical density (OD) ratios of OD_{664} to OD_{665} of the pigment extracts were <1.5, a threshold value used to indicate the algae were in poor health or senescent (Baird et al. 2017). The concentrations of AFDM were all were >10 $g \cdot m^{-2}$ and did not differ significantly.

Composition of Algal Assemblages

We identified 186 taxa of algae: 92 taxa of soft-bodied algae (Appendix 3) and 94 taxa of diatoms (Appendix 4). The most abundant soft-bodied algae sampled (Table 2) was the filamentous Rhodophyta *Audouinella hermannii* (Roth) Duby (16.0%) followed by the filamentous cyanobacterium *Leptolyngbya foveolarum* (Mont.) Anagn. and Komárek (11.4%). The most abundant diatom sampled (Table 3) was *Achnanthidium rivulare* Potapova and Ponander (10.4%) followed by *Navicula minima* Grun. (7.6%).

Percent composition of algae groups such as diatoms, cyanobacteria, and Chlorophyta differed dramatically between sites (Table 4). Cyanobacteria were the most abundant at the uppermost site and diatoms were most abundant at the lowermost site. Both the soft-bodied algal and diatom assemblages were distinct across sites (Table 5). The most similar assemblages of soft-bodied algae and diatoms were the assemblages at the two uppermost sites, the two sites with the lowest and most similar concentrations of TP of water samples (Table 1). The most dissimilar assemblages of soft-bodied algae and diatoms were those at the sites with the greatest differences in concentrations of TP, the uppermost site and the site immediately downstream of the wastewater facility the sites.

Metrics and Indices for Algae Assemblages

Richness of soft-bodied algal taxa increased downstream (Table 6). The similar values for the Shannon diversity index for soft-algae assemblages among sites were due partially to similar evenness. The highest value for the algae trophic index (ATI) for the assemblage of soft-bodied algae at site three indicates that this

Table 2. Most abundant soft-bodied algal taxa at sites sampled in the Harpeth River. Numbers in parentheses represent percent composition. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

Site 1	Site 2	Site 3	Site 4
Phormidium diguetii (29)	*Leptolyngba foveolarum* (25)	*Audouinella hermannii* (19)	*Leptolyngbya angustissimum* (24)
Phormidium fragile (16)	*Audouinella hermannii* (22)	*Cladophora glomerata* (19)	*Audouinella hermannii* (23)
Leptolyngbya foveolarum (11)	*Phormidium diguetii* (13)	*Oedogonium* sp. (10)	*Leptolyngbya foveolarum* (8)

Table 3. Most abundant diatom taxa at sites sampled in the Harpeth River. Numbers in parentheses represent percent composition. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

Site 1	Site 2	Site 3	Site 4
Achnanthidium rivulare (16.5)	*Achnanthidium rivulare* (15.0)	*Navicula cryptotenella* (8.6)	*Navicula minima* (11.4)
Cymbella affinis (16.5)	*Psammothidium* sp. (7.4)	*Melosira varians* (5.9)	*Achnanthidium rivulare* (7.6)
Achnanthidium minutissimum (11.0)	*Achnanthidium minutissimum* (7.3)	*Navicula minima* (5.4)	*Navicula cryptotenella* (5.2)

assemblage was most impacted by eutrophication.

Greater values for the Shannon diversity index for the diatom assemblages (Table 7) relative to the soft-algae assemblages at the same sites is due largely to greater the evenness of the diatom assemblages. Values for the pollution tolerance index for diatom assemblages (PTI) at the all the sites are <2.6, which indicate eutrophic conditions. Values for the organic pollution index (OPI) > 20 and the siltation index (SI) > 40 for the diatom assemblages at Sites 3 and 4 were above the threshold values used to designate assemblages as impacted by organic pollution and siltation, respectively.

DISCUSSION The high concentrations of benthic chl *a* at all four sites indicate eutrophic conditions based on Dodds et al. (1998) classification of temperate streams with chl *a* concentrations >70 $mg \cdot m^{-2}$ as eutrophic. The concentrations of AFDM at all four sites also indicate eutrophic conditions. All sites had AFDM values >10 $g \cdot m^{-2}$, a threshold value indicative of eutrophic environments based on earlier studies (O'Brien and Wehr 2010, Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017). The eutrophic concentrations of TP at the sites upstream of the Franklin Wastewater Treatment Facility likely reflect the heavy anthropogenic activities in the watershed and naturally high concentrations of P in the limestone bedrock (TDEC 2005). Concentrations of approximately 180 $\mu g\ TP \cdot L^{-1}$ are suggested to be a more realistic expectation of moderate levels of TP of surface waters in the Nashville Basin (TDEC 2005) as opposed to the boundary value of <75 $\mu g\ TP \cdot L^{-1}$ suggested by Dodds et al. (1998) to designate lotic systems as mesotrophic.

The TN:TP ratios at all four sites are well below TN:TP ratios hypothesized to indicate potential N limitation of algae growth. For example, N may be considered limiting when the TN:TP ratio is <10, and by P when the TN:TP ratio is >17 (Dodds 2003). The low TN:TP ratios may have precluded the potential for significant differences of concentrations of benthic chl *a* and AFDM among sites upstream and downstream of the wastewater treatment facility. Earlier studies of determinations of chl *a* from four cobbles per stream site were adequate to demonstrate significant increases of chl *a* at sites immediately downstream of wastewater

Table 4. Percent composition of algae groups at sites sampled in the Harpeth River. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

	Site 1	Site 2	Site 3	Site 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	0	0.9	1.5
Rhodophyta	0	15.6	7.0	4.8
Cryptophyta	0	0.1	0	0
Euglenophyta	0.1	0	0.1	0

Table 5. Percent similarity of soft-bodied algae and diatom assemblages between sites sampled in the Harpeth River. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

	Site 2	Site 3	Site 4
Soft-bodied algae			
Site 1	47	15	17
Site 2	—	34	41
Site 3		—	39
Diatoms			
Site 1	58	32	45
Site 2	—	45	49
Site 3		—	56

Table 6. Metrics and indices for soft-bodied algal assemblages at sites sampled in the Harpeth River. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

	Site 1	Site 2	Site 3	Site 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

treatment plants just outside the Nashville Basin (Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017). However, we acknowledge that four cobbles per sampling site is a relatively small number to estimate chl-*a* concentration given the spatial heterogeneity of small rivers and may have contributed to the absence of significant differences. In addition, environmental factors such as canopy angle and river morphological characteristics influence periphyton biomass.

High values for the algae trophic index (ATI) for soft-bodied algal assemblages and low values for the pollution tolerance index for diatom assemblages at the sites downstream of the Franklin Wastewater Treatment Facility indicate that additional nutrient enrichment of the Harpeth River as the river flows through Franklin impacts the structure of these assemblages. These index values demonstrate that the assemblages of soft-bodied algae and diatoms sampled downstream of the wastewater treatment facility have a greater relative abundance of taxa tolerant of eutrophic conditions. The higher values for the ATI at the lower three sites were due largely to the high abundances of *Audouinella hermannii*. The highest value for the ATI at site three was due largely to the high abundance of *Cladophora glomerata* (L.) Kütz. *Audouinella hermannii* and *C. glomerata* are assigned high trophic-indicator values for the ATI which indicate these taxa are abundant at eutrophic sites in Middle Tennessee (Grimmett and Lebkuecher 2017).

The high value for the pollution tolerance index (PTI) for diatom assemblages for the assemblage at the uppermost site is due largely from the abundance of *Achnanthidium* taxa (52%) and *Cymbella affinis* (16.5%). Both of these taxa are assigned eutrophication-tolerance values of three or four, given they are common in oligotrophic and mesotrophic lotic systems (KDOW 2008). The lowest PTI value for the assemblage at the site immediately below the wastewater treatment facility is due largely from the low abundance of *Achnanthidium* (8.3%; Appendix 4) and the greatest abundance of *Navicula minima* (11.0%). *Navicula minima* is an indicator of poor quality water and is assigned a eutrophication-tolerance value of 1.0 (KDOW 2008).

The PTI values $\leq$2.6 for the diatom assemblages 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. The PTI values for stream sites in Middle Tennessee considered some of the most nutrient impaired range from 2.3 to 2.0, whereas PTI values for stream sites in Middle Tennessee considered reference sites with good water quality range from 2.8 to 3.0 (Lebkuecher et al. 2011, Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017). The organic pollution index (OPI) values

Table 7. Metrics and indices for diatom assemblages at sites sampled in the Harpeth River. Sites 1 and 2 are upstream and Sites 3 and 4 are downstream of the Franklin Wastewater Treatment Facility.

	Site 1	Site 2	Site 3	Site 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

>20 for the diatom assemblages at the sites downstream of downtown Franklin suggest these assemblages may be impacted by organic pollution, yet are well below the threshold value of 40, which indicates severe impairment. Values well above 40 are common in reaches with very high concentrations of organics, such as Elk Fork Creek in the Red River Watershed in northern Middle Tennessee (Lebkuecher et al. 2011) and Jones Creek downstream of the Jones Creek Wastewater Treatment Plant near Dickson, Tennessee (Grimmett and Lebkuecher 2017).

The apparent impact on the diatom assemblages by siltation at the two lowermost sites sampled in the Harpeth River is typical for middle and lower reaches of lotic systems in agricultural regions. For example, diatom assemblages in two streams in morphologically similar watersheds in New Jersey with 1% and 28% agriculture had mean siltation index values of 18 ± 7 and 43 ± 4, respectively (Belton et al. 2005). Siltation index values are most informative when comparing values from stream sites with similar regional conditions given that soil erodibility and land use affects the composition of diatom assemblages. Siltation index 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, were 54 at the watershed's reference site and 78 at the site most impacted by siltation (Lebkuecher et al. 2011).

We do not know the reason why the uppermost site was dominated by cyanobacteria and the lowermost site was dominated by diatoms. 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 lower abundance cyanobacteria and the greater abundance of Chlorophyta at the sites downstream of downtown Franklin sites relative to the sites upstream are consistent with earlier studies that demonstrated cyanobacteria biomass and diversity were lower at river sites with higher concentrations of soluble reactive phosphorus of water samples in central Spain (Perona et al. 1998, Douterelo et al. 2004). Similar lower abundances of cyanobacteria relative to Chlorophyta at sites with higher concentrations of nutrients occur in other Middle Tennessee streams (Grimmett and Lebkuecher 2017).

The greater number of the same taxa among the diatom assemblages relative to the soft-algae assemblages at the sites studied in 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 diatom assemblages between sites sampled in August was far more consistent, ranging from 44% to 66%, relative to the similarity of soft-bodied algal assemblages, which ranged from 16% to 57%. In a different study of eight sites in eight streams in Middle Tennessee, percent similarity for diatom assemblages between sites sampled in August ranged from 25% to 67% and for soft-bodied algal assemblages, from 2% to 39% (Grimmett and Lebkuecher 2017).

Noteworthy and unexpected taxa identified in the Harpeth River by this study include *Chilomonas* sp., a nonphotosynthetic cryptomonad, *Chroomonas* sp., a photosynthetic cryptomonad, and *Paulinella chromatophora* Lauterborn, a filose thecamoeba. Cryptomonads are much more common in oligotrophic lentic water, although two sp. of *Cryptomonas* were documented in Middle Tennessee streams with relatively good quality water (Lebkuecher et al. 2015, Grimmett and Lebkuecher 2017). *Paulinella chromatophora* was not known to occur in the southeastern United States, possesses primitive, cyanobacteria–like plastids, and is frequently referenced as evidence for the endosymbiotic origin of plastids (Graham et al. 2016). 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).

CONCLUSIONS This study documents the composition of soft-algal and diatom assemblages needed to help monitor the integrity of periphyton in the upper and middle reaches of the Harpeth River. High concentrations of TP of water, benthic chl *a*, and AFDM at sites upstream of Franklin are likely a result of extensive anthropogenic activities in the watershed and naturally high concentrations of P in the limestone bedrock. Additional eutrophication of river sites downstream of the Franklin Wastewater Treatment Facility is indicated by increased concentrations of TP of water samples, increased values for the algae trophic index for soft-algal assemblages, and decreased values for the pollution tolerance index for diatom assemblages. The results indicate that additional degradation of water quality as the Harpeth River flows through Franklin alters the composition of algal assemblages.

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