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PERIPHYTON CHARACTERISTICS ARE A MORE ACCURATE INDICATOR OF TROPHIC STATUS RELATIVE TO WATER-COLUMN CHARACTERISTICS OF A SHALLOW LAKE IN DUNBAR CAVE STATE PARK IN MIDDLE TENNESSEE

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ABSTRACT

Swan Lake in Dunbar Cave State Park is impaired by nutrient enrichment as evidenced by nuisance biomass levels of macrophytes and mats of surface algae. Concentrations of total phosphorus and total nitrogen of water were below those expected in a eutrophic environment, most likely due to high demand. Other characteristics of the water column including chlorophyll *a* concentration, biochemical oxygen demand, and primary production were also below levels expected in a eutrophic environment and presumably reflect the low concentration of phosphorus and nitrogen of the water. Periphyton characteristics accurately indicate the eutrophic status of the lake and include a high concentration of benthic chlorophyll *a*, high concentration of ash-free dry mass of benthic organics, and a high relative abundance of soft algal and diatom taxa listed as eutrophic indicators. The results indicate that (1) periphyton characteristics best characterize the trophic status of Swan Lake relative to water-column characteristics and that (2) assessment and monitoring of eutrophic lentic habitats should include measurements of periphyton characteristics.

Phosphorus (P) enrichment frequently increases primary production and is a major cause for the degradation of aquatic communities worldwide (Chonova et al. 2019). High rates of primary production, high concentrations of chlorophyll (chl) *a,* and changes of organism composition are hallmarks of eutrophication (Harris and Piccinin 1977). The primary objectives of this research were to characterize the water quality and biological integrity of Swann Lake in Dunbar Cave State Park such that changes can be monitored. We used multiple approaches to achieve the objectives including determinations of (1) nutrient concentrations of water, (2) pigment concentrations of water and periphyton, (3) biochemical oxygen demand of water, (4) primary production on artificial substrates, and (5) composition of benthic soft-algal and diatom assemblages.

The above ground origin of Swan Creek is the entrance to Dunbar Cave in Dunbar Cave State Park in Clarksville, Tennessee. The creek is dammed approximately 0.3 km downstream of the cave entrance forming Swan Lake. The lake is approximately 35 ha and averages approximately 3 m deep. Swan Creek is part of the Lower Cumberland River Watershed which is in the Western Pennyroyal Karst (71e) and Western Highland Rim (71f) Level IV Ecoregions. The geologic base of the watershed is Mississippian-age limestone and includes some chert, shale, siltstone, sandstone, and dolomite. The soils are a thin loess mantle, highly erodible, and very fertile (Baskin et al. 1997). Forests are Western Mesophytic and consist largely of *Quercus* and *Carya* species. Over 50% of the Lower Cumberland River Watershed is used to produce agriculture products including tobacco, corn, soybean, and livestock (TDEC 2012). The cumulative effects of erosion, agricultural runoff, livestock access to streams, and poorly functioning sewage systems result in poor quality water in most of the tributaries in the watershed, including Swan Creek. The negative impact of eutrophic conditions of Swan Lake is obvious by the nuisance biomass levels of the aquatic macrophyte *Myriophyllum spicatum* L. and surface algal mats.

Methods

Water-column characteristics of Swan Lake were determined on September 9, 2019, following the methods described by Lebkuecher et al. (2018a). Photosynthetic photon flux density (PPFD) was measured at the surface and at 0.25-m depths at 2 locations (2 replicates) with a spherical underwater quantum sensor coupled to a Li-Cor quantum meter (Li-Cor Cooperate, Lincoln, Nebraska). These data were used to calculate the vertical extinction coefficient of light (n"; Lind et al. 1992): n" = (ln $PPFD_{surface}$ - ln $PPFD_{depth}$)/depth. The value of n" is an expression of the PPFD-depth slope on a logarithmic axis and is largely a function of turbidity; the higher the n" value, the greater the vertical extinction rate of light as it penetrates the water column. Concentrations of total phosphorus (TP) and total nitrogen (TN) of water samples collected with a Van Dorn sampler at 0.25 m depth were determined using a Lachat QuickChem 8500 Flow Injection Analyzer. [TP] was determined using the persulfate-digestion and the ascorbic-acid method (Baird et al. 2017). [TN] was determined by the persulfate-digestion and cadmium-reduction method (Baird et al. 2017).

Concentration of chl *a* concentration and turbidity of the water at a 0.25-m depths at six different locations (six replicates) were determined using a portable fluorometer and nephelometer (AlgaeChek Ultra, model RS232, Modern Water Incorporated, New Castle, Delaware). The fluorometer measures the intensity of fluorescence at 685 nm emitted from chl *a* upon excitation by low emission diodes (470 nm). The nephelometer measures the concentration of suspended particulates expressed as nephelometric turbidity units calculated from the intensity of scattered light reflected from a source beam due to particles in the water.

Primary production of the water was determined using a periphytometer holding glass microscope slides (25- by 75-mm) vertically 5 cm below the surface. The periphytometer was deployed on Aug. 30, 2019, and retrieved Sept. 9, 2019. No significant rainfall occurred during the deployment and most days were partly cloudy. Periphyton were removed from both sides of 12 slides using a razor blade (two slides per six replicates) into 100 mL of deionized water. The periphyton were suspended in water by swirling and concentrations of chl *a* determined using the AlgaeChek portable fluorometer probe described above.

The effect of organic matter in the water column on oxygen consumption by microorganisms was estimated by the biochemical oxygen demand 5-day assay (BOD_5 assay) as described by Baird et al. (2017). Water samples were retrieved from a depth of 0.25 m using a Van Dorn sampler. Samples were transferred to two borosilicate-glass bottles with ground-glass stoppers. Initial dissolved oxygen (DO) concentration was determined using a portable DO meter (model MW600, Milwaukee Instruments Incorporated, Rocky Mount, North Carolina). The bottles were incubated for 5 days in darkness at 25 ^{O}C and the concentration of DO consumed recorded as BOD_5 (mg DO $\cdot$ $L^{-1} \cdot 5$ d^{-1}).

Periphyton chl *a* concentration, ash-free dry mass of benthic organics, and algal composition were determined from five cobbles removed from upper littoral zone (approximately 0.25 m depths) following the methods of Lebkuecher et al. (2015). Phytoplankton richness was estimated from multiple samples collected with phytoplankton nets (120-µm pore size) and Van Dorn samplers. Phytoplankton were preserved in 1 % glutaraldyde and concentrated by settling in darkness. Algae were identified to the lowest taxon possible using references listed in Grimmett and Lebkuecher (2017). Indices including the algae trophic index of soft algae assemblages (ATI, Grimmett and Lebkuecher 2017) and the pollution tolerance index for diatom assemblages (PTI, KDOW 2008) were used to infer the impact of trophic status on benthic algal assemblages. The 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 (Grimmett and Lebkuecher 2017). The trophic-indicator values are the abundance-weighted averages of benthic [chl *a*] listed in Grimmett and Lebkuecher (2017). The pollution tolerance index for diatom assemblages (PTI; KDOW 2008) 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 for taxon j, and N = total number of individuals assigned a eutrophication-tolerance value and tallied to calculate the index. The eutrophication-tolerance values range from one (taxon very tolerant to eutrophic conditions) to four (taxon very intolerant to eutrophic conditions) and are based on decades of autecological studies (KDOW 2008). Although trophic indicator values assigned to taxa for both the ATI and PTI were determined from evaluations of streams and rivers, these indices were used because indices specific to lotic environments have yet to be developed.

Results and discussion

The physical characteristics of Swan Lake (Table 1) are typical of lentic environments in Tennessee. For example, the slightly basic pH is similar to other aquatic systems in the ecoregion given the limestone geologic base. The relatively low n" of Swann Lake on 9 Sept. 2019 most likely reflects the lack of significant precipitation, thus lack of sediment runoff during the previous two weeks. The majority of the light-extinction studies from a variety of natural freshwater lakes and reservoirs with different morphologies and chemistries report n" values near 1.3, a value which has been adopted as typical for purposes of comparison (Renolds 1990). Values of n" $\geq$ 1.8 indicate unusually high concentrations of suspended matter (Luettich et al. 1990). The relatively low concentration of suspended particles in Swan Lake is verified by the low value for the nephelometric turbidity units (NTU). For example, NTU values above 25 indicate the water column contains an unhealthy concentration of suspended particles (USEPA 1986).

Table 1. Physical characteristics of Swan Lake on Sept. 9, 2019.

pH at 0.25 m at 9:30 AM CST	7.1
Light extinction coefficient (n")	1.0 ± 0.1
Nephelometric turbidity units	21 ± 5
Total phosphorus ($\mu g \cdot L^{-1}$)	20
Total nitrogen ($\mu g \cdot L^{-1}$)	841
Total phosphorus to total nitrogen ratio	42

The concentration of total phosphorus (TP) of water collected from Swan Lake is in the range suggested by Carlson and Simpson (1996) to designate lakes as mesotrophic (> 12 $\mu g\ TP \cdot L^{-1}$ to < 25 $\mu g\ TP \cdot L^{-1}$). The high ratio of total nitrogen (TN) to TP suggests P limits photoautotroph growth in Swan Lake. For example, P may be considered limiting when the TN:TP ratio by mass is > 33 (Carlson & Simpson 1996). The ratio of TN to TP of Swann Lake was expected given P limits algae biomass in most freshwaters. The concentration of P is a primary criterion used to designate trophic status of aquatic habitats although several studies have demonstrated that eutrophic environments with high concentrations of algae may have low water [P] as a result of high P demand (Grimmett & Lebkuecher 2017).

The low concentration of chl *a* of the water column of Swan Lake (Table 2) indicates that the productivity of the water column was relatively low. For example, concentrations of water column chl *a* > 7.3 μg/L are indicative of eutrophic conditions (Carlson & Simpson 1996). The rates of photoautotrophic periphyton production (Table 2) are substantially lower than rates considered typical of eutrophic environments and support the conclusions from analyses of the concentration of chl *a* of the water column that the productivity of the water column is low. For example, primary production values $\leq$ 0.3 mg chl $a \cdot m^{-2} \cdot day^{1}$ are typical of aquatic environments with good quality water in Middle Tennessee (Lebkuecher et al. 2009).

The mean value for the biochemical oxygen demand 5-day assay (BOD_5 assay) is typical for those determined from samples without excessive concentrations of organics. BOD_5 values from water without excessive concentrations of organics range from near 0 to 8 mg O_2 consumed $\cdot L^{-1} \cdot 5\ d^{-1}$, while values of wastewater from wastewater treatment plants are often above 19 mg O_2 consumed $\cdot L^{-1} \cdot 5\ d^{-1}$ (Delzer & McKenzie 2003; Yun & An 2016).

Table 2. Biomass characteristics, OD_{664} to OD_{665} ratio, and biochemical oxygen demand (means $\pm$ S.E.) of Swan Lake.

Water column chlorophyll *a* ($\mu g \cdot L^{-1}$)	4.3 ± 0.2
Primary production on artificial substrates (mg chlorophyll *a* $\cdot m^{-2} \cdot day^{-1}$)	0.1 ± 0.0
Biochemical oxygen demand (mg $0_2 \cdot L^{-1} \cdot 5\ days^{-1}$)	2.6 ± 0.3
Benthic (cobble) chlorophyll *a* ($mg \cdot m^{-2}$)	91.2 ± 29.1
OD_{664} to OD_{665} ratio	1.6 ± 0.02
Ash-free dry mass of benthic organics ($g \cdot m^{-2}$)	12.1 ± 3.0

Concentrations of benthic chl *a* were > 70 $mg \cdot m^{-2}$, a value suggested by Dodds et al. (1998) to designate aquatic systems as eutrophic. The OD_{664} to OD_{665} ratio of pigment extracts indicate the photoautotrophic periphyton collected from Swan Lake were in good physiological condition. Because pigment extracts from healthy algae may have no detectable pheophytin *a* determined by optical density (OD) measurements using a spectrophotometer, the chl *a* to pheophytin *a* ratio is indicated as the ratio of OD_{664} to OD_{665}. Pheophytin *a* is a chl *a* degradation product. An OD_{664} to OD_{665} ratio of 1.7 is interpreted as no detectable pheophytin *a* present and ratios < 1.5 indicate the algae contained high concentrations of pheophytin *a* (Baird et al. 2017).

The concentrations of ash-free dry mass of benthic organic matter was > 10 $g \cdot m^{-2}$, a value indicative of eutrophic environments (O'Brian & Wehr 2010). This result supports the conclusions from the measurements of the concentrations of benthic chl *a* that demonstrate an unhealthy concentration of benthic photoautotrophic periphyton biomass. The high concentrations of benthic chl *a* and ash-free dry mass of benthic organic matter indicate that periphyton characteristics of Swan Lake are a more accurate indicator of the lake's eutrophic status relative to water-column characteristics measured such as nutrient concentration of water, chl *a* concentration of water, and primary production rate on artificial substrates suspended in the water column.

We identified 69 taxa of soft algae (Appendix 1). The most abundant benthic soft alga sampled was the filamentous cyanobacterium *Leptolyngbya angustissimum* (West & West) Anagn. & Komárek (28.1 %) followed by *Oedogonium* sp. (12.0 %) and *Cladophora glomerata* (L.) Kütz. (10.9 %). The taxa richness of the benthic soft-algal assemblage was lower relative to the phytoplankton assemblage (Table 3). The low Shannon diversity index for the benthic soft-algal assemblage in Swan Lake is typical for soft algal assemblages in Middle Tennessee (Lebkuecher et al. 2018b) is due largely to the low evenness value. The high value for the algae trophic index (ATI) for the benthic soft algal assemblage indicates this assemblage is impacted by eutrophication. ATI values correspond with Dodds et al. (1998) suggested classification of [benthic chl *a*] $\geq$ 70 $mg \cdot m^{-2}$ as eutrophic. The high value for the ATI is due partially to the high abundance of *Cladophora glomerata* (L.) Kütz. (10.9 %) which has a high trophic-indicator value used to calculate the ATI given *C. glomerata* is common at eutrophic sites (Grimmett & Lebkuecher 2017).

We identified 38 taxa of benthic diatoms (Appendix 2). The most abundant diatom sampled was *Achnanthidium minutissimum* (Kütz.) Czarn. (28.1 %) followed by *Achnanthidium* sp. (9.6 %), and *Cocconeis placentula* Ehrenb. (7.9 %). The higher value for the Shannon diversity index for the diatom assemblage (Table 4) relative to the soft algal assemblage (Table 3) is due largely to the greater

evenness value for the diatom assemblage. Greater values for evenness and the Shannon diversity index of diatom assemblages relative to soft algal assemblages are typical of benthic communities in Middle Tennessee (Lebkuecher et al. 2018b). The low value for pollution tolerance index (PTI) for the diatom assemblage indicates the assemblage is impaired by nutrient enrichment. A value for the PTI < 2.6 is a suggested value to indicate an assemblage is substantially impacted by eutrophic conditions (Lebkuecher et al. 2018b). The low PTI value for the diatom assemblage in Swan Lake results largely from the high percent compositions of *Navicula* and *Nitzschia* taxa, most of which are assigned low trophic-indicator values for the PTI given they are typically more abundant in eutrophic habitats (KDOW 2008).

Table 3. Metrics and indices for the benthic soft-algal assemblage and phytoplankton richness of Swan Lake.

Index	Index value
Taxa richness of benthic soft algae	41
Taxa richness of phytoplankton	49
Shannon diversity index for benthic soft algae	2.3
Evenness for benthic soft algae	0.6
Algae trophic index for benthic soft algae	77

Table 4. Metrics and indices of the benthic diatom assemblage sampled from cobbles in Swan Lake.

Index	Index value
Taxa richness	41
Shannon diversity index	2.8
Evenness	0.7
Pollution tolerance index	2.6

Conclusions

Concentrations of total phosphorus and total nitrogen of water sampled from Swan Lake were below those expected in a eutrophic environment, most likely due to high demand. Other water-column characteristics including chl *a* concentration, biochemical oxygen demand, and primary production on suspended substrates were also below levels expected in a eutrophic environment and likely reflect the low concentration of water-column nutrients. Periphyton characteristics including a high concentration of chl *a*, high concentration of ash-free dry mass of benthic organics, and values for algal indices of the benthic assemblages accurately indicate the trophic status of Swan Lake. The results indicate that periphyton characteristics best characterize the trophic status of Swan Lake relative to water-column characteristics and that assessment and monitoring of eutrophic lentic habitats should include measurements of periphyton characteristics.

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Appendix 1. Percent composition of benthic soft-algal taxa sampled from cobbles, benthic soft-algal taxa sampled from cobbles present (P) observed independently of determinations of percent composition, and phytoplankton taxa present (P) sampled from the water column in Swan Lake listed in alphabetical order by phylum.

Chlorophyta	**Percent composition of benthic soft-algal taxa**	**Phytoplankton taxa present (P)**
Aphanochaete polychaete (Hansg.) Fritsch.		P
Bulbochaete sp.	0.71	P
Chlamydomonas patellaria Whitford		P
Chlamydomona gloeogama Korshikov		P
Chlamydomonas sp.	0.02	
Chlorococcum humicola (Nägeli) Rabenh.	0.04	
Cladophora glomerata (L.) Kütz.	10.91	
Coelastrum microporum Nägeli	0.36	P
Coelastrum reticulatum (Dang.)		P
Cosmarium botrytis Menegh.	0.69	P
Cosmarium contractum Kirchn.		P
Cosmarium galeritium Nordst.	3.28	P
Cosmarium raciborskii (Racib.) Lagerh.	4.028	
Cosmarium sp.	0.02	P
Cosmarium subprotumidum Nordst.	0.09	P
Cosmarium turpinii Bréb.	0.07	P
Desmidium sp.		P
Gloeocystis gigas (Kütz.) Langerh.	3.74	P
Gloeocystis vesiculosa Nägeli	10.49	P
Mougeotia sp.	10.08	P
Oedogonium sp.	11.99	P
Oocystis sp.		P
Pandorina morum (Müller) Bory	0.47	P
Pediastrum araneosum (Racib.) G. M. Sm.	0.17	
Pediastrum integrum (Meyen 1829)		P
Pediastrum sp.	0.37	P

Pediastrum tetras (Ehrenb.)		P
Scenedesmus acuminatus (Lagerh.) Chodat	0.06	
Scenedesmus bijuga (Turp.) Lagerh.	0.51	P
Scenedesmus dimorphus (Turp.) Kütz.	0.04	
*Scenedesmus obliquus (*Turp.) Kütz.		P
Scenedesmus quadricauda (Turp.) Bréb.	0.07	
Scenedesmus sp.	0.06	
Selenastrum capricornutum Printz	0.06	P
Spirogyra sp.		P
Staurastrum obliculare (Erenb.) Ralfs	0.02	P
Staurastrum sp.		P
Sphaerocystis sp.		P
Sphaerocystis planktonia (Korsh.) Bourr.	0.34	
Volvox globulator (L.)		P
Zygnema sp.		P
Cyanobacteria		
Aphanocapsa elachista West and West		P
Chroococcus minor (Kütz.) Nägeli	0.04	
Chroococcus minutus Kütz.		P
Chroococcus sp.		P
Coelosphaerium sp.	0.21	
Dactylococcopsis raphidiodes (Hansg.) Chodat and Chodat		P
Komvophoron munitum (Skuja) Anagn. and Komárek		P
Leibleinia nordgaardii (Wille) Anagn. and Komárek	0.66	P
Leptolyngbya angustissimum (West and West) Anagn. and Komárek	28.11	P
Leptolyngbya sp.	4.68	P
Merispodedia tenuissima Lemmerm.	P	
Microcystis aeruginosa (Kütz.) Kütz.	0.02	
Microcystis incerta Lemmerm.	0.02	
Oscillatoria agardhii Gomont	0.09	
Oscillatoria limosa (Dylwin) C. Agardh		P
Oscillatoria sp.	0.24	P
Oscillatoria subtilissima Kütz. and De Toni	2.44	
Phormidium articulatum (Gardner) Anagn. and Komárek	0.37	
Phormidium diguetii (Gomont) Anagn. and Komárek	0.32	
Phormidium sp.	7.49	P
Synechocystis sp.	0.02	
Dinophyta		
Peridinium sp.		P
Euglenophyta		
Euglena sp.		P
Cryptophyta		
Chroomonas nordstedtii Hansg.		P

Chrooomonas sp.		P
Cryptomonas erosa Erenb.		P
Ochrophyta		
Dinobryon sp.		P
Lagynoin sp.	P	

Appendix 2. Percent composition of benthic diatom taxa sampled from cobbles and benthic diatom taxa sampled from cobbles present (P) observed independently of determinations of percent composition in Swan Lake listed in alphabetical order.

Taxon name	Percent composition
Achnanthidium gracillimum Lange-Bert.	2.31
Achnanthidium minutissimum (Kütz.) Czarn.	28.05
Achnanthidium rivulare Potapova and Ponander	5.28
Achnanthidium sp.	9.57
Cocconeis placentula Ehrenb.	7.92
Cocconeis placentula var. euglypta Ehrenb.	0.33
Cymatopleura solea (Bréb. and Godey) W. Sm.	0.33
Eunotia pectinalus (Kütz.) Rabenh.	P
Eunotia sp.	0.99
Frustulia vulgaris (Thwaites) De Toni	0.33
Gomphoneis olivacea (Horn.) Daws.	0.33
Gomphonema acutinatum Ehrenb.	0.66
Gomphonema angustatum (Kütz.) Rabenh	1.98
Gomphonema gracile Ehrenb.	0.33
Gomphonema minutum Ag.	0.66
Gomphonema parvulum (Kütz.) Kütz.	0.33
Gomphonema pseudoaugur Lange-Bert.	0.33
Gomphonema truncatum Ehrenb.	0.33
Melosira varians Ag.	6.60
Navicula capitatoradiata Germ.	4.62
Navicula cryptocephala Kutz.	1.65
Navicula crytotenella Lange-Bert.	P
Navicula lanceolata (Ag.) Ehrenb.	0.33
Navicula menisculus var. *upsaliensis* (Grun.) Grun.	0.66
Navicula sp.	1.98
Navicula veneta Kütz.	1.98
Nitzschia acicularis (Kütz.) W. Sm.	0.66
Nitzchia amphibia Grun.	1.65
Nitzschia angustata (W. Sm.) Grun.	0.33
Nitzschia capitellata Hust.	2.64
Nitzschia fonticola Grun.	0.66
Nitzschia frustulum (Kütz.) Grun.	1.65
Nitzschia inconspicua Grun.	1.32
Nitzschia sinuata var. tabellaria Grun.	0.66
Nitzschia flexa Schumann	P
Nitzschia sociabilis Hust.	1.98
Synedra delicatissima W. Sm.	2.31

Thalassiosira weissflogii (Grun.) G. Fryxell and Hasle	0.33
Ulnaria sp.	0.66
Ulnaria ulna (Nitzsch.) Compère	0.66
Ulnaria capitata (Erenb.) Compère	6.60