To the Graduate Council:

I am submitting herewith a thesis written by Robert Gerald Middleton entitled “Cattle access affects periphyton community structure in Tennessee farm ponds.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

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Dean of the Graduate School

(Original signatures are on file with official student records.)

Cattle Access Affects Periphyton Community Structure in Tennessee Farm Ponds

A Thesis
Presented for the
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Degree
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Robert Gerald Middleton
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ABSTRACT

Cattle farming is vital to the economy of the United States. Frequently, cattle are given access to ponds and streams for water. The relative impacts of cattle access in natural water sources on the periphyton community have been rarely investigated. Periphyton is the basis of the aquatic food web, and community composition can serve as a bioindicator of pollution. Thus, my objectives were to quantify the effects of cattle access in aquatic lentic systems on periphyton community structure and biovolume, identify taxa that were associated with cattle access, and identify abiotic mechanisms that might be driving assemblage changes. I conducted my research in 4 cattle-access (CA) and 4 no-access (NA) farm ponds on the University of Tennessee Plateau Research and Education Center from May 2005 – April 2006. Periphyton community composition and water quality were measured every 2 weeks using standard environmental monitoring procedures. I documented 181 new periphyton taxonomic records (7 phyla, 52 families, and 132 genera) in Cumberland County, Tennessee. Periphyton species richness was greatest in NA ponds. Mean biovolume of pollution-sensitive diatoms (e.g., *Achnanthidium minutissimum, Cymbella* sp., *Eunotia* sp., *Fragilaria crotonensis* and *Tabellaria fenestrata*) was greater in NA ponds. In contrast, pollution-tolerant diatoms (e.g., *Gomphonema* sp. and *Navicula* sp.) and non-diatoms (e.g., *Oscillatoria* sp. and *Scenedesmus* sp.) were more abundant in CA ponds. Turbidity, pH, conductivity, and concentrations of the total Kjeldahl nitrogen, total phosphorus, potassium, periphyton phosphorus, silicon, iron, magnesium and aluminum

were greater in CA ponds. Thus, changes in water quality associated with cattle-access likely mediated changes in the periphyton community. Considering that changes in periphyton community composition can destabilize higher trophic levels, I recommend that cattle farmers take advantage of USDA conservation programs that provide funds for fencing cattle from watersheds and developing alternate water sources. My results also provide evidence that monitoring the periphyton community is a reliable technique to detect water pollution from cattle.

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CHAPTER I

INTRODUCTION

Agricultural pollution has resulted in severe disturbance of watersheds and biogeochemical cycles worldwide, resulting in changes to the structure and function of aquatic food webs (Vitousek et al. 1997, Smith et al. 1999, Grimm et al. 2008). Lentic (i.e., standing water) systems are often the most heavily impacted, because slow hydrologic turnover results in the accumulation of nutrients, contaminants and toxins (López-Flores et al. 2003). Maintaining environmental quality in lentic systems (e.g., wetlands) is a major conservation initiative, because many have been destroyed (Tori et al. 2002, Bledzki 2008). For example, at least 50% of the original wetlands in the conterminous United States have been drained (Dahl and Johnson 1991). Wetlands provide habitat for more species than aquatic or terrestrial systems, and they serve as important sites for nutrient cycling, floodwater storage and groundwater recharge (Johnson 1991, Mitsch and Gosselink 2000). Although federal and state laws protect the dredging and filling of wetlands (Mitsch and Gosselink 2000), human land use, such as agriculture, can affect water quality and resident biota. Identifying land-use practices that negatively impact abiotic and biotic characteristics of lentic systems is fundamental to conceiving and implementing science-based conservation strategies.

Cattle farming is a major agricultural industry in the United States (USEPA 2000). There are currently 950,000 cattle farms in the United States, with approximately 93.7 million head (USDA 2010 b,c). Many livestock farms contain wetlands or ponds, and cattle are given access to them to drink water or graze vegetation (Belsky et al. 1999).

Currently, few or no states have laws that restrict cattle-access in wetlands. Cattle can negatively impact aquatic systems by reducing water quality through deposition of nitrogenous wastes (Line 2003). On average, cattle defecate 12X per day (Miner et al. 1992, Hoorman 2005), with one herd member excreting 136 kg of nitrogen, 20 kg of phosphorus and 75 kg of potassium annually (Brown and Archer 1987). Nutrients in livestock excreta can result in eutrophication (Line 2003, Dunne et al. 2005), which can have negative impacts on resident fish and wildlife. Organic pollution causes rapid growth of microorganisms, which increases biochemical oxygen demand and can result in dissolved oxygen deficits for aquatic life (Hooda et al. 2000). These trophic level changes are frequently detected first via changes in algal community composition and biomass. In particular, the periphyton community is sensitive to changes in water quality (Wehr and Sheath 2003, Smol 2008).

Periphyton is a microcommunity assemblage of algae, fungi, bacteria and other organisms that colonize benthic substrates in aquatic ecosystems (Stevenson et al. 2002, Carr et al. 2005). Taxa interactions may be positive or negative. For example, a study of stream biofilms in Canada suggested that bacteria and algae coexist in a mutually dependent association (Carr et al. 2005). In contrast, Dor and Benzion (1980) reported that the growth of the green algae *Chlorella vulgaris* and *Scenedesmus quadricauda* were inhibited by bacteria such as *Citrobacter and Aeromonas* (Dor 1978). Algal inhibition, rather than stimulation, by bacterial activity is expected in ponds receiving large inputs of animal waste (Toerien et al. 1984). Thus, human land use (e.g., cattle farming) might affect periphyton assemblages through mediating competitive interactions.

An important component of the periphyton community is diatoms (Bacillariophyceae), which are unicellular photosynthetic protists with frustules constructed of silicon sequestered from the water column (Round et al. 2007). This physiological requirement of diatoms makes them major players in the global biogeochemical cycle of silicon (Treguer et al. 1995, Yool and Tyrrell 2003, Connolly et al. 2008). Diatom genera exist within narrow environmental conditions (e.g., light, pH, conductivity, water chemistry), and are excellent bioindicators of water quality (Lange-Bertalot 1979, Sabater et al. 1987, Gold et al. 2003). Non-diatom algae in periphyton communities typically consist of filamentous and unicellular green algae, filamentous and unicellular blue-green bacteria, desmids, dinoflagellates, euglenoids, and phytoplankton that may become trapped in the periphyton matrix. Many periphyton taxa demonstrate clear individual tolerances or sensitivities to stressors, such as elevated concentrations of metals and nutrients (Genter et al. 1988, St-Cyr 1997, Medley and Clements 1998). Thus, community composition of periphyton can be useful in identifying degraded water conditions (Genter et al. 1988).

There are many hypotheses for possible mechanisms that may be responsible for periphyton community shifts, many of which are related to anthropogenic stressors. These include water contamination, global climate change, solar radiation, habitat degradation, or a combination of these factors (Stoermer and Smol 1999, Steinman et al. 2003, Hill and Fanta 2007). Additionally, a variety of abiotic and biotic water quality variables may drive responses in periphyton communities such as light, metals, invertebrate grazers, temperature, pH, conductivity, dissolved oxygen, spates (i.e., floods) and turbidity.

Except for studies conducted in the Florida Everglades (Goldsborough and Robinson 1996, McCormick and Scinto 1999, Pan et al. 2000), investigations into anthropogenic mechanisms driving shifts in periphyton assemblages remain largely unexplored in the southeastern United States, and none exist for Tennessee wetlands. To my knowledge, no studies have directly investigated the impacts of allowing cattle access in lentic systems on the periphyton community. Given the widespread nature of cattle farming in the United States and elsewhere, there is a need to quantify the effects of allowing cattle access in watersheds on periphyton. Identifying periphyton taxa associated with cattle-access sites also will be useful for aquatic environmental monitoring programs. Herein, I examine the direct and indirect effects of allowing continuous cattle access in Cumberland Plateau farm ponds on the periphyton community. Chapter II is written in manuscript style hence contains Introduction, Methods, Results and Discussion sections. I also summarize my results and provide some thoughts on conservation strategies and future research directions. Although not a specific research objective, my research resulted in a comprehensive list of diatom and non-diatom algal taxa for Cumberland County, Tennessee. Possible publication outlets for my research include *Freshwater Biology*, *Hydrobiologia*, and *Journal of Applied Phycology*.

CHAPTER II

IMPACTS OF CATTLE ACCESS IN FARM PONDS ON PERIPHYTON COMMUNITIES

Introduction

Agriculture is fundamental to sustaining global human populations and it is a critical component of the economy in the United States (Benson et al. 1998). The United States is the leading producer of beef in the world (USDA 2010a). There are currently 950,000 cattle farms in the United States, with approximately 93.7 million head (USDA 2010b,c). Tennessee is #15 among U.S. states in cattle production, with nearly 47,000 farms and 2.04 million head (USDA 2010a). The majority of these operations use farm ponds, streams, and wetlands to provide water for cattle. On average, beef cattle drink 48 L/day (Parish and Rhinehart 2008), thus the availability of water can be a limiting factor to cattle farming (Lardy and Stoltenow 1999, Landefeld and Bettinger 2002).

There are over 190,000 farm ponds in Tennessee (Hill 1999, TWRA 2009). These are important sites for watering cattle, and they provide habitat for various fish and wildlife (Daborn et al. 1993, McCollum et al. 1998, Knutson et al. 2004). Cattle can negatively impact aquatic communities by altering water quality (Belsky et al. 1999, Schmutzer et al. 2008). Common water quality changes in cattle-access wetlands include increases in turbidity, specific conductivity, nitrogenous compounds, and phosphorous (Carpenter et al. 1998, Hooda et al. 2000, Schmutzer et al. 2008). Nutrient inputs can cause algal blooms, increase biological oxygen demand, and decrease available dissolved oxygen (Anderson and Garrison 1997, Sabater et al. 2000, Dunne et al. 2005). Changes in algal biomass and taxa composition can cause trophic cascades that have irreversible effects on aquatic community structure and function (Bourassa and Cattaneo 2000, Chase 2003, Jones and Sayer 2003).

On average beef cattle may defecate up to12X per day and 2 kg per defecation (Brown and Archer 1987, Hoorman 2005, Oudshoorn et al. 2008). When given access to surface water for drinking, cattle defecate 11% of the time in the water (Bagshaw 2002), which is equivalent to about 3 kg of fecal addition per animal per day. Cattle feces contain high levels of nitrogen and phosphorous: 0.34 and 0.092 kg /1000 kg of live animal weight per day, respectively (Hubbard et al. 2004). Thus, nitrogen and phosphorus pollution are commonly cited as negative impacts of cattle access in streams and wetlands on resident flora and fauna (Rouse et al. 1999, Arbuckle and Downing 2001, McCormick et al. 2001).

Few studies have examined the impacts of cattle access on the algal communities in wetlands (Vymazal and Richardson 1995, Scott et al. 2005). Schmutzer (2007) found that cattle access in Tennessee farm ponds resulted in a 21 – 600% increase in biomass of filamentous algae, with the pollution-tolerant *Hydrodictyon* more prevalent in cattle ponds compared to control ponds where *Spirogyra* was dominant. In contrast, Scrimgeour and Kendall (2002) reported that allowing cattle access in Canadian streams reduced algal biomass by 50 – 300%. No other studies have directly investigated the effects of cattle on algal communities, but several studies have reported changes in periphyton community composition associated with elevated nitrogen and phosphorus in runoff from agricultural fields (McCormick and Stevenson 1998, Pan et al. 2000, McCormick et al. 2001). Collectively, these studies indicate that agricultural land use may impact periphyton community structure.

In Tennessee, several periphyton studies have been conducted but those were in lotic systems (Vaughan 1979, Mulholland and Rosemond 1992, Johansen et al. 2007). To my knowledge, no studies have been published that examined the impacts of agricultural stressors on the periphyton community in Tennessee lentic systems. In 2005 and 2006, a field study was conducted at the University of Tennessee Cumberland Plateau Research and Education Center (PREC) that examined the impacts of cattle access in farm ponds on water quality, algal biomass, aquatic invertebrates, and larval and postmetamorphic amphibians (Schmutzer et al. 2008, Burton et al. 2009). This study provided a unique opportunity to examine concurrently the impacts of cattle on periphyton. Thus, my objectives were to quantify the effects of cattle access on periphyton community structure and biovolume, identify diatom and non-diatom taxa that were associated with cattle ponds hence could be used as future bioindicators of pollution, and identify abiotic mechanisms that might be driving treatment differences. Also, given that no records of periphyton taxa existed for Cumberland County, TN, an ancillary objective was to generate a genera list of all detected taxa.

Methods

<u>Study Area</u>

From May 2005 – April 2006, I sampled periphyton communities at eight constructed farm ponds located at the University of Tennessee Plateau Research and Education Center (PREC) on the Cumberland Plateau near Crossville, Tennessee (36^{o} 00' 59" N, 85^{o} 07' 57" W; Fig. 1, this and all following figures and tables can be found in the Appendix). The PREC is located in the Appalachian Plateau physiographic province, and the residual surface soils are shallow and sandy (Smith 2000, Newell 1986). The ponds ranged from 0.15 – 1.29 ha (Table 1), were permanently flooded (<2 m deep), contained hydric soils and emergent shoreline vegetation (e.g., rushes, sedges, cattails, Burton et al. 2009); thus, my study ponds could be classified as persistent emergent wetlands (USACE 1987). Ponds PREC 1 and PREC 3 had very little overstory canopy or emergent macrophytes, PREC 4 had heavy canopy shading from trees, and PREC 6 had moderate canopy shading with some emergent macrophytes along the shoreline. Ponds PREC 2, 5, 7 and 8 were open canopy but with emergent macrophytes providing some shade.

Cattle (Black Angus, Gelbvieh, and Balancer breeds) were allowed 12-month access to PREC 1 – 4 at an average density of 19 head per 0.1 ha surface area of pond water (Gray et al. 2007). Cattle were not allowed access to PREC 5 – 8 in over 10 years (Burton et al. 2009). However, there was a natural drainage that connected PREC 1 and 5, with PREC 1 upslope and approximately 300 m from PREC 5 (Fig. 1). Ponds PREC 6 and 7 were hydrologically linked via 50-m drainage, with PREC 6 higher in the watershed than PREC 7 (Fig. 1). Despite these hydrologic connections, I assumed each PREC pond represented an independent experimental unit of treatment responses.

Climate conditions on PREC during my study were typical of mid-latitudinal United States, with a mean low of 0.8°C in December 2005 and mean high of 22.7°C in August 2005 (Table 2). There were 11 storms where rainfall exceeded 2.5 cm in 24 hrs and one storm on 8 April 2006 that produced 9.5 cm of rain in 24 hrs. In contrast, very little precipitation fell from September – November 2005 (Table 2), which resulted in pond levels dropping significantly. In particular, the water level in PREC 5 dropped over 0.76 m.

<u>Sample Collection</u>

I deployed artificial substrates to quantify benthic algae in my study ponds to reduce the variability that is often associated with sampling natural substrates (Patrick 1968, Deniseger et al. 1986, Gold et al. 2002). Although artificial substrata may differ from natural substrates, they are frequently used to measure benthic algae (Cattaneo and Amireault 1992, Wunsam et al. 2002) because: (1) there is a known time of colonization (i.e., usually <1 month), (2) similar communities colonize artificial and natural substrates, and (3) artificial substrates allow consistent measurement of the benthic algal structure (Meier et al. 1983, Murdock and Dodds 2007). Additionally, for designed experiments such as mine, the results in each treatment are relative. Thus, I assume that use of artificial substrates provided a reasonable estimate of periphyton community structure in cattle-access and no-access ponds.

My artificial substrates were constructed of standard red masonry bricks with 24 unglazed, ceramic tiles (5.76 cm^2 each) that were semi-permanently affixed with silicon glue to the brick (Hill and Boston 1991, Hill and Middleton 2006, Fig. 2). I placed four artificial substrates in each of the 8 ponds on untreated plywood (1.8 [L] x 0.3 [W] x 0.1 [H] m) to prevent the bricks from sinking into the mud. Each artificial substrate was stabilized with nails on the side of the brick and spaced at 0.5-m intervals. The board was placed in the water along an randomly generated azimuth that bisected the pond and at 2.5 m from and perpendicular to the shoreline. As water levels dropped or rose, I moved the board so that it remained at 2.5 m from the shoreline to standardize light conditions throughout the study. The board was submersed using sandstone rocks as ballasts. The 0.5-m spacing resulted in each successive brick at greater depth progressing toward the center of the pond (Figs. 2 and 3). Placement of bricks at different depths potentially allowed for colonization of different taxa at different light regimes. At the 4 cattle-access ponds, heavy-gauge wire field fencing was placed around the artificial substrates to prevent cattle from damaging them. The fencing encircled the bricks with at least a 1 m buffer between the bricks and fence, the fence had large mesh (5 x 7.5 cm), and there was no top. Because it is highly unlikely that enclosures impacted light intensity or water quality around bricks, it was reasonable to assume that the fencing had minimal impacts on periphyton responses in CA ponds.

I deployed artificial substrates in early April and allowed 30 days for periphyton recruitment and colonization prior to initial sample collections (Gold et al. 2002, KDOW 2008). Thereafter, I sampled all ponds every 2 weeks from May 2005 – April 2006. To avoid disturbing sediments in the pond water while sampling, I installed local sandstone rocks between the shore and artificial substrates, which functioned as footpads. I also changed waders between ponds to avoid cross contamination. During each sample trip, I randomly selected 1 tile from each of the 4 artificial substrates per pond (n = 32 samples/trip). After the tile was carefully dislodged from the brick with a knife, it was placed upright in pre-labeled 50-mL plastic containers with sealable caps containing 10 ml of distilled water. Thus, periphyton that may have sloughed off the tiles in the sample vial during transport was retained as part of the sample slurry. Samples were placed in an ice cooler for transport. Upon returning to the laboratory, samples were stored in dark refrigeration at 4°C until processing (≤ 24 hours, Flotemersch et al. 2006). Depth from the water surface to each brick was measured before leaving the pond.

During the course of my research (April 2005 – May 2006), I made 24 sampling trips (2 trips per month x 12 months) collecting 32 samples per trip (4 samples per pond x 8 ponds), thus generating a total of 768 periphyton samples. Due to this large number of samples, I processed only 1 sample trip/month from 2 bricks (shallowest and deepest), thus total sample size was 2 samples/pond/month x 8 ponds x 12 months or 192 total samples examined. I processed the same sample trip per month so there was exactly 4 weeks between each trip. All samples remained in cold storage preserved in

Lugol's solution (i.e., 3 drops per vial) until processing. Fresh Lugol's was added every 90 d (Jørgensen and Vollenweider 1988).

Water Quality and Available Light

To investigate possible mechanisms associated with changes in periphyton taxa, I measured various water quality variables and the amount of light penetrating the water column every 2 weeks from May 2005 – April 2006. Dissolved oxygen, temperature, conductivity, turbidity, and pH were measured at a probe depth of 15 cm using a Horiba® U-10 meter (Horiba, Ltd., Irvine, CA) within 1 m of the substrates. I used a LI-COR® 250A light meter coupled with a submersible LI-190SA quantum sensor (LI-COR Environmental, Lincoln, Nebraska, USA) to measure photosynthetic light; sampling occurred between 1000 – 1400 hrs. I measured relative light intensity (µmol photons$\cdot m^{-2} \cdot s^{-1}$, Dodds 1992, Ensminger et al. 2005) at the ground surface adjacent to each pond and underwater at the bottom immediately adjacent to the top of each artificial substrate.

In August 2005, I collected water samples from each pond and tested for chemical oxygen demand, ammonia nitrogen, total NO_3 and NO_2 nitrogen, total Kjeldahl nitrogen, total phosphorus, sulfate, dissolved residue, suspended residue, total residue, alkalinity, and the metals Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, Pb, Si, and Zn. Laboratory methods for testing concentration of metals included EPA 200.7 (metals and trace elements by inductively coupled plasma/atomic spectrometry), EPA 200.9 (trace elements in water by graphite furnace atomic absorption, GFAA), EPA 245.1 (cold vapor CVAA for Hg), and EPA 370.1 (Si by colorimetry). Methods for measuring nutrient concentrations included EPA 350.1 (ammonia by automated colorimetry), EPA 351.2 (semi-automated colorimetry), EPA 353.2 (nitrate-nitrite nitrogen by colorimetry), EPA 354.1 (nitrite by spectrophotometry), and EPA 365.4 (phosphorus by automated colorimetry, USEPA 2010). Other standard laboratory methods included EPA 160.2 (non-filterable residue by oven-drying) and EPA 410.1 (COD by titrimetry). The laboratory minimum detectable levels (MDLs) for statistically significant variables were total Kjeldahl nitrogen = 0.5 mg/L, potassium = 0.3 mg/L, total phosphorus = 0.10 mg/L, Si = 0.2 mg/L, Mg = 0.02 mg/L, Fe = 0.025 mg/L, Al = 0.10 mg/L, COD = 5 mg/L, and total residue = 10 mg/L. Simultaneous with my sampling, Schmutzer et al. (2008) measured ammonia nitrogen, nitrite, nitrate and phosphate once per month at the identical ponds, which I compared in the Discussion.

I also measured periphyton phosphorus, because phosphorus is an essential nutrient limiting periphyton growth (McCormick et al. 2001, Notestein et al. 2003). I used 4 water samples per pond collected in July 2005, November 2005, January 2006, and April 2006 for periphyton phosphorous analyses. The quantification of phosphorus requires several pretreatment steps including the conversion of the phosphorus to dissolved orthophosphate followed by colorimetric determination of dissolved orthophosphate with the Uvikon spectrophotometer (North Star Scientific, Ltd., Leeds, UK, Hauer and Lamberti 2007). The phosphorus analyses were conducted at the laboratory of Dr. Walter Hill, Illinois Natural History Survey, Champaign, Illinois, USA.

To estimate periphyton phosphorus, a 5-mL aliquot was taken from each of 32 samples. Samples were weighed in tared aluminum pans, dried at 60° C for 8 hrs to estimate dry mass, and ashed in a muffle furnace at 500° C for 4 hrs to obtain ash-free dry mass (AFDM, Hauer and Lamberti 2007), which is a measurement of the organic matter in each sample (USEPA 1995). Percent organic content is the ratio of the AFDM to dry mass. Aluminum pans (with dried periphyton) were retained and kept in cool, dark storage until phosphorus analysis. Dry mass and AFDM were used to estimate phosphorus concentration using the colorimetric ascorbic acid technique (APHA 1998, Hauer and Lamberti 2007).

Laboratory Processing

To increase processing efficiency while determining if there were interactive effects of water depth with taxa responses, I processed samples from the shallowest and deepest artificial substrates (i.e., 0.5 and 2 m from shore). Periphyton was brushed from tiles and carefully rinsed with 20 mL deionized water into a clean laboratory pan. Using a clean funnel, the resultant algal slurry was poured into 30-mL dark brown Nalgene® HDPE bottles. The slurry was preserved with 3 drops of Lugol's solution and kept in cold storage (4℃) until counting (Wunsam et al. 2002, Hill et al. 2009). For statistical analyses, I enumerated the algal component of periphyton to genus using a Zeiss® (Carl Zeiss Microimaging, Inc., Thornwood, NY, USA) inverted microscope at 400X magnification (Utermöhl 1958, Gillett et al. 2009). Several periphyton studies have reported that genera classifications explain a larger portion of environmental variance than species classification (Chessman et al. 1999, Wunsam et al. 2002, Bellinger et al. 2006). I enumerated genera abundance of algal taxa within 10 fields-of-view (FOV). If <500 cells were observed in 10 FOV, then I tallied genera until 500 cells were counted. I stopped enumerating if 500 cells were not counted after 100 FOV. Algal filaments were also counted, where a 10-µm segment was considered equivalent to 1 cell for consistent enumeration (Alverson et al. 2003, Brierley et al. 2007, KDOW 2008). The minimum number of algal cells counted was 112 in PREC 7. I assumed that counting diatom and non-diatom cells provided a reasonable estimate of algal community structure in cattle-access and no-access ponds.

To generate a species list of periphyton taxa (i.e., algal component) for Cumberland County, I identified algae routinely collected from the artificial substrates and from water column samples (phytoplankton). Species identifications were accomplished by acid-cleaning samples, mounting with Naphrax on slides, and examining at 1000X with an oil immersion objective (Fore and Grafe 2002, Cattaneo et al. 2004, KDOW 2008).

Taxa counts were used as measures of relative abundance. Phycologists argue that cell counts do not give a true evaluation of composition (Wetzel 2001), because periphyton taxa vary greatly in size (e.g., coccoid blue-green = <5 μm^3, large diatoms = >250,000 μm^3). Thus, I converted counts to biovolume by estimating cell volume using standard geometric formulae that best approximated cell shape. Cell dimensions were measured using an ocular micrometer from at least 10 cells per taxon (Hillebrand et al. 1999, Sun and Liu 2003, Hill and Fanta 2007). Biovolume (μm^3 cm^2) was estimated by multiplying the density (# cells/cm^2) and cell volume (μm^3) of a taxon (Hill et al. 2009). Biovolumes for poorly preserved or rarely encountered taxa were calculated from autecological data provided by the Philadelphia Academy of Sciences (Charles et al. 2002).

Periphyton Metrics

I used the Kentucky Diatom Bioassessment Index (i.e., Kentucky Index) to determine water quality scores calculated from taxonomically-derived metrics to make inferences on the environmental conditions at the PREC ponds (Winter and Duthie 2000, KDOW 2008, KDOW 2009). The Kentucky Index is similar to the indices for fish and macroinvertebrates in streams (Karr 1981, Hilsenhoff 1987) in that it is a multimetric index. The six metrics I used were: (1) total number of diatom taxa, (2) Shannon-Wiener diversity, (3) pollution tolerance index, (4) siltation index (% relative abundance of *Navicula* + *Nitzschia* + *Surirella*), (5) *Fragilaria* group richness, and (6) *Cymbella* group richness. Details on the Kentucky Index can be found in KDOW (2008). In general, this index is based on previous research that demonstrated diatom composition changes in response to pollution. For example, the “pollution tolerance index” is a measure of the overall tolerance level of the community to environmental stress. A healthy population will include diatoms at all tolerance levels (i.e., pollution tolerant vs. pollution sensitive), however, the number of tolerant diatoms should be relatively low compared to sensitive taxa (KDOW 2008). The mean of the six metrics is the final Kentucky Index score that characterizes the periphyton assemblage and ecological integrity of each pond (Bahls 1993, Griffith et al. 2002, KDOW 2008). I used default tolerance scores for genera from the Kentucky diatom master taxa list for my determinations of diatom status as either pollution tolerant or pollution sensitive (KDOW 2008). It has been well documented that diatom generic-level taxonomy is an effective tool for environmental bioassessments of impacted aquatic systems (Chessman et al. 1999, Hill et al. 2001, Wang et al. 2006). Kentucky Index scores from 75 – 100 = excellent, 62 – 75 = good, 51 – 62 = fair, and 0 – 51 = poor water quality (KDOW 2008). For example, if a cattle-access pond (e.g., 50 score) had a lower Kentucky Index compared to a no-access pond (e.g., 75 score), it would indicate impaired water quality at the former. Thus, I assume the application of the Kentucky Index provided a reasonable assessment of periphyton community structure in cattle-access and no-access ponds.

Statistical Analyses

Response variables for my analyses included periphyton genera richness and diversity (Shannon-Wiener, Morin 1999), biovolume of periphyton genera, the Kentucky Index, periphyton phosphorus, and several water quality variables. I used a 3-way repeated-measures analysis-of-variance (ANOVA) to test for differences in richness, Shannon-Wiener diversity (Morin 1999), biovolume, and the Kentucky Index between cattle-access and no-access ponds (Montgomery 2005). Variables were averaged between sampling weeks within months, and month used as the repeated effect. I included brick location (shallow vs. deep) as an effect, thus temporal (month) and depth effects were tested. If there was an interaction between effects, I ran analyses by month (Montgomery 2005). For descriptive purposes, I also constructed stacked bar and pie charts, and qualitatively compared the proportion of the most dominant diatom and non-diatom taxa based on total counts between cattle treatments and among months.

For water variables that were measured every two weeks, I averaged values by pond for each month and I used a 2-way repeated-measures ANOVA to test for differences between treatments and among months. For variables that were measured once in August 2005, a 1-way ANOVA was used to test for differences between cattle-access and no-access ponds. All analyses were performed $\alpha = 0.05$ using PROC GLM in SAS® (SAS Institute, Cary, NC; Little et al. 1991).

Given that the Kentucky Index represented a community response to potential environmental stressors in my experiment, I used multiple linear regression with stepwise selection (variable entry and stay $\alpha = 0.05$) to identify possible abiotic mechanisms driving community composition. Possible explanatory variables included water quality variables that were measured each sample period (pH, temperature [°C], turbidity [NTU], conductivity [mS/cm^{-1}], and dissolved oxygen [mg/L]) and those analyzed from the August 2005 water samples (Al, Cd, Cu, Fe, Pb, Mn, Zn [µg/L]; alkalinity, ammonia, Ca, chemical oxygen demand, K, Mg, Na, dissolved residue, suspended residue, total residue, Si, sulfate, total nitrogen, total Kjeldahl nitrogen, total phosphorus [mg/L]). Given the unbalanced design of the water quality data (i.e., August

2005 variables measured once), I averaged the Kentucky Index and all water variables that were sampled more than once across sample periods for each pond prior to the analysis. Thus, $n = 8$ for these analyses.

Results

Taxa Responses

I documented 181 periphyton taxonomic records (7 phyla, 52 families, and 132 genera) for Cumberland County, Tennessee (Table 3). Mean genera richness of periphyton averaged four more genera in no-access (NA; $\bar{x} = 28.9$, SD = 4.8) ponds than in cattle-access (CA; $\bar{x} = 25.2$, SD = 4.4) ponds ($F_{1,138} = 32.1$, $P < 0.001$). The Kentucky diatom index in NA ponds ($\bar{x} = 84.3$, SD = 5) was 20% greater than in CA ponds ($\bar{x} = 70.2$, SD = 3.6; $F_{1,138} = 20.8$, $P < 0.001$). No differences were detected in genera diversity between CA and NA ponds ($F_{1,138} = 0.73$, $P = 0.4$). Mean genera diversity was greatest in April ($\bar{x} = 2.57$, SD = 0.31) and lowest in June ($\bar{x} = 2.10$, SD = 0.33, $F_{11,138} = 2.33$, $P = 0.01$). No additional statistical differences were detected in periphyton community metrics.

Based on total counts (cells/cm^2) from May 2005 to April 2006, the 10 most dominant taxa were the diatoms *Achnanthidium minutissimum, Fragilaria*, *Gomphonema*, *Navicula cryptocephala*, and *Nitzschia*, and the non-diatoms *Cosmarium*, *Oedogonium*, *Oscillatoria*, *Scenedesmus*, and *Trachelomonas* (Fig. 4). In NA ponds, A*. minutissimum* and *Fragilaria* were the most common taxa whereas in CA ponds *Scenedesmus* and *Gomphonema* were most common (Fig. 4). Total periphyton cell counts were greatest December – June, with *Fragilaria* and *Nitzschia* most common during cooler months in NA and CA ponds (Figs. 5 and 6). *Scenedesmus* was most prevalent during warmer months in CA ponds (Fig. 6). Across periphyton phylums, diatoms (Phylum Bacillariophyta) were most prevalent (66%) in NA ponds (Fig. 7), while non-diatom algae were most common in CA ponds (Fig. 8). Phylum Chlorophyta composed the majority of non-diatom algae (Figs. 7 and 8).

Based on cell counts converted to biovolume, the 10 taxa exhibiting the highest biovolume included 2 diatoms (*Gomphonema* and *Nitzschia*), 5 chlorophytes (*Cosmarium, Oedogonium, Pediastrum, Spirogyra* and *Staurastrum*), the cyanobacterium *Gloeotrichia*, the dinoflagellate *Peridinium* and the euglenoid *Trachelomonas volvocina* (Fig. 9). The biovolume for these taxa were integrated into diatom and non-diatom groups representing <20% and >80% respectively of the periphyton composition in both treatments (Figs. 10 and 11). The total mean biomass of all taxa in CA ponds = 40.39 x 10^9 $\mu m^3/cm^2$, whereas in NA ponds = 33.84 x 10^9 $\mu m^3/cm^2$. Mean diatoms in CA ponds was 5.49 x 10^9 $\mu m^3/cm^2$ while in NA ponds was 6.10 x 10^9 $\mu m^3/cm^2$. In contrast, mean biomass for non-diatoms = 34.90 x 10^9 $\mu m^3/cm^2$ in CA ponds and for non-diatoms = 27.74 10^9 $\mu m^3/cm^2$ in NA ponds.

Mean relative biovolume of 26 periphyton taxa were statistically different between CA and NA ponds (Table 4). Taxa that exhibited the greatest magnitude (>10X) in mean differences between treatments were *Aphanothece*, *Planktothrix*, *Schroederia setigera*, *Bulbochaete*, *Desmidium*, *Eunotia*, *Quadrigula closterioides*, *Rhipidodendron splendidum*, *Tabellaria fenestrata*, and *Xanthidium antilopaeum*. The first three taxa had greater-biovolume in CA ponds; the biomass was greater for the others in NA ponds. Other taxa of interest due to their known association with degraded water quality conditions included the diatoms *Navicula cryptocephala* and *N. radiosa*, the filamentous cyanobacterium *Oscillatoria* and the euglenoid *Trachelomonas.* Biomass of these taxa was 1.6X – 4.5X (66 – 351%) greater in CA ponds. Mean biovolume of the diatoms *Fragilaria* and *A. minutissimum* was 1.6X – 3.1X (62 – 209%) greater in NA wetlands, which are known to be associated with high water quality (Table 4). Based upon statistically significant taxa (Table 4), the total mean biomass in CA ponds = 4.46 x 10^8 $\mu m^3/cm^2$ and in NA ponds = 8.29 x 10^8 $\mu m^3/cm^2$. Mean biomass of diatoms in CA ponds = 0.73 x 10^8 $\mu m^3/cm^2$, and in NA ponds = 1.49 x 10^8 $\mu m^3/cm^2$. Mean biomass of non-diatom algae = 3.73 x 10^8 $\mu m^3/cm^2$ in CA ponds and in NA ponds = 6.80 x 10^8 $\mu m^3/cm^2$.

Month and treatment effects interacted for eight taxa, thus analyses were separated by month (Table 4). For these taxa, treatments were not different every month. For example, the mean biovolume of the diatom *Navicula radiosa* was on average 2.2X greater in CA ponds, but only during May. Mean biovolume of the unicellular chlorophyte *Scenedesmus* was on average 4.3X greater in CA ponds during March, May, June, July, and August only (Table 4).

I detected a depth effect in mean biovolume for two taxa: the coccoid chlorophyte *Chlorella* and the diatom *Nitzschia.* Mean biovolume of *Chlorella* was 3.3X greater on tiles attached to shallow bricks ($\bar{x}$ = 4.58 x 10^5 $\mu m^3/cm^2$, SD =1.35 x 10^6 $\mu m^3/cm^2$) compared to deep bricks ($\bar{x}$ = 1.38 x 10^5 $\mu m^3/cm^2$, SD = 2.81 x 10^5 $\mu m^3/cm^2$; $F_{1,138}$ =

4.69, $P = 0.03$). Mean biovolume of *Nitzschia* was 1.5X greater on shallow bricks ($\bar{x}$ = 3.84 x 10^8 $\mu m^3/cm^2$, SD = 5.78 x 10^8 $\mu m^3/cm^2$) compared to deep bricks ($\bar{x}$ = 2.45 x 10^8 $\mu m^3/cm^2$, SD = 2.92 x 10^8 $\mu m^3/cm^2$; $F_{1,138} = 5.92$, $P = 0.02$). Mean depth at shallow and deep bricks was 9.7 (SD = 3.9) and 24.8 (SD = 13) cm, respectively, which was statistically different ($F_{1,14} = 9.8$, $P = 0.007$). Although mean photosynthetic light was 14% greater at shallow bricks ($\bar{x}$ = 348.7, SD = 349.8 µmol photons$\cdot m^{-2}\cdot s^{-1}$) compared to deep bricks ($\bar{x}$ = 307.3, SD = 316.2 µmol photons$\cdot m^{-2}\cdot s^{-1}$), no statistical differences were detected ($F_{1,61} = 1.23$, $P = 0.27$).

Water Quality

Several water quality variables were different between CA and NA ponds (Table 5). The pH, specific conductivity, and turbidity were 7% – 4.8X greater in CA ponds compared to NA ponds. For the variables that were measured from August water samples, Kjeldahl nitrogen, total phosphorus, total residue concentration, and chemical oxygen demand were 2.5 – 3.7X greater in CA ponds. Concentrations of several metals also were different between CA and NA ponds. Silicon, aluminum, iron, magnesium, and potassium were 47% – 5.7X greater in CA ponds compared to NA ponds. Periphyton phosphorus was infinitely greater in CA ponds (Table 5). No additional differences in water variables were detected between CA and NA ponds, including the average intensity of photosynthetic active radiation in the water column (NA: $\bar{x}$ = 348.7 µmol photons $\cdot m^{-2}\cdot s^{-1}$, SD = 349.8, and CA: $\bar{x}$ = 307, SD = 316.2 µmol photons $\cdot m^{-2}\cdot s^{-1}$, $F_{1,70} = 0.96$, $P > 0.33$).

Only one water quality variable (concentration of potassium) was retained in the stepwise multiple linear regression model ($F_{1,6} = 22.7$, $P = 0.003$, Fig. 12). Potassium explained 79% of the variation in the Kentucky Index among ponds. The concentration of potassium was strongly negatively correlated with the Kentucky Index (Fig. 12).

Discussion

I documented 181 new periphyton taxonomic records (7 phyla, 52 families, and 132 genera) for Cumberland County, Tennessee. To my knowledge, there has been only one other study in the Cumberland Plateau region that specifically examined benthic algal assemblages. Vaughan (1979) investigated fish and diatom communities in New River and its tributaries (Scott County, Tennessee) to assess the impacts of coal mining activities on water quality. Thus, my results contribute to the understanding of periphyton communities in Tennessee.

Periphyton Responses

I found that cattle access significantly influenced the community composition and biovolume of some periphyton taxa. Periphyton genera richness in no-access (NA) ponds was 15% greater than in cattle-access (CA) ponds, which was equivalent to approximately 4 additional genera per pond at NA sites. The Kentucky Index, which is a measure of environmental pollution with low values indicating degraded conditions, was 20% greater in NA ponds compared to CA ponds. These results are concerning because periphyton are the basis of many aquatic food webs (Lowe and Pan 1996). No studies have previously examined the impacts of cattle-induced organic pollution on periphyton communities in Tennessee wetlands; however, several studies elsewhere have documented the effects of nutrient enrichment associated with agriculture on periphyton community structure (e.g., Rader and Richardson 1992, Pan et al. 2000, McCormick et al. 2001). Collectively, these studies indicate that excessive nutrients result in degraded water quality and increased impacts on algal communities (Smith et al. 1999, Stoermer and Smol 1999, Carrick and Lowe 2007). Algal communities exposed to environmental stressors are typically composed of fewer pollution-tolerant species (Schelske and Stoermer 1971, Whitton et al. 1991, Harrison 2001). Thus, my study suggests that cattle access in farm ponds degraded water quality and reduced periphyton biovolume of some taxa.

Based upon total cell counts, my results indicated that the periphyton community in NA ponds was composed mostly of diatoms (Phylum Bacillariophyta) whereas non-diatom algae dominated the community in CA ponds. The phylum Chlorophyta composed the majority of non-diatom algae. Several studies have reported that organic enrichment can shift algal communities from domination by diatoms to non-diatom taxa such as Chlorophyta and Cyanophyta (Patrick 1977, Steinman et al. 1991, Leland 1995). One possible mechanism may be secondary silicon limitation associated with nutrient enrichment (Schelske and Stoermer 1971,1972, Carrick et al. 1988, Carrick and Lowe 2007). The optimum nutritional requirement for diatom frustule development in aquatic systems is related to the Redfield-Brzezinski nutrient ratio (i.e., C:Si:N:P = 106:15:16:1, Brzezinski 1985). Thus, because of this delicate nutritional balance, even modest levels of P enrichment can induce Si limitation, which in turn, causes a shift from diatom flora to chlorophyte and cyanobacteria species (Carrick and Lowe 2007). Chemical stress from high nutrient concentrations and competitive interactions between diatoms and non-diatom algae are other possible mechanisms driving this assemblage shift (Palmer 1969, Zhang and Prepas 1996, Finkel et al. 2009).

In contrast, my biovolume results indicated that the periphyton community in both NA and CA ponds was composed mostly of non-diatom algae (e.g., chlorophytes, cyanophytes, euglenoids). The biovolume of diatoms was greater in NA ponds than in CA ponds. I found that mean relative biovolume of 26 periphyton taxa were statistically different between CA and NA ponds. The diatom *Navicula cryptocephala* and the chlorophyte *Scenedesmus* had the greatest biomass in CA ponds. The greatest differences in mean biovolume (≥10X) between treatments for CA ponds were for the cyanobacteria *Aphanothece* and *Planktothrix* and the chlorophyte *Schroederia setigera*. Additionally, the cyanobacterium *Oscillatoria* and unicellular euglenoids *Euglena*, *Phacus* and *Trachelomonas volvocina* exhibited greater biovolume in CA ponds. These results provide additional evidence of the prevalence of non-diatom periphyton in CA ponds.

Collectively, the above taxa in CA ponds are typically associated with degraded water quality and organic pollution (Palmer 1969, Lange-Bertalot 1979, Hill et al. 2000). However, the result that the diatom *N. cryptocephala* biovolume was greater in CA ponds is surprising because it is considered to be a pollution-sensitive species (Kelly et al. 2005, KDOW 2008). In contrast, studies investigating the effects of organic enrichment on benthic diatoms in streams and a lake determined that *N. cryptocephala* was tolerant of pollution (Guerrero and Rodriguez 1991, Rakowska 2004). Other researchers have considered high proportions of the genus *Navicula* (including *N. cryptocephala*) within a population to generally suggest degraded water quality (Palmer 1969, Biggs et al. 1998). The chlorophyte *Scenedesmus* is frequently abundant in nutrient-rich waters and considered to be a strong indicator of degraded water conditions (Theng 2003, Jafari and Gunale 2006, Szeląg-Wasielewska 2007). Palmer (1969) classified *Scenedesmus* as one of the top eight taxa tolerant of organic pollution. He also classified the filamentous cyanophyte *Oscillatoria* in this category (Palmer 1969), which was more abundant in CA ponds. *Oscillatoria* often forms floating dense mats in response to organic pollution and out-competes other algae by producing cyanotoxins, which inhibit growth and can reduce survival (Viaggiu et al. 2004, Jafari and Gunale 2006). Similarly, filamentous cyanophyte *Planktothrix* and the coccoid cyanophyte *Aphanothece* form dense aggregations, produce cyanotoxins, and are classified as pollution-tolerant taxa (Lepistö et al. 2004, Carrasco et al. 2007). Several investigators consider the euglenoids (*Trachelomonas, Euglena, and Phacus)* as taxa that are tolerant of excessive organic pollution (e.g., Palmer 1969, Jafari and Gunale 2006). Euglenoids are particularly abundant in eutrophic lakes and reservoirs surrounded by agricultural land where runoff of organic nutrients is high, and they may reach bloom proportions in ponds exposed to animal feces (Round and Palmer 1966, Say and Whitton 1980, Rosowski 2003). Ecological information is scarce about the unicellular chlorophyte *Schroederia setigera* except that it is a monotypic genus in the

United States, and is found in the phytoplankton of ponds and lakes (Shubert 2003). My results suggest these taxa are pollution-tolerant species.

In contrast, NA ponds were dominated mostly by periphyton taxa that are not associated with degraded water quality. The most common diatom taxa based on cell counts in NA ponds were *Achnanthidium minutissimum* and *Fragilaria crotonensis*. However, based on my statistically significant biovolume results, the most common (i.e., greater biomass) diatoms in NA ponds were *Navicula cryptocephala* and *Fragilaria crotonensis.* The greatest differences in mean biovolume between treatments (>10X) occurred for *Bulbochaete*, *Desmidium*, *Eunotia*, *Quadrigula closterioides*, *Rhipidodendron splendidum*, *Tabellaria fenestrata*, and *Xanthidium antilopaeum*. The diatom *Achnanthidium minutissimum* has been recognized as a pollution-sensitive species (KDOW 2008). However, others have suggested that it can tolerate moderate levels of organic pollution in rivers (Palmer 1969, Jafari and Gunale 2006). My results provided support *A. minutissimum* as a pollution-sensitive species (at least in enriched Tennessee farm ponds), but its classification may depend on the type of aquatic system (e.g., lentic vs. lotic). As mentioned previously, the diatom *N. cryptocephala* is considered to be a pollution-sensitive species by some researchers (Genter et al. 1987, Kelly et al. 2005, KDOW 2008), but in other studies, *N. cryptocephala* demonstrated a tolerance to organic pollution (Palmer 1969, Guerrero and Rodriguez 1991, Rakowska 2004). My results indicate that the diatom *Fragilaria crotonensis* exhibited greater biovolume in NA ponds than CA ponds, suggesting it is sensitive to organic pollution. This is another surprising result because *F. crotonensis* has been reported to increase in response to elevated phosphorus concentrations (Stoermer et al. 1978) and organic pollution (Jafari and Gunale 2006), and is a dominant summer taxon in the phytoplankton of eutrophic lakes (Wehr and Sheath 2003). Consistent with my result as a pollution-sensitive taxon, other researchers have demonstrated the *Fragilaria* group to be a pollution-sensitive genus (Bahls 1993, KDOW 2008). The unicellular chlorophyte *Desmidium* and the free-floating diatoms *Eunotia* and *Tabellaria fenestrata* are known to be associated with oligotrophic or reference sites conditions, and are classified as a pollution-sensitive taxa (Coesel et al. 1978, Yung et al. 1986, Neustrupa et al. 2009). Other species of *Eunotia* are known to attach to substrates with mucilaginous stalks (Wehr and Sheath 2003). The two diatom taxa may out-compete other algae by forming chains or zig-zag filaments suspended in the water column (Kociolek and Spalding 2003, Kelly et al. 2005). Very little information is available on the ecological roles of the colonial flagellate *Rhipidodendron splendidum* and the chlorophytes *Bulbochaete* sp. *Quadrigula closterioides*, and *Xanthidium antilopaeum* (Gerrath 2003, Lepistö et al 2004 and Naz et al. 2008). My results suggest these taxa are pollution sensitive.

The filamentous green algae *Spirogyra* had the greatest biovolume among taxa in NA ponds suggesting it is sensitive to agricultural pollution from cattle. During a concurrent PREC study, Schmutzer (2007) reported that dry biomass of *Spirogyra* was greater in NA ponds compared CA ponds. Wehr and Sheath (2003) characterize *Spirogyra* as proliferating in more oligotrophic systems along with epiphytic diatoms. In contrast, Palmer (1969) classified *Spirogyra* as somewhat tolerant of organic pollution.

My results suggest that pollution associated with cattle in farm ponds may limit the growth of *Spirogyra*.

Although total proportion of diatom taxa (approximately 62% composition) exceeded abundance of non-diatom taxa (38%) across treatments, biovolume of diatoms (20%) was much less than non-diatoms (80%). Other studies have reported that non-diatom algae (in addition to diatoms) are critical in nutrient cycling and carbon sequestration in aquatic systems (Mulholland et al. 1991, Smith et al. 1999). Thus, my results suggest non-diatoms may have played a more significant role in the food chain dynamics in the PREC pond system.

<u>Water Quality</u>

Differences in water quality likely contributed to periphyton trends observed between CA and NA ponds. Turbidity, total suspended solids, and conductivity in CA ponds were 60% – 4.8X greater than in NA ponds likely due to cattle depositing feces and disturbing sediments. Particulate matter in the water column may hinder growth of periphyton by covering benthic substrates or reducing the photic zone (Robel 1961, Dieter 1991, Birkett et al. 2007). Given that I found no significant differences in light intensity penetrating the water column between pond treatments, the former mechanism may have been most important at my study sites. A negative association between periphyton richness, turbidity and conductivity has been reported in other studies (Biggs 1995, Lavoie et al. 2004, Greenwood and Lowe 2006). My results indicated that genera richness decreased in the cattle ponds which had higher turbidity and conductivity compared to the no-access ponds.

Nutrients including total Kjeldahl nitrogen, potassium, and periphyton phosphorus were 2.85X – 4.85X (185 – 384%) greater and total phosphorus was infinitely greater in CA ponds compared to NA ponds likely due to fecal inputs from cattle. Brown and Archer (1987) reported that on average one cow annually excretes 136 kg of nitrogen, 20 kg of phosphorus and 75 kg of potassium. In a concurrent PREC study, Schmutzer et al. (2008) determined that ammonia, nitrite and nitrate were elevated (5-216%) in CA ponds. In a laboratory culture study, Admiraal (1977) found that ammonia concentrations >0.5 mg/L inhibited the growth of several species of diatoms, and that inhibition of photosynthesis by ammonia was strongly enhanced under conditions of high irradiance and pH. Other research confirmed a significant decline of diatom populations in response to concentrations of ammonia >0.06 mg/L (Hürlimann and Schanz 1993, Blanco et al. 2008). Nitrogen can be a limiting nutrient in aquatic systems and promote periphyton growth (Lin et al. 2008); however at high levels, it also can be toxic to algal taxa, especially diatoms (Nõges et al. 2008). My results suggest that elevated nutrient levels due to cattle defecation and urination in CA ponds affected benthic algal community composition, structure and biovolume.

High levels of phosphorous also can impact the periphyton community. Mean phosphorus in CA ponds were 0.27 mg/L, which is higher than levels necessary to observe shifts in the periphyton community. A transition from oligotrophic to eutrophic periphyton taxa was documented in the Florida Everglades at total phosphorus concentrations of 0.01 – 0.02 mg/L (McCormick et al. 1996, Pan et al. 2000, Vis et al. 2008). During flow-through laboratory stream experiments, Hill and Fanta (2008) observed that periphyton growth increased hyperbolically with increasing phosphorus, reaching a threshold of growth saturation between 0.022 – 0.082 mg/L. Thus, at my study ponds, phosphorus likely played a role in structuring periphyton communities.

I found the periphyton phosphorus concentrations in my CA ponds = 0.00517 mg P/mg ash free dry mass (1489 mg P/kg dry mass) and in NA ponds = 0.00233 mg P/mg ash free dry mass (1078 mg P/kg dry mass). My periphyton phosphorus results are within the range of recent research findings in stream periphyton (0.0023 – 0.0369 mg P/mg ash free dry mass, Fanta et al. 2010). Periphyton phosphorus concentrations in highly enriched areas of the Florida Everglades are reported at levels >4000 mg P/kg dry mass and <200 mg P/kg dry mass in reference areas (McCormick and Stevenson 1998). Given this is a wetland comparison between a natural marsh (Florida) and constructed farm ponds (Tennessee), both of which have been impacted by agricultural pollution, my results of elevated periphyton phosphorous (i.e., converted to mg P/kg dry mass) in impacted sites are consistent with the Florida Everglades study.

Concentrations of several metals (silicon, iron, aluminum and magnesium) were 1.46X – 5.71X (46 – 471%) greater in CA ponds. Silicon is a major limiting nutrient for diatom growth and primary production (Martin-Jézèquel et al. 2000). I expected that silicon would be limiting in nutrient-enriched wetlands, because Carrick and Lowe (2007) predicted that modest levels of nutrient enrichment can induce secondary silicon limitation from increased diatom growth. However, mean Si concentration was 4.5 mg/

L at cattle-access sites. Concentrations of Si <0.50 mg/L is considered limiting (Schelske et al. 1986, Wetzel 2001). Thus, I suspect that nutrient concentrations in CA ponds affected the response of individual pollution-tolerant and pollution-sensitive taxa, exceeded their chemical tolerance thresholds, and facilitated growth of competing non-diatom periphyton that tolerate organic pollution (McCormick and Stevenson 1998). The change in the periphyton community to fewer diatoms may have contributed to greater silicon in CA ponds.

Iron and aluminum concentrations in CA ponds also were greater than in NA ponds. These metals may have been elevated in CA ponds from cattle continuously stirring sediments while wading in ponds. High concentrations of Fe and Al (>1.0 and >0.087 mg/L, USEPA 2009) can negatively impact periphyton biomass (Gensemer 1990, Sheldon and Skelly 1990, Niyogi et al. 1999). Total biomass for statistically significant taxa in NA ponds (biovolume = 8.29×10^8 um^3/cm^2) was greater than in CA ponds (biovolume = 4.46×10^8 um^3/cm^2). For Fe and Al, my results exceeded EPA thresholds as Fe and Al concentrations were 2.58 and 0.78 mg/L in CA ponds respectively, and Al was 0.14 mg/L in NA ponds. Besser et al. (2001) reported that high concentrations of aluminum and iron in periphyton were associated with reduced biomass suggesting degradation of benthic habitats by deposition of colloidal Al and Fe oxides may cause reduced primary production. During laboratory mesocosm experiments, Al was found to inhibit the growth of the diatom, *Cyclotella meneghiniana*, at a concentration of 0.81 mg/L (Rao and Subramanian 1982). The green alga, *Selenastrum capricornutum*, was inhibited at Al concentrations >0.46 mg/L in laboratory experiments (Peterson et al. 1974). Thus, elevated concentrations of Al and Fe may have impacted periphyton biomass and taxa composition.

In my study, magnesium was found to be greater in CA ponds (1.7 mg/L) than in NA ponds (1.1 mg/L). Magnesium is required by chlorophyllous plants as the Mg prophyrin component of chlorophyll molecules and as a micronutrient in enzymatic transformations of algae, fungi and bacteria (Wetzel 2001). Low available Mg has been implicated as potentially inhibiting phytoplankton productivity in oligotrophic lakes (Goldman 1960, Sudhakar et al. 1994). In contrast, concentrations of 0.42 M $MgSO_4$ and 0.22 $MgCl_2$ decreased abundance of the coccoid chlorophyte *Chlorella* by 50% in laboratory experiments (Trelease and Selsam 1939). Thus, concentrations of Mg may have impacted periphyton biomass and taxa composition in my study ponds.

I built a model using stepwise multiple linear regression to relate water quality to the Kentucky Index. The only variable that was retained was potassium, and it had a strong negative correlation with the index. Potassium is an important nutrient for autotrophs to sustain photosynthesis and for active transport of nutrients and essential metals through the plasmalemma into the cytosol (i.e., ionic osmosis across cell membranes, Overnell 1975, Bhattacharyya and Volcani 1980, Reynolds 2006). As discussed earlier, potassium compounds can have negative effects on diatom assemblages at concentrations of 73 – 400 mg/L (Parker et al. 1997, Mischke et al. 2006). At my ponds, mean potassium levels ranged from 3.2 (NA) – 15.5 (CA) mg/L. Potassium in most freshwater systems normally exists between 1 – 5 mg/L (Mohleji and Verhoff 1980), which is slightly lower concentrations than my CA pond results. It is known that potassium salts inhibit growth of diatoms (Parker et al. 1997, Zheng et al. 2005, Mischke et al. 2006). However, potassium also is a major cellular cation in cyanobacteria, and K^+ starvation may cause multiple metabolic impairments that limit photosynthesis and growth (Alahari and Apte 1998). Thus, elevated potassium can negatively affect diatoms while positively impacting cyanobacterium, which supports the periphyton changes that I observed between CA and NA ponds. The strong relationship (R^2 = 0.79) between the Kentucky Index and potassium concentration suggests that potassium may be a good variable to include in future bioassessments, where large variation in potassium levels are expected (e.g., watersheds impacted by organic pollution) and diatoms are used as an indicator community.

Water Depth

I found that biomass of the coccoid chlorophyte *Chlorella* was greater on the artificial substrate placed at 0.5 m from shore (biovolume = 4.58×10^5 um^3/cm^2) compared to the one placed at 2 m from shore (biovolume = 1.38×10^5 um^3/cm^2). The biomass for *Chlorella* was greater in CA ponds (biovolume = 3.25×10^5 um^3/cm^2) than in NA ponds (biovolume = 2.77×10^5 um^3/cm^2). The biomass for *Nitzschia* was greater on the artificial substrate placed at 0.5 m from shore (biovolume = 3.84×10^8 um^3/cm^2) compared to the one placed at 2 m from shore (biovolume = 2.45×10^8 um^3/cm^2). However, the biomass for *Nitzschia* in NA ponds (biovolume = 3.32×10^8 um^3/cm^2) was greater than in CA ponds (biovolume = 3.00×10^8 um^3/cm^2). Mean depth among ponds at the shallow and deep substate was 10 and 25 cm, respectively, which was statistically different. Mean photosynthetic light at the shallow and deep substrate were 348 and 307 µmol photons $\cdot m^{-2} \cdot s^{-1}$, respectively, but statistical differences were not detected. The photosynthesis-irradiance responses of periphyton were investigated in developmental sequences from three stream locations with differed in canopy cover (Hill and Boston 1991). They reported that saturation of photosynthesis occurred between 130 – 385 µmol photons $\cdot m^{-2} \cdot s^{-1}$, and that photosynthesis was slightly inhibited at 600 µmol photons $\cdot m^{-2} \cdot s^{-1}$. Photosynthesis of most benthic algal assemblages saturates above 100 µmol photons $\cdot m^{-2} \cdot s^{-1}$ (Hill 1996). Thus, I assume that photosynthesis of benthic algae in both of my treatments reached saturation because both >100 umol photons$\cdot m^{-2} \cdot s^{-1}$. Because I did not record continuous light data, I am reluctant to conclude that the slight differences between my CA and NA mean light data limited algal growth.

Chlorella is a coccoid, colonial chlorophyte that is characterized by Palmer (1969) as an indicator taxon tolerating organic pollution. Several studies have reported that changes in light intensity can significantly affect periphyton growth (Kim and Richardson 2000, Hill and Fanta 2008, Ragni et al. 2008). The greater abundance of *Chlorella* on the shallow substrate suggests this taxon is especially sensitive to light limitation, considering this relationship was not observed with other taxa and statistical differences in light intensity between shallow and deep substrates were not detected. Alternatively, a factor that interacted with light intensity or depth (e.g., competitive exclusion by other taxa, nutrient concentration and depredation) may have impacted the relative abundance of *Chlorella* at deeper substrates. My results suggest *Chlorella* is a pollution-tolerant taxon because it exhibited greater biomass in CA ponds.

Many species of the genus *Nitzschia* are recognized as indicators of organic enrichment or pollution of the water in which they are found (Lowe 1974, Wehr and Sheath 2003, Jafari and Gunale 2006) and environmental disturbances (siltation index, Bahls 1993). Hence, it is surprising that the biovolume of *Nitzschia* was slightly greater in NA ponds than in CA ponds. Perhaps an explanation is that many *Nitzschia* species are nitrogen heterotrophs and have the ability to capitalize on available organic nitrogen molecules available in the water (Wehr and Sheath 2003). Another reason is that substantial populations of *Nitzschia* can be found in the upper few cm of loose lake sediment (epipelic and epipsammic substrata, Moss 1977, Wehr and Sheath 2003). The pond bottom substratum in all 8 of my study ponds consist of clayey-silty-sandy muck which are likely highly colonized by *Nitzschia* regardless of cattle presence.

<u>Other Potential Mechanisms</u>

Changes in shoreline vegetation associated with cattle access also may have had direct effects on the periphyton community. Two concurrent studies found that shoreline vegetation was 25 – 85% greater and detrital biomass was 5 – 22X greater in NA ponds (Schmutzer et al. 2008, Burton et al. 2009). Submersed shoreline vegetation and detritus are important attachment sites for many periphyton species (Vădineanu et al. 1992, Kelly and Hawes 2005, Messyasz and Kuczyńska-Kippen 2006). In the Florida Everglades, production by some algae (mainly filamentous chlorophytes) is increasing while the original mat matrix may be losing considerable quantities of detrital (and bacterial) biomass, in addition to cyanobacteria and their calcite precipitate (Gaiser et al. 2006).

Changes in water quality and shoreline vegetation also may have had indirect effects on the periphyton community through affecting trophic level interactions. For example, tadpole species richness and relative abundance were lower in my CA ponds compared to the NA ponds (Schmutzer et al. 2008). Changes in water quality from cattle also affect aquatic invertebrate populations (Braccia and Voshell 2007, Schmutzer 2007). Tadpoles and aquatic macroinvertebrates are known consumers of periphyton (Hill and Knight 1988, Kupferberg 1997). Consumers in higher trophic levels can have profound impacts on biomass and species composition of lower-level consumer and primary producer assemblages (Power 1992, Carpenter and Kitchell 1993, Leibold 1996). Further, some emergent plant species share a near-symbiotic relationship with certain periphytic algae communities (Eminson and Moss 1980, Blindow 1987). Disruption of these delicate biological associations may induce trophic changes that affect periphyton assemblages (Chick et al. 2008). Bottom-up effects also can occur on higher trophic levels as periphyton community composition and biomass change in response to decreases in water quality (Rosemond et al. 1993, Sinistro 2010). The potential for these types of trophic-level interactions emphasize the importance of promoting land-use practices that reduce or eliminate impacts on the native periphyton community.

Conclusions and Conservation Recommendations

My study provided evidence that cattle grazing in wetlands affected periphyton genus richness, abundance, biovolume, and water quality on the Cumberland Plateau, Tennessee. I compared periphyton communities between cattle-access (CA) and no-access (NA) farm ponds. Decreases in water quality associated with fecal deposition and mechanical disturbances by cattle likely induced stress that caused changes in periphyton community. My study provided evidence that periphyton community composition is a reliable bioindicator of pollution associated with livestock access to watersheds. My study suggests that periphyton community responses were driven by elevated concentrations of nutrients, metals, conductivity and turbidity due to cattle access. Also, the loss of emergent hydrophytes in CA ponds may have contributed to changes in the periphyton community structure compared to NA ponds.

Periphyton species richness was greater in NA ponds. Specifically, mean abundance and biovolume of pollution-sensitive diatoms (e.g., *Achnanthidium minutissimum, Cymbella* sp., *Eunotia* sp., and *Tabellaria fenestrata*) was greater in NA ponds. In contrast, pollution-tolerant diatoms (e.g., *Gomphonema* sp. and *Navicula* sp.) and non-diatoms (e.g., *Oscillatoria* sp., *Scenedesmus* sp., and *Trachelomonas volvocina*) were more abundant and exhibited greater biovolume in CA ponds. These trends were likely driven by high nutrient (e.g., total Kjeldahl nitrogen, total P, periphyton P) and metal concentrations (e.g., Al and Fe) in CA ponds, which can affect periphyton by exceeding metals and nutrients tolerance thresholds or impacting competitive interactions among periphyton species. Although diatoms comprised over half of the total abundance of periphyton in cell counts, biovolume of non-diatom algae was 4X greater than diatoms. Thus, non-diatom algae likely play a more significant role in the energy dynamics of farm ponds (i.e., the food chain).

Turbidity, pH, conductivity, and concentrations of the total Kjeldahl nitrogen, total phosphorus, potassium, and periphyton phosphorus were greater in CA ponds compared to NA ponds. Livestock degrade water quality by grazing, defecating and urinating in surface water (Platts 1991, Belsky et al. 1999, Hooda et al. 2000), which often results in eutrophication (Griffith et al. 2002, Dunne et al. 2005). Increased sedimentation and turbidity are additional stressors associated with cattle disturbance (Mulkey 1977, Campbell and Allen-Diaz 1997). Deposition of nitrogenous waste in wetlands causes change in algal community structure (Rader and Richardson 1992, McCormick and O'Dell 1996, Havens et al. 1999), which can affect other trophic levels (Wohl and Carline 1996, Scrimgeour and Kendall 2003, Braccia and Voshell 2007). Silicon, iron, magnesium and aluminum also were in greater concentrations in CA ponds. Elevated concentrations of iron and aluminum are known to inhibit periphyton production (Gensemer 1990, Sheldon and Skelly 1990, Niyogi et al. 1999). My results for Fe (2.58 mg/L) and Al (0.78 mg/L) exceeded EPA thresholds (Fe = >1.0 and Al = >0.087 mg/L) in CA ponds known to negatively impact periphyton. I hypothesize that multiple variables interacted and exceeded survival thresholds of various pollution-sensitive periphyton taxa. Results from my study suggests that elevated metals (Al, Fe), nutrients (P, N, K and periphyton phosphorus), turbidity, conductivity, and loss of facultative wetland plants were likely mechanisms driving periphyton responses and thereby the differences in periphyton community structure, composition and biovolume between CA and NA ponds.

Due to the potential negative impact of cattle on periphyton communities, I recommend fencing cattle from natural water sources. Ideally, at least a 10-m buffer should be created to promote growth of shoreline vegetation and reduce the effects of nutrient inputs from cattle (Chalmers 2000). Excluding cattle 10 – 16 m from streams in North Carolina lowered weekly phosphorous loads by 76% (Line et al. 2000). I recommend providing alternate water sources (e.g., well water, or gravity fed spring or pond water) for cattle to reduce the need to use surface water (Nader et al. 1998). Farmers are encouraged to participate in government programs (e.g., Wetlands Reserve Program, Conservation Reserve Program) that provide financial assistance for incorporating best management land-use practices that help maintain herd health and minimize environmental impacts (USDA National Resources Conservation Service 2005). Several programs are available that can provide cost-share assistance for fencing and alternate water sources for cattle.

Fencing cattle from farm ponds also helps protect human and cattle health by promoting non-toxic periphyton communities. In my study, blue-green algae (Cyanophyta) were most common in CA ponds. Strains of several cyanobacterial genera produce high levels of hepatotoxins (microcystins, nodularins, cylindrospermopsin) and neurotoxins (e.g., anatoxin-a, saxotoxins; Carmichael et al. 1997, Rapala et al. 1997, Rinta-Kanto and Wilhelm 2006). Several studies have reported cattle mortality associated with blue-green algae hepatotoxicosis (Fitzgerald and Poppenga 1993, Puschner et al. 1998). Humans also can be impacted by cyanobacteria. For example, a major outbreak of gastrointestinal illness in 5000 residents was attributed to contamination of the water supply by *Schizothrix, Plectonema, Phormidium,* and *Lyngbya* in Sewickley, Pennsylvania (Falconer 1994). Thus, elevated densities of cyanobacteria in natural water sources are a beef production and human health concern (USEPA 1998).

Research Needs

Additional research is needed to fully understand the impact that cattle may have on the periphyton community. In particular, mechanisms of periphyton response need to be identified. I recommend taking an aquatic mesocosm approach where periphyton communities are established in 1000-L outdoor tanks and cattle feces is added daily at different concentrations. Fecal concentrations should be related to a typical range of herd sizes in Tennessee. Nutrients and metals in the wet feces and the water column could be measured to gain a greater appreciation of the relationship between these water quality variables and periphyton community composition. Mesocosm studies could be coupled with laboratory experiments that expose representative periphyton taxa to different concentrations of nutrients and metals to identify tolerance thresholds. Ultimately, survival and relative abundance estimates from mesocosm and lab experiments could be used to model periphyton community dynamics under different cattle-access regimes. I suspect that a certain amount of cattle access in wetlands would have minimal impacts on periphyton assemblages or may be beneficial. More complex studies could incorporate higher trophic levels to investigate food web interactions or use different levels of shading to simulate vegetation reduction. Additional field experiments could include investigating periphyton responses to different cattle-access scenarios such as partial exclosure from a farm pond or rotational grazing. A useful technique study would be investigating differences in periphyton colonization between artificial and natural substrates.

My study has contributed to the knowledge (181 new taxonomic records) and understanding of periphyton communities and their response to agricultural stressors in Cumberland Plateau wetlands. Voucher specimens are stored at the Tennessee Department of Environment and Conservation, Department of Energy Oversight Division, 761 Emory Valley Road, Oak Ridge, Tennessee. Information from my research has been used in the development of the Tennessee Department of Environment and Conservation (TDEC), Division of Water Pollution Control's standard operating procedures for sampling of periphyton communities in Tennessee streams (TDEC 2010). These procedures are being used to identify water bodies in Tennessee that are impaired due to nutrients. To date, more than 1,500 stream miles have been identified as impaired in Tennessee due to nutrients (TDEC 2004).

The Kentucky Index model that I developed may be useful for biologists conducting bioassessments in streams and wetlands. Potassium concentrations explained a substantial amount of variation (80%) in the Kentucky Index. Thus, biologists could use my model (Figure 12) to predict values of the Kentucky Index using potassium measurements in water sources instead of enumerating diatom taxa. Given that my model was developed across a wide range of potassium concentrations (1 – 19 mg/L), it likely has widespread applicability in farm pond systems.

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APPENDIX

Table 1. Size, depth and vegetation characteristics at cattle-access (CA) and no-access (NA) ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006.

Pond	Land Use	Ha	Depth (cm)[a]		canopy
			Brick 1	Brick 2	
1	CA	0.14	7.5	15.3	open
2	CA	0.28	10.1	20.2	open, macrophytes
3	CA	0.61	13.3	35.0	open
4	CA	0.23	5.1	8.3	shaded
5	NA	1.04	10.9	12.3	open, macrophytes
6	NA	0.25	16.7	35.2	shade, macrophytes
7	NA	0.25	6.4	26.3	open, macrophytes
8	NA	0.3	7.8	45.7	open, macrophytes

[a]Depths measured every two weeks from May 2005 – August 2006 and averaged; bricks 1 and 2 were 0.5 and 2 m from the shoreline respectively.

Table 2. Monthly temperature and precipitation results from weather station at the University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006.

	Temperature (℃)			Precipitation (cm)			
Date	Mean Monthly	Mean Max	Mean Min	Mean Monthly	Total Monthly	Storm Date	Storm Event Total (>2.54 cm, 1 or more consecutive days)
May-05	15	21.1	7.9	0.19	6.14	20 May	5.21
Jun-05	20.4	25.7	15.3	0.27	7.52		
Jul-05	22.6	27.2	18.5	0.63	19.66	12-15 Jul	2.44,1.22,4.27,1.65
						1 Aug	3.35
Aug-05	22.7	28.0	18.0	0.33	9.47	18-19 Aug	0.68,1.90
Sep-05	19.7	26.4	12.9	0.19	5.89	26-27 Sep	0.91,0.31
Oct-05	12.4	18.8	6.4	0.11	3.53		
						15-16 Nov	2.67,2.62
						21-22 Nov	0.18,2.41
Nov-05	8.2	14.3	1.3	0.42	12.72	29 Nov	3.83
Dec-05	0.6	5.6	-4.1	0.31	9.6	15 Dec	3.53
						17 Jan	4.42
Jan-06	5.4	10	-0.1	0.52	16.02	22-23 Jan	2.03,1.55
Feb-06	2	7	-3.3	0.29	8.08		
Mar-06	7.8	13.3	1.6	0.29	9.17	14 Mar	3.33
						3 Apr	3.25
						8 Apr	9.47
						21-22 Apr	1.52,2.41
Apr-06	15	20.7	8.3	0.65	19.51	25-26 Apr	0.23,2.41

Table 3. New periphyton taxonomic records for Cumberland County, Tennessee, University of Tennessee Plateau Research and Education Center, May 2005-April 2006.

Taxa	Family	Phylum
Acanthosphaera	Micractiniaceae	Chlorophyta
Achnanthes lanceolata	Achnanthaceae	Bacillariophyta
Achnanthidium minutissimum	Achnanthaceae	Bacillariophyta
Actinastrum hantzschii	Scenedesmaceae	Chlorophyta
Amphora ovalis	Cymbellaceae	Bacillariophyta
Amphora arcus	Cymbellaceae	Bacillariophyta
Anabaena circinalis	Nostocaceae	Cyanophyta
Anabaena spirodes	Nostocaceae	Cyanophyta
Anacystis	Chroococcaceae	Cyanophyta
Ankistrodesmus falcatus	Oocystaceae	Chlorophyta
Aphanocapsa	Chroococcaceae	Cyanophyta
Aphanothece	Chroococcaceae	Cyanophyta
Arthrodesmus convergens	Desmidiaceae	Chlorophyta
Arthrodesmus octocorne	Desmidiaceae	Chlorophyta
Asterionella	Fragilariaceae	Bacillariophyta
Aulacoseira granulata	Coscinodiscaceae	Bacillariophyta
Basicladia	Cladophoraceae	Chlorophyta
Biddulphia laevis	Biddulphiaceae	Bacillariophyta
Botrydium granulatum	Botrydiaceae	Chrysophyta
Bulbochaete	Oedogoniaceae	Chlorophyta
Caloneis	Naviculaceae	Bacillariophyta
Campylodiscus hibernicus	Surirellaceae	Bacillariophyta
Ceratium	Ceratiaceae	Pyrrhophyta
Ceratoneis arcus	Fragilariaceae	Bacillariophyta
Chaetophora	Chaetophoraceae	Chlorophyta
Characium	Chlorococcaceae	Chlorophyta
Chlorococcum	Chlorococcaceae	Chlorophyta
Chlamydomonas	Chlamydomonadaceae	Chlorophyta
Chlorella	Oocystaceae	Chlorophyta
Chroococcus	Chroococcaceae	Cyanophyta
Cladophora	Cladophoraceae	Chlorophyta
Closteriopsis	Oocystaceae	Chlorophyta
Closteridium	Oocystaceae	Chlorophyta
Closterium	Desmidiaceae	Chlorophyta

Table 3 (continued).

Taxa	Family	Phylum
Cocconeis pediculus	Achnanthaceae	Bacillariophyta
Cocconeis placentula	Achnanthaceae	Bacillariophyta
Coelastrum	Coelastraceae	Chlorophyta
Colonial Greens	Oocystaceae	Chlorophyta
Coleochaete	Coleochaetaceae	Chlorophyta
Coscinodiscus	Coscinodiscaceae	Bacillariophyta
Cosmarium bioculatum	Desmidiaceae	Chlorophyta
Cosmarium circulare	Desmidiaceae	Chlorophyta
Cosmarium reniforme	Desmidiaceae	Chlorophyta
Craticula	Naviculaceae	Bacillariophyta
Crucigenia	Scenedesmaceae	Chlorophyta
Cryptomonas	Cryptomonadaceae	Cryptophycophyta
Cyclidiopsis	Euglenaceae	Euglenophyta
Cymatopleura elliptica	Surirellaceae	Bacillariophyta
Cymatopleura solea	Surirellaceae	Bacillariophyta
Cymbella lanceolata	Cymbellaceae	Bacillariophyta
Cymbella naviculiformis	Cymbellaceae	Bacillariophyta
Cymbella tumida	Cymbellaceae	Bacillariophyta
Cymbella turgidula	Cymbellaceae	Bacillariophyta
Denticula	Epithemiaceae	Bacillariophyta
Desmidium	Desmidiaceae	Chlorophyta
Diatoma vulgare	Fragilariaceae	Bacillariophyta
Dictyosphaerium pulchellum	Dictyosphaeriaceae	Chlorophyta
Dinobryon sertularia	Ochromonadaceae	Chrysophyta
Diplochloris	Oocystaceae	Chlorophyta
Diploneis elliptica	Naviculaceae	Bacillariophyta
Elakatothrix	Coccomyxaceae	Chlorophyta
Encyonema	Cymbellaceae	Bacillariophyta
Entomoneis	Entomoneidaceae	Bacillariophyta
Epithemia turgida	Epithemiaceae	Bacillariophyta
Euastrum	Desmidiaceae	Chlorophyta
Eudorina	Volvocaceae	Chlorophyta
Euglena intermedia	Euglenaceae	Euglenophyta
Euglena sanguinea	Euglenaceae	Euglenophyta
Eunotia bilunaris	Eunotiaceae	Bacillariophyta
Eunotia curvata	Eunotiaceae	Bacillariophyta

Table 3 (continued).

Taxa	Family	Phylum
Eunotia formica	Eunotiaceae	Bacillariophyta
Eunotia rhomboidea	Eunotiaceae	Bacillariophyta
Eunotia serra	Eunotiaceae	Bacillariophyta
Excentrosphaera viridis	Oocystaceae	Chlorophyta
Fragilaria capucina	Fragilariaceae	Bacillariophyta
Fragilaria crotonensis	Fragilariaceae	Bacillariophyta
Fragilaria vaucheriae	Fragilariaceae	Bacillariophyta
Frustulia rhomboides	Naviculaceae	Bacillariophyta
Gloeocystis gigas	Gloeocystaceae	Chlorophyta
Gloeotrichia	Rivulariaceae	Cyanophyta
Gomphonema acuminatum	Gomphonemataceae	Bacillariophyta
Gomphonema angustatum	Gomphonemataceae	Bacillariophyta
Gomphonema olivaceum	Gomphonemataceae	Bacillariophyta
Gomphonema truncatum	Gomphonemataceae	Bacillariophyta
Gymnodinium	Gymnodiniaceae	Pyrrhophyta
Gyrosigma acuminatum	Naviculaceae	Bacillariophyta
Haematococcus lacustris	Chlamydomonadaceae	Chlorophyta
Hantzschia amphioxys	Nitzschiaceae	Bacillariophyta
Hydrodictyon	Hydrodictyaceae	Chlorophyta
Kirchneriella	Oocystaceae	Chlorophyta
Mastogloia	Naviculaceae	Bacillariophyta
Melosira varians	Coscinodiscaceae	Chrysophyta
Merismopedia	Chroococcaceae	Cyanophyta
Menoidium	Euglenaceae	Euglenophyta
Meridion circulare	Fragilariaceae	Bacillariophyta
Micrasterias apiculata	Desmidiaceae	Chlorophyta
Micrasterias radiata	Desmidiaceae	Chlorophyta
Microcystis	Chroococcaceae	Cyanophyta
Microspora	Microsporaceae	Chlorophyta
Monoraphidium	Oocystaceae	Chlorophyta
Mougeotia	Zygnemataceae	Chlorophyta
Navicula capitata	Naviculaceae	Bacillariophyta
Navicula cryptocephala	Naviculaceae	Bacillariophyta
Navicula lanceolata	Naviculaceae	Bacillariophyta
Navicula petersenii	Naviculaceae	Bacillariophyta
Navicula radiosa	Naviculaceae	Bacillariophyta
Neidium	Naviculaceae	Bacillariophyta

Table 3 (continued).

Taxa	Family	Phylum
Netrium	Mesotaeniaceae	Chlorophyta
Nitzschia acicularis	Nitzschiaceae	Bacillariophyta
Nitzschia filiformis	Nitzschiaceae	Bacillariophyta
Nitzschia linearis	Nitzschiaceae	Bacillariophyta
Nitzschia sigmoidea	Nitzschiaceae	Bacillariophyta
Oedogonium	Oedogoniaceae	Chlorophyta
Oocystis	Oocystaceae	Chlorophyta
Oscillatoria	Oscillatoriaceae	Cyanophyta
Pachycladon umbrinus	Oocystaceae	Chlorophyta
Pediastrum boryanum	Hydrodictyaceae	Chlorophyta
Pediastrum simplex	Hydrodictyaceae	Chlorophyta
Penium	Desmidiaceae	Chlorophyta
Peridinium	Peridiniaceae	Pyrrhophyta
Phacus acuminatus	Euglenaceae	Euglenophyta
Phormidium	Oscillatoriaceae	Cyanophyta
Pinnularia acuminata	Naviculaceae	Bacillariophyta
Pinnularia mesolepta	Naviculaceae	Bacillariophyta
Pinnularia subcapitata	Naviculaceae	Bacillariophyta
Pinnularia viridis	Naviculaceae	Bacillariophyta
Placoneis elginensis	Naviculaceae	Bacillariophyta
Planktothrix	Oscillatoriaceae	Cyanophyta
Planktosphaeria	Oocystaceae	Chlorophyta
Pleurotaenium	Desmidiaceae	Chlorophyta
Quadrigula closterioides	Oocystaceae	Chlorophyta
Raphidiopsis curvata	Hammatoideaceae	Cyanophyta
Reimeria sinuata	Gomphonemataceae	Bacillariophyta
Rhipidodendron splendidum	Amphimonididae	Craspedophyta
Rhoicosphenia curvata	Achnanthaceae	Bacillariophyta
Rhopalodia gibba	Epithemiaceae	Bacillariophyta
Scenedesmus armatus	Scenedesmaceae	Chlorophyta
Scenedesmus dimorphus	Scenedesmaceae	Chlorophyta
Scenedesmus obliquus	Scenedesmaceae	Chlorophyta
Schroederia setigera	Characiaceae	Chlorophyta
Scytonema	Scytonemataceae	Cyanophyta
Selenastrum gracile	Oocystaceae	Chlorophyta
Sirogonium	Zygnemataceae	Chlorophyta

Table 3 (continued).

Taxa	Family	Phylum
Sorastrum americanum	Hydrodictyaceae	Chlorophyta
Sphaerocystis	Palmellaceae	Chlorophyta
Sphaeroplea annulina	Sphaeropleaceae	Chlorophyta
Spinoclosterium curvatum v. spinosum	Desmidiaceae	Chlorophyta
Spirogyra	Zygnemataceae	Chlorophyta
Spirulina	Oscillatoriaceae	Cyanophyta
Spirotaenia	Mesotaeniaceae	Chlorophyta
Staurastrum curvatum	Desmidiaceae	Chlorophyta
Staurastrum natator v. crassum	Desmidiaceae	Chlorophyta
Staurastrum paradoxum	Desmidiaceae	Chlorophyta
Staurastrum	Desmidiaceae	Chlorophyta
Stigeoclonium tenue	Chaetophoraceae	Chlorophyta
Stauroneis anceps	Naviculaceae	Bacillariophyta
Stauroneis phoenicenteron	Naviculaceae	Bacillariophyta
Stauroneis smithii	Naviculaceae	Bacillariophyta
Surirella brebissonii	Surirellaceae	Bacillariophyta
Surirella oblonga	Surirellaceae	Bacillariophyta
Surirella ovalis	Surirellaceae	Bacillariophyta
Surirella minuta	Surirellaceae	Bacillariophyta
Surirella robusta	Surirellaceae	Bacillariophyta
Surirella splendida	Surirellaceae	Bacillariophyta
Synedra acus	Fragilariaceae	Bacillariophyta
Synedra ulna	Fragilariaceae	Bacillariophyta
Synura uvella	Synuraceae	Chrysophyta
Tabellaria fenestrata	Fragilariaceae	Bacillariophyta
Tabellaria tabulata	Fragilariaceae	Bacillariophyta
Tabellaria fasciculata	Fragilariaceae	Bacillariophyta
Tetraedron	Oocystaceae	Chlorophyta
Tetraspora	Tetrasporaceae	Chlorophyta
Tetradesmus	Scenedesmaceae	Chlorophyta
Tetrastrum	Scenedesmaceae	Chlorophyta
Trachelomonas volvocina	Euglenaceae	Euglenophyta
Treubaria	Oocystaceae	Chlorophyta
Tribonema	Tribonemataceae	Chrysophyta
Ulothrix	Ulotrichaceae	Chlorophyta

Table 3 (continued).

Taxa	Family	Phylum
Volvox	Volvocaceae	Chlorophyta
Xanthidium	Desmidiaceae	Chlorophyta
Zygnema	Zygnemataceae	Chlorophyta

Table 4. Mean biovolume of periphyton from cattle-access and no-access wetlands at the University of Tennessee Plateau Research and Education Center, Crossville, Tennessee, May 2005 – April 2006. (Units are 10^6 um^3/cm^2).

	Cattle-access		No-access				
Taxa[a,b,c]	$\bar{x}$	SE	$\bar{x}$	SE	*F*	*P*	Interactions
ACHN	2.5502	0.3678	7.8838	0.9797	31.78	<0.001	NI
APHA	0.0267	0.0094	0.0022	0.0042	6.11	0.01	NI
AULA	2.3009	0.6542	0.6371	0.1157	7.54	0.007	NI
BULB	0.0501	0.4866	1.6616	0.7755	5.43	0.02	NI
CYMB	3.2095	0.5521	9.0542	1.1484	21.72	<0.001	NI
DESM	0.8771	0.6533	19.6791	7.8070	5.68	0.02	NI
DINO	0.2101	0.1378	5.3227	1.7424	8.69	0.004	NI
EUGL	23.1931	3.5824	10.7038	1.6753	7.5-25.9	0.01-0.02	M (4,5,7,11)
EUNO	2.4731	0.7838	25.7336	4.6906	21.80	<0.001	NI
FRAG	27.4656	0.51455	44.5335	7.8007	4.49	0.04	NI
FRUS	5.8387	1.0716	2.4680	0.3617	9.79	0.009	M (1)
NAVI	0.6810	0.1894	0.3159	0.0901	5.74	0.03	M (5)
NAV2	30.6732	3.5791	11.0354	1.3438	5.3-10.4	0.01-0.04	M (1,2,5,6,8)
OSCI	7.2633	0.9053	4.3598	0.8907	4.13	0.04	NI
PHAC	5.0280	1.3516	2.2662	0.3037	5.08	0.04	M (9)
PLKO	1.5177	0.6971	0.0660	0.0475	4.26	0.04	NI
QUAD	0.0016	0.0016	0.0496	0.0213	5.30	0.02	NI
RHIP	0.0777	0.0777	4.0671	1.4670	7.21	0.008	NI
SCEN	30.2675	5.0636	7.0189	1.5182	5.03-7.4	0.02-0.04	M (4,5,6,8)
SCHR	2.7910	0.7594	0.2831	0.1058	6.04-6.2	0.03	M (6,8)
SPIR	222.2960	44.8892	504.8299	96.2103	8.85	0.004	NI
STAU	13.1509	3.0076	79.7661	13.8824	23.27	<0.001	NI
SURI	0.0405	0.0166	0.2494	0.0648	8.68	0.004	NI

Table 4 (continued).

Taxa[a,b,c]	Cattle-access		No-access				
	$\bar{x}$	SE	$\bar{x}$	SE	*F*	*P*	Interactions
TABE	0.5714	0.4714	47.9306	13.0159	11.73	0.001	NI
TRAC	63.3367	13.4063	14.0321	1.7106	12.92	0.001	NI
XANT	0.5687	0.4452	25.6827	9.5350	6.94	0.009	NI

[a]Periphyton taxa identified to genus and species when possible, except for COGR, which is an unidentified coccoid green alga.

[b]ACHN = *Achnanthidium minitissimum*, APHA = *Aphanothece*, AULA = *Aulacoseira granulata*, BULB = *Bulbochaete*, COGR = Coccoid Green Algae (*Chlorella*), CYMB = *Cymbella*, DESM = *Desmidium*, DINO = *Dinobryon*, EUGL = *Euglena*, EUNO = *Eunotia*, FRAG = *Fragilaria crotonensis*, FRUS = *Frustulia rhomboides*, GLTR = *Gloeotrichia*, NAVI = *Navicula radiosa*, NAV2 = *Navicula cryptocephala*, NITZ = *Nitzschia*, OSCI = *Oscillatoria*, PHAC = *Phacus*, PLKO = *Planktothrix*, QUAD = *Quadrigula closterioides*, RHIP = *Rhipidodendron splendidum*, SCEN = *Scenedesmus*, SCHR = *Schroederia setigera*, SPIR = *Spirogyra*, STAU = *Staurastrum*, STIG = *Stigeoclonium*, SURI = *Surirella*, TABE = *Tabellaria fenestrata*, TRAC = *Trachelomonas volvocina*, XANT = *Xanthidium antilopaeum*.

[c]NI = no interaction, M = interaction with month; treatment effect for months indicated parenthetically, where months are numbered sequentially from 1 = January to 12 = December.

Table 5. Mean levels of water quality variables between cattle-access and no-access ponds, University of Tennessee Plateau and Research Center, Tennessee, May 2005 – April 2006.

	Cattle-access			No-access				
Variable[a]	*n*[b]	$\bar{x}$	SE	*n*	$\bar{x}$	SE	*F*	*P*
pH	47	7.76	0.19	47	7.23	0.14	7.38	0.008
Turbidity	47	163.51	26.75	47	34.15	9.92	20.14	<0.001
Conductivity	47	129.25	5.51	47	81.25	8.56	22.20	0.003
COD	4	51.00	6.57	4	20.75	0.75	20.93	0.004
TotalKN	4	3.00	0.33	4	0.82	0.17	33.44	0.001
TotalP	4	0.27	0.11	4	0	0	6.33	0.04
ResidueT	4	217.75	53.09	4	62.00	8.03	8.41	0.03
Si	4	4.50	0.64	4	2.00	0.41	10.71	0.02
Al	4	779.00	145.5	4	136.3	78.9	15.08	0.008
Fe	4	2582.50	298.42	4	460.53	230.27	7.91	0.03
Mg	4	1.67	0.06	4	1.14	0.28	12.21	0.01
K	4	15.45	1.76	4	3.18	0.84	12.21	0.01
PeripP	16	31.89	4.93	16	11.18	2.96	11.75	0.002

[a]Turbidity = NTUs (nephelometric turbidity units); Conductivity = mS cm^{-1}; COD (chemical oxygen demand), Al and Fe = µg/L; TotalKN = total Kjeldahl nitrogen (mg/L), TotalP = total phosphorus, ResidueT =total suspended solids), Si, Mg, and K = mg/L; and PeripP = periphyton phosphorus (reported here in µg; as µg P/mg AFDM in text).

[b] *n* = 47 (1 samples/pond/month x 8 ponds x 12 months); *n* = 4 (1 sample per pond in August 2005); *n* = 16 (4 samples/treatment x 4 months (representing 4 quarters).

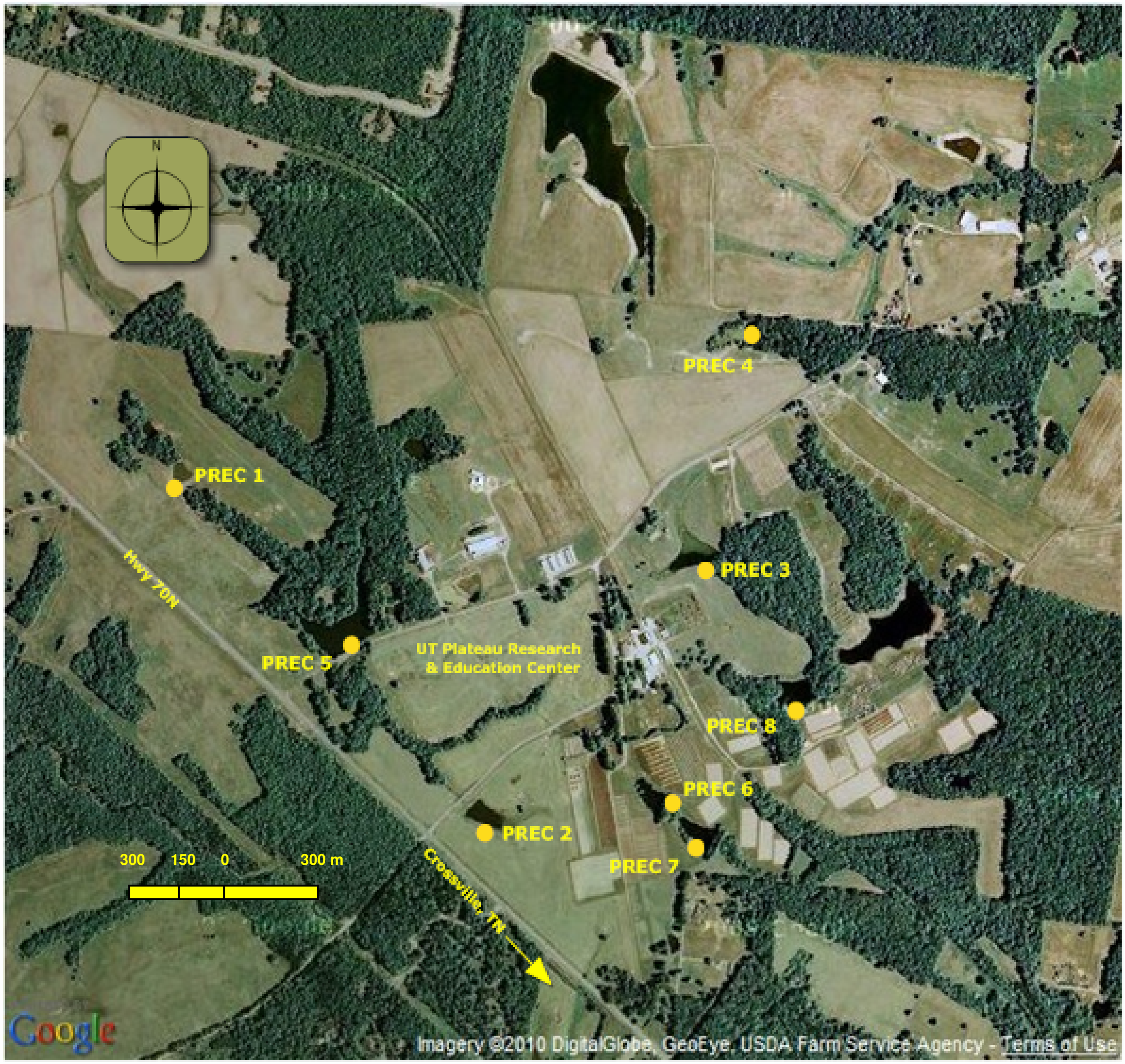

Figure 1. Experimental ponds PREC 1 – 8 at the University of Tennessee Plateau Research and Education Center in Crossville, Tennessee. PREC 1 – 4 ponds had cattle-access while cattle were excluded from PREC 5 – 8 ponds.

shoreline.

Figure 2. Artificial substrate for periphyton colonization, University of Tennessee Plateau Research and Education Center, Tennessee, May 2004 – April 2006.

Figure 3. Artificial substrate deployment in study ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. Substrates were spaced 0.5 m and the board placed 2 m from and perpendicular to the shoreline.

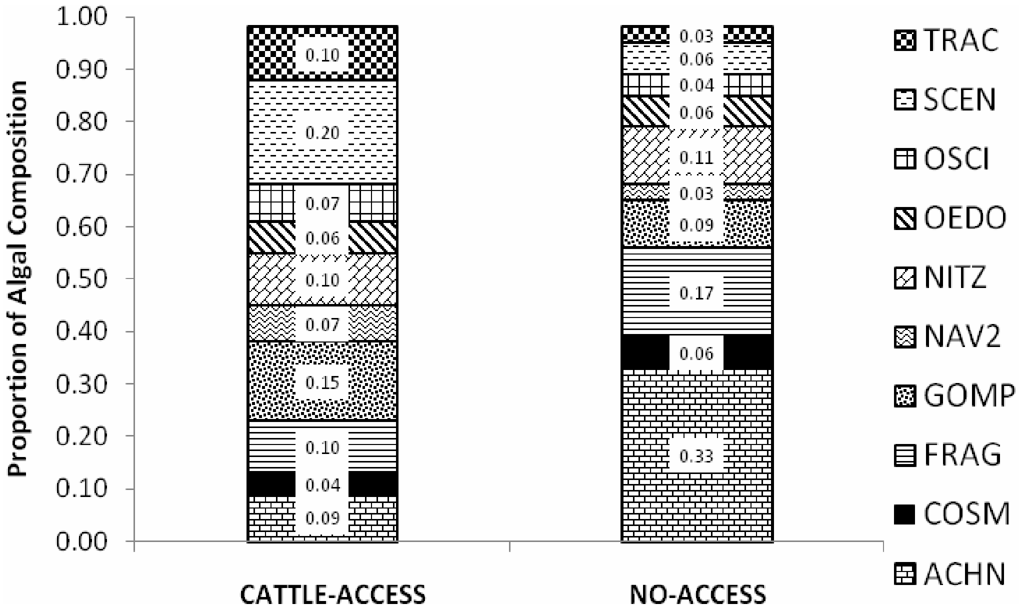

Figure 4. Proportion of the 10 most abundant alga taxa from total counts in cattle-access and no-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. ACHN = *Achnanthidium minutissimum*, COSM = *Cosmarium*, FRAG = *Fragilaria crotonensis*, GOMP = *Gomphonema*, NAV2 = *Navicula cryptocephala*, NITZ = *Nitzschia*, OEDO = *Oedogonium*, OSCI = *Oscillatoria*, SCEN = *Scenedesmus*, TRAC = *Trachelomonas volvocina*.

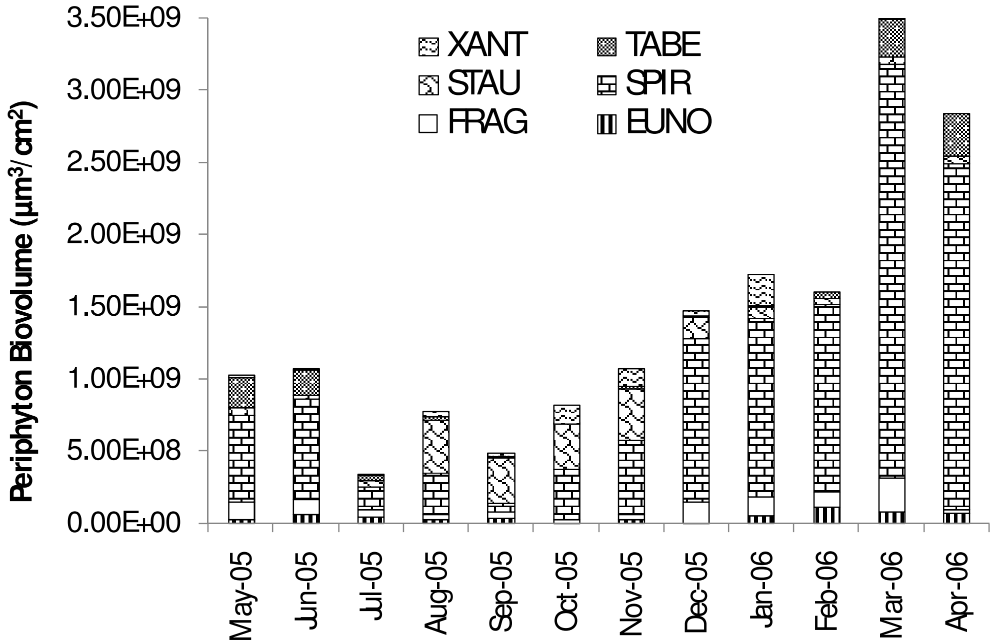

Figure 5. Changes in periphyton biovolume (um^3/cm^2) for the dominant taxa in no-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. EUNO = *Eunotia*, FRAG = *Fragilaria crotonensis*, SPIR = *Spirogyra*, STAU = *Staurastrum,* TABE = *Tabellaria fenestrata*, and XANT = *Xanthidium antilopaeum.*

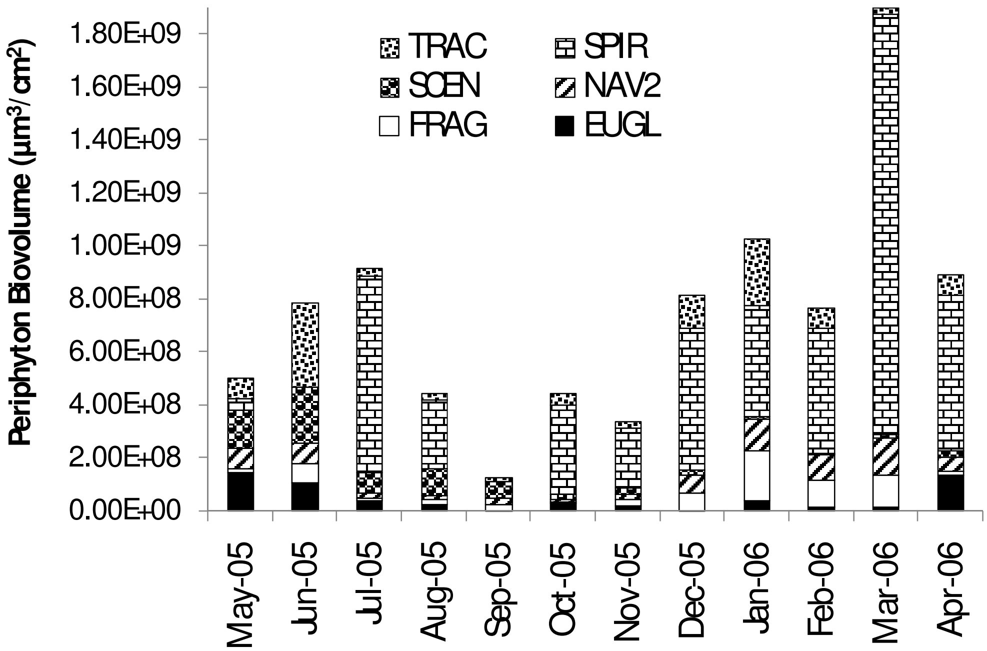

Figure 6. Changes in periphyton biovolume (um^3/cm^2) for the dominant taxa in cattle-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. EUGL = *Euglena,* FRAG = *Fragilaria crotonensis*, NAV2 = *Navicula cryptocephala*, SCEN = *Scenedesmus*, SPIR = *Spirogyra*, TRAC = *Trachelomonas volvocina*.

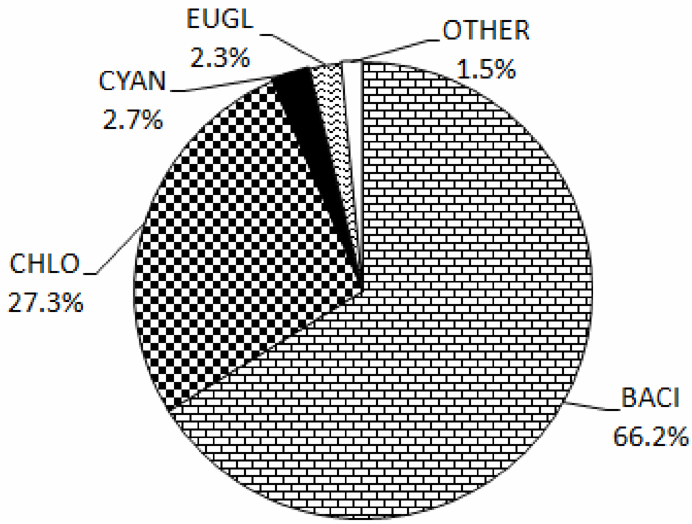

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Figure 7. Composition of the dominant periphyton phyla in no-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. BACI = Bacillariophyta, CHLO = Chlorophyta, CYAN = Cyanophyta (Cyanobacteria), EUGL = Euglenophyta, OTHER = Chrysophyta, Craspedophyta, Cryptophycophyta, Pyrrhophyta.

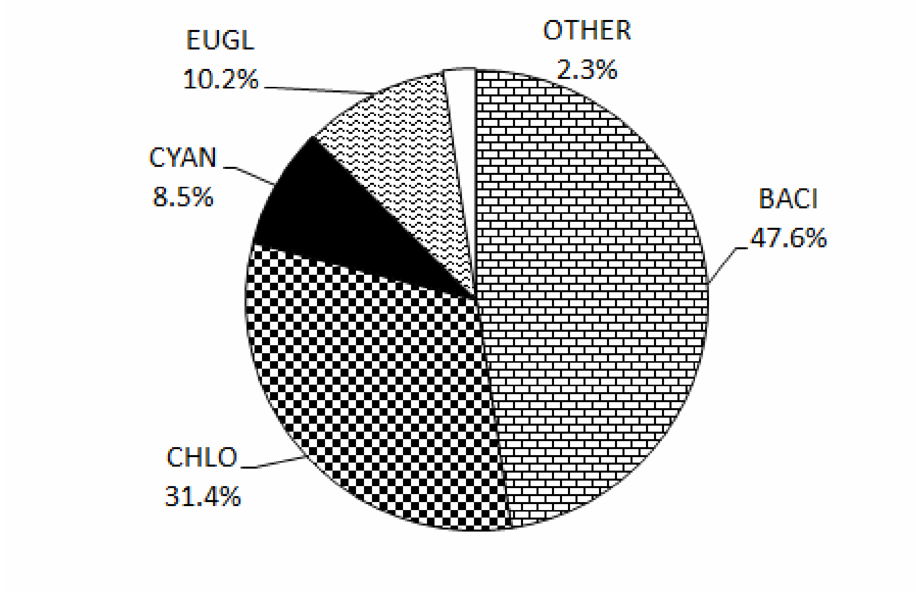

Figure 8. Composition of the dominant periphyton phyla in cattle-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. BACI = Bacillariophyta, CHLO = Chlorophyta, CYAN = Cyanophyta (Cyanobacteria), EUGL = Euglenophyta, OTHER = Chrysophyta, Craspedophyta, Cryptophycophyta, Pyrrhophyta.

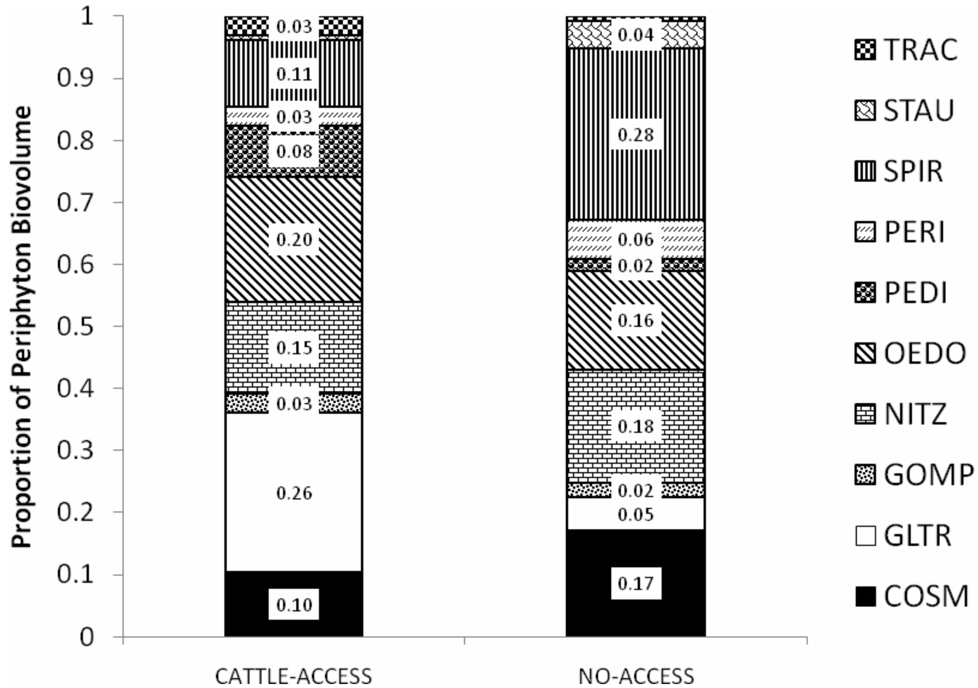

Figure 9. Proportion of the 10 dominant periphyton taxa based on biovolume (um^3/cm^2) calculated from total counts in cattle-access and no-access ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. COSM = *Cosmarium*, GLTR = *Gloeotrichia*, GOMP = *Gomphonema*, NITZ = *Nitzschia*, OEDO = *Oedogonium*, PEDI = *Pediastrum*, PERI = *Peridinium*, SPIR = *Spirogyra*, STAU = *Staurastrum*, TRAC = *Trachelomonas volvocina*.

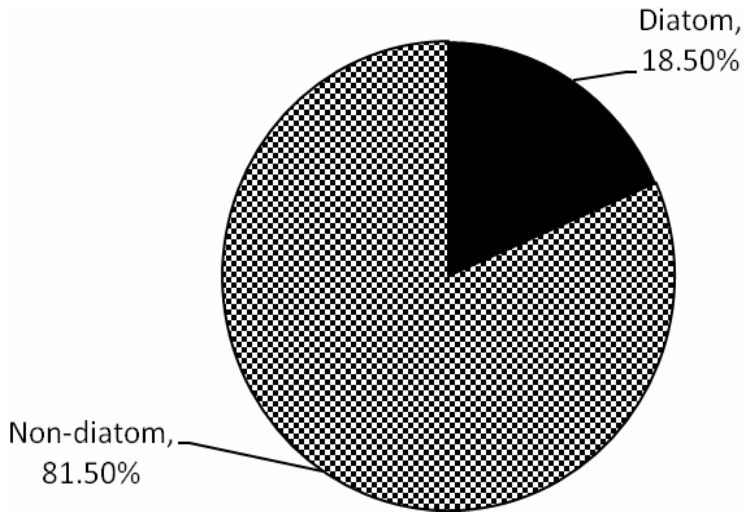

Figure 10. Changes in periphyton distribution comparing the relative biovolume (um^3/cm^2) results between diatom and non-diatom algae in no-access ponds at the University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006.

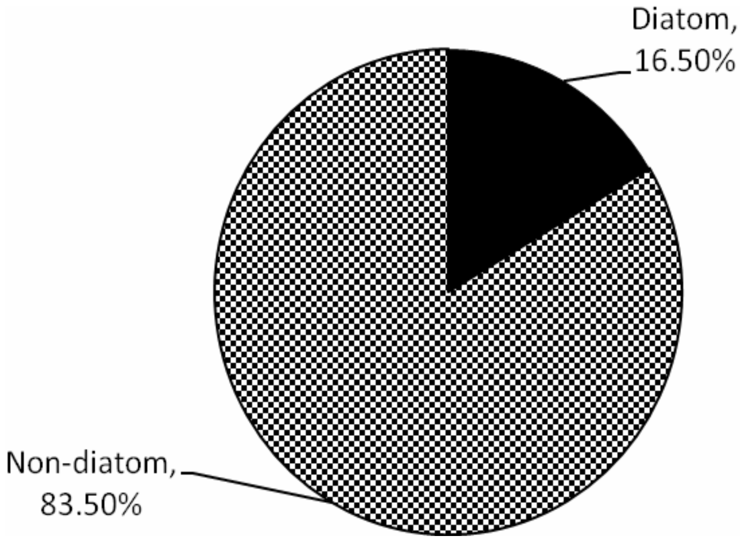

Figure 11. Changes in periphyton distribution comparing the relative biovolume (um^3/cm^2) results between diatom and non-diatom algae in cattle-access ponds at the University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006.

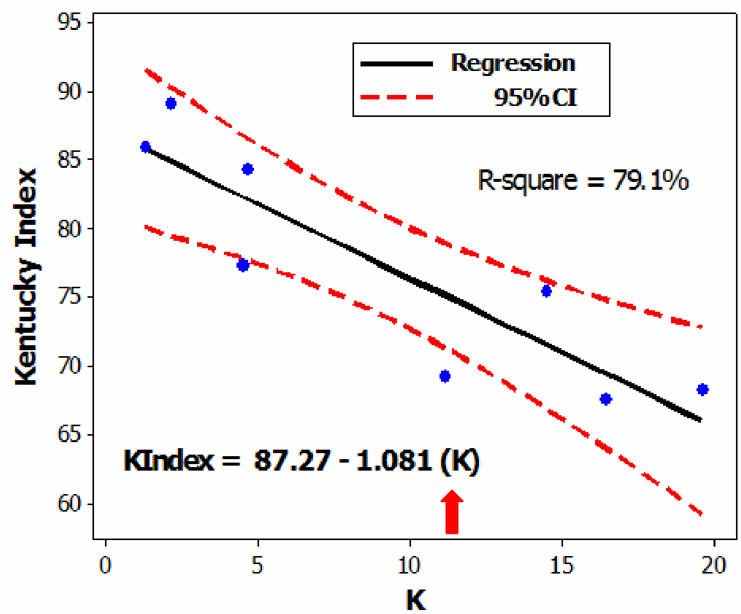

Figure 12. Relationship between the Kentucky Index (KDOW 2008) and potassium concentrations (mg/L) at eight study ponds, University of Tennessee Plateau Research and Education Center, Tennessee, May 2005 – April 2006. Red arrow indicates the World Health Organization potassium threshold level for drinking water (12 mg/L, WHO 2009).

VITA

Robert Gerald Middleton (Gerry) was born in Athens, TN on February 15, 1949. He was raised in Copperhill, TN, and later attended grade, junior high and high school in Etowah, TN. He graduated from McMinn Central High School, Englewood, TN in 1967. From there, he went on to Tennessee Tech University, Cookeville, TN and received his B.S. degree in Geology in 1972. After graduation, Gerry worked as a consulting geologist for Kenwill, Inc. in Maryville, TN, and a geologist for the Tennessee Valley Authority in Chattanooga, TN. Since 1993, he has served as a licensed professional geologist and environmental specialist with the Tennessee Department of Environment and Conservation in Oak Ridge, TN. Recent job responsibilities include environmental sampling, algal and macroinvertebrate taxonomy and biomonitoring, and field botany (threatened and endangered plant species). Gerry returned to college in 2001 taking botany courses at TennesseeTech and the University of Tennessee. In 2005, he was admitted to the University of Tennessee graduate school and went on to receive his M.S. in Wildlife and Fisheries Science in 2010. Future goals include teaching, staying active through hiking and volunteering in state and national parks, continued algal and macroinvertebrate research, publishing findings, and assisting university departments involved with environmental and biological programs.