5-2015

Epiphytic Diatom Community Structure in a Karst Riverine System

Gregory John Barren
Western Kentucky University, gregory.barren363@topper.wku.edu

EPIPHYTIC DIATOM COMMUNITY STRUCTURE IN A KARST RIVERINE SYSTEM

A Thesis
Presented to
The Faculty of the Department of Biology
Western Kentucky University
Bowling Green, KY

In Partial Fulfillment
Of the Requirements for the Degree
Masters of Science

By
Gregory John Barren

May 2015

EPIPHYTIC DIATOM COMMUNITY STRUCTURE IN A KARST RIVERINE SYSTEM

Date Recommended April 15, 2015

Dr. Scott Grubbs, Director of Thesis

Dr. Albert Meier

Dr. Ouida Meier

Dean, Graduate Studies and Research 4-25-15 Date

Acknowledgements

I would like to acknowledge several people who have helped me complete my thesis. First, I would like to thank my advisor Scott Grubbs who has guided and assisted me through the entire process. His patience and accessibility were greatly appreciated. I would also like to thank my committee members Albert Meier and Ouida Meier for their invaluable inputs. I would like to thank John Andersland and Richard Schultz for their great ideas and for providing me with resources necessary for my research. I would like to thank Megan Grandinetti and Elizabeth Malloy for both their assistance in the field and help passing time in the lab. Funding for this research was provided by a WKU ARTP Collaborative Grant awarded jointly to the Center for Biodiversity Studies and the Hoffman Environmental Research Institute, a WKU RCAP 1 Award (awarded to Scott Grubbs), and the WKU Green River Preserve. Finally, I would also like to thank Kelly Derham, Olivia Boyd, Jennie Dumaine, Sarah Goodyear, Charlotte Hacker and Andrea Sejdic and others who have made my time at WKU memorable.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

EPIPHYTIC DIATOM COMMUNITY STRUCTURE IN A KARST RIVERINE SYSTEM

Gregory John Barren May 2015 62 Pages

Directed by: Dr. Scott Grubbs, Dr. Albert Meier, and Dr. Ouida Meier

Department of Biology Western Kentucky University

The goal of this study was to assess the epiphytic diatom community structure of two host species along a karst gradient in the upper Green River, Kentucky to a gain a better understanding of the role of diatoms in the food web. The host species studied were *Podostemum ceratophyllum* and *Cladophora.* Percent cover of *P. ceratophyllum* and *Cladophora* were quantified in the four study reaches. The host species were sampled near-shore and mid-channel in each reach in September and October of 2013. After diatoms were extracted from the host and enumerated the density and diversity were quantified. Twelve genera were identified with > 91% of the community in each reach being *Cocconeis*. The second most abundant genus was *Achnanthes* or *Navicula* depending on the reach. The density and diversity of diatoms increased longitudinally going downstream. Exceptions to this trend occurred when high flow events disturbed the community. Within reaches there were no differences in diatom diversity in near-shore and mid-channel habitats. Diatom density in near-shore and mid-channel habitats was only different in the most downstream reach. *Cladophora* had a community twice as dense as *P. ceratophyllum*, but less diverse. The results of this study indicate that there are longitudinal differences in diatom communities in the upper Green River and host species are an important factor in determining the community composition. The importance of epiphytic diatoms in the food web, however, remains unclear.

Introduction

Diatoms are a diverse group of algae that are distributed globally. They are found mostly in marine and freshwater environments, but in some cases can be found in terrestrial environments. Within these environments they can be found in various habitats. For example, they can be found suspended in the water column, growing on benthic substrate, or growing on the surface of macrophytes and macroalgae. Diatoms are important because they are a significant contributor to global gross primary productivity (Scala and Bowler, 2001), they can act as ecosystem engineers (Gerbersdorf et al., 2009), and because they form the base of many aquatic food webs (Finlay et al., 1999; Mayer and Likens, 1987).

Diatoms are highly productive, accounting for about 40% of the oceans primary productivity (Scala and Bowler, 2001). Marine phytoplankton fixes about 45 GT of carbon•yr^{-1} and under favorable conditions they can fix about 2000 g•m^{-2} annually (Scala and Bowler, 2001; Falkowski et al., 1999). In addition to carbon they are also important in the cycling of silica, phosphorous, and nitrogen by fixing these nutrients and then settling to the benthos after cell senescence (Smetacek, 1985).

In some systems diatoms are considered ecosystem engineers because they stabilize sediments and prevent erosion with sticky exudates that bind the sediment together, which allows other organisms to colonize those habitats (Gerbersdorf et al., 2009). Some diatoms are also capable of modifying the environment by increasing the biologically accessible nitrogen (N) available through N-fixation (Furey et al., 2012). N-fixation is carried out by cyanobacteria that live symbiotically in some diatom genera (e.g., *Epithemia* and *Rhopalodia)* (Furey et al., 2012). When the standing crop of these

genera is large enough they can be a significant source of biologically accessible N, especially in N-limited systems (Furey et al., 2012).

Diatoms are also important because they form the base of many aquatic food webs. In open canopy streams where light availability is high diatoms can be a significant food source for many herbivorous macroinvertebrates (i.e., grazers) because of their high productivity (Finlay et al., 1999; Vannote et al., 1980). In closed canopy streams diatoms are generally considered less important because they are less productive and less abundant than terrestrial detritus (Vannote et al., 1980). Despite being less productive they can still be a significant food source for grazers (Finlay et al., 1999). For example, in a closed canopy stream in New Hampshire diatoms made up ca. 50% of the diet of *Neophylax aniqua* (Trichoptera) and supported ca. 75% of their growth (Mayer and Likens, 1987).

Within open and closed canopy systems the effect of grazers on diatom communities may be more or less important than other factors (e.g., nutrients) (Lange et al., 2011; Miralto et al., 1999; Dudley, 1992). Lange et al. (2011) found that grazing significantly affected only one of 13 benthic species, whereas nutrient enrichment significantly affected 11 species. When the grazing intensity is high enough however, they can remove most epiphytic diatoms with the exception of low profile taxa (e.g., *Cocconies)* (Dudley, 1992). For example under high grazing intensity in Montana's Madison River ca. 75% of epiphytic diatoms were removed from the host (Dodds, 1991).

Epiphytic diatom communities are important in the food web when macroalgae and macrophytes are present (Cattaneo and Kalff, 1980). Some macroalgae (e.g., *Cladophora*) can be an unfavorable food source due to its chemical composition (Dodds,

1991), and some macrophytes may be only consumed by macroinvertebrates at the end of the growing season when they undergo senescence (Cattaneo and Kalff, 1980). In systems with a lot of the benthic surface covered by macroalgae and macrophytes epiphytes may sustain macroinvertebrate communities for most of the growing season (Cattaneo and Kalff, 1980).

Cladophora is a common macroalgae that has been studied in many epiphytic diatom studies (Ferreiro et al., 2013; Furey et al., 2012; Malkin et al., 2009; Mpawenayo and Mathooko, 2005; Marks and Power, 2001; Benenati, 1998; Bergey et al., 1995; Shannon et al., 1994; Dudley, 1992; Hardwick, 1992; Moore, 1977; Jansson, 1967). *Cladophora* is distributed globally, present across a broad variety of marine and freshwater habitats (Dodds and Gudder, 1992). It can have a complex structure with many branching filaments or a simple structure depending on the environment (Bergey et al., 2008). *Cladophora* can be found as floating mats in standing water systems (i.e., ponds), but it typically attached to bottom substrates (e.g., rocks) in many types of shallow habitats (Millner and Sweeney 1982). Attached algae, including *Cladophora*, often are the most important photosynthetic species and contribute the most primary production activity in running water systems (Power et al., 2009). Riverine *Cladophora* growth often is highly seasonal, with rapid growth during summer (Power et al. 2009) and particularly during periods of low precipitation.

Macrophytes increase the heterogeneity of benthic habitats and offer a complex three-dimensional habitat for epiphyte colonization. *Podostemum ceratophyllum* Michx. 1803 is a riverine macrophyte that can be highly productive in shallow, swift-flowing riffles in the eastern United States (Hutchens et al., 2004; Hill and Webster, 1984), is

indicative of high quality aquatic systems (Hill and Webster, 1984; Meijer, 1976), and provide stable habitat for macroinvertebrate communities (Tinsley, 2012; Hutchens et al., 2004). This macrophyte can form dense mats, increasing the stability, surface area, and anchoring of benthic substrates (Hutchens et al., 2004) due to the spreading configuration of the root-like holdfast structures (Hill and Webster, 1984). The epiphytic diatom communities hosted by *P. ceratophyllum* have not yet been quantified in the literature.

Epiphyte communities can be shaped by the species and growth form of the host. In general as the habitat complexity increases there is greater epiphytic biomass (Ferreiro et al., 2013; Knapp and Lowe, 2009). In a study on bryophyte epiphytes, liverwort leaves were observed to offer little protection against scouring flow and had lower densities than moss (Knapp and Lowe, 2009). Other hosts, such as *Cladophora*, offer little protection from scouring flow but can have a higher density of epiphytes than more complex macroalgae, such as *Bangia atropurpurea* (Lowe et al., 1982). Sloughing of the cell wall causes the lower density on *B. atropurpurea* (Lowe et al., 1982). The density of diatoms on *Cladophora* can exceed 600 diatoms•μg^{-1} dry mass (DM) of *Cladophora* in productive systems (Malkin et al., 2009).

In some systems there tends to be an increase in diversity with increasing complexity due to more niche availability, but this is not always the case with epiphytic diatoms (Ferreiro et al., 2013; Bazzaz, 1975). Ferreiro et al. (2013) found no increased diversity with increasing macrophyte complexity in a nutrient rich stream. Diatom diversity may be more related to colonization rates after disturbances (Marks and Power, 2001). Early colonizers on *Cladophora* predominantly belong to one genus, but the community diversifies with time (Marks and Power, 2001).

Diatoms on these hosts are also going to be influenced by several abiotic factors, including nutrient availability, temperature, and flow. Nutrient concentrations have been shown to affect diatom community composition, but they have varying effects on the diversity ranging from no difference to highly significant differences (Frankovich et al., 2006; Snyder et al., 2002; Marks and Power, 2001) The three nutrients that may be limiting to diatom productivity are nitrogen, phosphorous, and silica. The extents to which these nutrients limit growth differ amongst freshwater communities and species (Hecky and Kilham, 1988). Lange et al. (2011) found that in a 3rd-order, nutrient-poor stream benthic diatom species were affected by nitrate and phosphate additions. Eleven of the 13 species studied responded significantly to nitrate and phosphate treatments, eight of them positively and three of them negatively (Lange et al., 2011). Silica limitations can occur when diatom biomass is high (Malkin et al., 2008). This can result in decreased thickness of individual frustules within the community (Malkin et al., 2008).

Another important environmental variable is water temperature. As temperature increases the general trend is for a decrease in cell volume and an increase in growth rate, but this has many exceptions (Montagnes and Franklin, 2008). Epiphytic diatom communities in the tailwaters of the Glen Canyon Dam showed a significant response to water temperature increasing from 12 to 18°C (Blinn et al., 1989). The 12°C water was dominated by *Rhoicosphenia curvata* and *Diatoma vulgare* (Blinn et al., 1989). Both of these species declined as water temperature increased while *Cocconeis pediculus* and *Achnanthes minutissim* become the dominant species (Blinn et al., 1989). The change in composition may have been due an increase in grazers in the warmer temperatures or a

physiological limitation (Blinn et al., 1989). In general, there is an increase in diatom diversity as the temperature increases (Smith and Manoylov, 2013).

Flow (i.e., water velocity and discharge) can affect the composition of diatom communities (Biggs et al., 2002). In fast-flowing water diatom communities are dominated by species that are able to adhere to benthic substrates via a gelatinous excretion (Patrick and Reimer, 1966). Diatom biomass increases going from slow to moderate flow because of increased transfer of nutrients with limited scouring (Biggs et al., 2002). High flow conditions, however, may induce scouring and decrease biomass (Biggs et al., 2002). Lamb and Lowe (1987) found that diatom periphyton diversity and density were greater in slow flow relative to fast flow. The density in slow flow was three times as great due in part to vertical growth, which offers more substrate for diatoms to colonize (Lamb and Lowe, 1987). In general, only low profile taxa are able to withstand high flow while high profile and motile taxa are scoured (Passy, 2007)

The flow of a system can also affect the diatom communities by increasing scouring seston (Webster et al., 1987). High flow can suspend benthic particles as well as introduce colluvium to a river during flood events (Webster et al., 1987). The increase in seston and water velocity during high flow increases the scouring of diatoms leaving behind only low profile taxa (Biggs et al., 2002). Flood events have been shown to decrease the biomass of periphyton communities (Francoeur and Biggs, 2006). Epiphytic communities typically have the lowest resistance to high discharge when compared to epilithic and epipelic communities, but they have the highest resilience (Soininen and Eloranta, 2004). After a flood event Soininen and Eloranta (2004) observed the epiphytic community return to a pre-flood state faster than the epilithic and epipelic communities.

The goal of this study was to quantitatively assess epiphytic diatom community structure in the upper Green River, Kentucky in an attempt to understand the community's role in food webs. The diets of organisms in food webs are often traced through stable isotope analysis, but this is difficult to do for epiphytic diatoms because it can be problematic to separate the diatom from the host tissue (Angradi, 1994). Studying the community composition can give a better idea of the role of epiphytic diatoms as a food resource for grazers. Epiphytic community structure was quantified with density and diversity measures. Three questions were addressed:

1) Are there differences in epiphytic diatom communities between reaches with increasing karst development? The upper Green River transitions longitudinally from a siliciclastic-carbonate basin upstream to a carbonate-dominated basin downstream. The downstream reaches have markedly higher nutrient levels **(**Penick et al., 2012). I hypothesized that river reaches with higher percent cover of macroalgae and macrophytes would have greater density and diversity of epiphytic diatoms because of less edge effects. The edges of clusters of macroalgae and macrophytes would be prone to scouring seston, leaving behind only low profiled epiphyte species with low community diversity. Hence, this scouring affect will limit the success of high profile taxa

2) Is there a difference in diatom communities between shallow and deeper habitats? I hypothesized that mid-channel habitats would have lower diversity and density because of increased water velocity that can scour diatoms. Scouring flow may only

allow low profile taxa to grow and may decrease the density if the velocity is high enough.

3) Is there a difference in diatom communities between hosts? The two hosts that were examined were *Cladophora* and *P. ceratophyllum*. These hosts offer differing degrees of protection due to their structure. *Podostemum ceratophyllum* has a more complex structure and therefore was expected to have greater epiphyte diversity due to more niche availability and protection from scouring flow. *Podostemum ceratophyllum* is also less prone to scouring than *Cladophora*. This was expected to result in less diversity on *Cladophora* because it was possible that only quickly colonizing genera were present at the time of collection. Even though *Cladophora* is a filamentous alga and offers little in the way of protection from scouring currents I expected it to have a greater density because it has more surface area available for diatoms than *P. ceratophyllum* of equal mass.

Methods

Study Reaches

This study was conducted in shallow run habitats along the upper Green River, Kentucky, USA (Figure 1). The Green River originates in central Kentucky and flows west, emptying into the Ohio River in northwestern Kentucky. Four study reaches downstream of Green River Lake were used for this research. Two upstream reaches (reaches 1 and 2) were positioned in a mixed siliciclastic-carbonate landscape and are underlain by Fort Payne Formation and Reef, Salem, and Warsaw Limestones from the Upper Mississippian (Kentucky Geologic Survey, 2015). The upstream reaches were

positioned in a 6th order section of the upper Green River. The downstream reaches were located in a carbonate-dominated landscape in Munfordville, KY (reach 3) and at the Western Kentucky University Green River Preserve (reach 4). Saint Genevieve and St. Louis limestones from the Upper Mississippian underlie reach 3 while St. Genevieve Limestone and Girkin Formation from the Upper Mississippian underlie reach 4 (Kentucky Geologic Survey, 2015). The downstream reaches were positioned in a 7th order section of the upper Green River. Hydrologic characteristics of the study reaches can be found in Table 1.

All of the study reaches had Quarternary-aged alluvium (Kentucky Geologic Survey, 2015). Each reach had an open canopy and a substrate that consisted of mostly pebbles and cobbles. In most years the most abundant macrophyte and macroalga present are *P. ceratophyllum* and *Cladophora (*Malloy, 2014; Penick, 2012*).*

Field method

At each reach all sampling was performed in a 50x15-m sub-reach with the exception of reach 1, which was not as wide as the other reaches. A 50 x 10-m sub-reach was used for reach 1. A flag was attached to a tree perpendicular to the start point at the upstream side of the reach to allow for the same sub-reach to be used each sampling period. The abundance of *P. ceratophyllum* and *Cladophora* was quantified using a percent cover line transact method that is commonly used to quantify the area covered by a species (Brown 1975; Madsen 1999; Fiala et al. 2006). Percent cover of hosts was quantified along ten randomly generated 15-m transects in each 50-m long sub-reach in September and October of 2013 and 2014. *Potamogeton* sp. and *Fontinalis* sp. were also present, but

were not sampled because they were not common. For each transect a tape measure was extended perpendicular to flow. Percent cover was quantified by measuring the amount of *Cladophora* and *P. ceratophyllum* that was directly under the extended tape measure.

Hosts were sampled in 1-m^2 quadrats at 1-m (near-shore) and 7.5-m (mid-channel) along each transect. In reach 1 they were sampled at 1-m and 5-m. Before collection, depth (cm) and velocity ($m \bullet s^{-1}$) were recorded at the 1-m and 7.5-m points or 1-m and 5-m points depending on the reach. These measurements were recorded using a Marsh McBirney FLO-MATE model 2000 Portable Flowmeter. Sampling consisted of collecting *Cladophora* and *P. ceratophyllum* located in the 1-m^2 quadrat, placing it in a Whirl-Pak bag, and then on ice for transfer to the lab. Enough host tissue was sampled to comprise $\geq$ 0.5-g of dried material.

Extraction Method

The method of diatom extraction was based on Al-Handal and Wulff (2008). Samples were dried for at least 48 hours at 70°C. After drying, 0.5-g of sample was placed into a centrifuge vial with 20-ml of 30% H_2O_2 for 24 hours to separate diatom frustules from the host. The plant tissue was first added to the centrifuge vial and then the H_2O_2 was added a few milliliters at a time. The gradual addition of H_2O_2 was done to prevent violent reactions that occurred in some samples, which resulted in tissue overflowing the vial. Once the entire 20-ml of H_2O_2 was added the cap was loosely screwed on to allow for pressure release. The vials were periodically inverted and lightly shaken to re-submerge plant tissue that was forced upward by O_2 production in the oxidation process. To separate the freed frustules from the host the vials were vortexed for two bursts of ten

seconds and then filtered through a 500-µm sieve into a beaker. The plant tissue soaked in the H_2O_2 formed a thick mat that trapped some of the diatoms. To extract as many diatoms from the original sample as possible the dense mat of plant tissue was placed back in the vial with 20-ml of DI water. The vial was then vortexed and filtered again using the same method. The process of placing the plant tissue back in the vial and vortexing was repeated three times. Three repetitions of this process were deemed sufficient in obtaining the vast majority of diatoms. A fourth shaking and filtering into a separate beaker resulted in negligible numbers. Once separated, the frustules were boiled in 30% H_2O_2 for 15-20 min or until the solution was clear in appearance. The boiled samples were then poured back into a clean centrifuge vial.

Counting Method

The counting method used was based on the protocol published by the USGS National Water-Quality Assessment Program (Acker, 2002). 0.1-ml aliquots from each host were examined under a Wild M40 inverted compound microscope at 400x with phase contrast. A WILDCO Palmer-Maloney (P-M) counting cell was used to enumerate the samples. A cover slip was first placed over the reservoir perpendicular to the P-M cell leaving the loading chambers open to allow filling. When the cover slip was in place the sample was pipetted into the reservoir through one of the filling chambers. As the sample entered the reservoir it pushed air out through the other filling chamber, which prevented air bubbles from forming in the reservoir. Once the aliquot was pipetted into the chamber the cover slip was rotated 90^o to cover both the loading chambers and reservoir. All diatom individuals were identified to genus, which is the most practical taxonomic level

for quantitative counts (Saunders et al., 2012; Acker, 2002). Diatoms were identified using Patrick and Reimer (1966). Although more modern guides exist, they sometimes lack supporting data and can be internally inconsistent (Wehr and Sheath, 2003).

Samples were either concentrated or diluted to fall in the range of 10–30 cells per field of view to make counts statistically reliable and prevent errors (Acker, 2002; Wetzel and Likens, 1991). The total number of diatoms that needed to be enumerated per aliquot to have statistically reliable results was 300 (Acker, 2002). If the sample had less than 10 units per field of view the sample was left to settle for two days and then decanted to concentrate. If the sample had greater than 30 diatoms per field of view it was diluted with DI water. The amount removed or added was documented to calculate changes in the concentration.

Fields of view were counted along transects on the P-M cell. To avoid the center of the P-M cell, where some clumping may occur, transects were selected in the top, bottom, left, or right third (Acker, 2002). Transects were started and finished 1-mm from the edge to avoid clumping areas (Acker, 2002). Once a starting point was established the horizontal stage adjustment was used to view fields at 1-mm increments. If at least 300 diatoms were not counted along the first transect then another transect was counted using the same method. Once a transect was started it was finished no matter when the 300 diatoms were counted to avoid counting bias. A tally counter was used to keep track of diatoms counted in each field of view.

The number of diatoms per field of view was recorded. Fragmented diatoms that had at least 2/3 of their frustule present were counted. Any diatoms with less than 2/3 of the frustule present were not counted. Often times girdle bands break away and maintain

the shape of the frustule, but contain no material (Patrick and Reimer, 1966; Palmer and Keeley, 1900). To avoid double counting girdle bands were not counted. Diatoms that were only partly in the field of view were counted if at least 2/3 of the frustule was visible. The genera *Fragilaria* and *Synedra* were lumped together because they are distinguished by the ability to form filaments (Patrick and Reimer, 1966). This growth pattern could not be observed due to the extraction method.

Statistical Methods

All analyses were performed in R (version 2.15.1, The R Project for Statistical Computing, 2012). The resampling procedure script used was provided by Collyer et al. (2015). All hypothesis tests below had an alpha value set at 0.05. The percent cover of *P. ceratophyllum* and *Cladophora* were quantified for each transect in each reach. The proportion data was then arc-sin transformed. The raw data were not normally distributed according to a Shapiro-Wilk test ($W = 0.87$, $p = 1.8 \times 10^{-11}$) so a non-parametric approach was used. Percent cover of each host was compared between reaches using a one-way ANOVA randomization procedure and pairwise comparisons with 10,000 permutations. The only assumption of this approach is independent observations. Comparisons were done for *P. ceratophyllum* with the September and October samples combined because it was not expected to fluctuate between months due to its slow growth rate and scour resistance (Argentina et al., 2010; Philbrick and Novelo, 2004). Comparisons for *Cladophora* were done between months and years because it is often scoured and can have rapid growth (Zulkifly et al., 2013).

The density of diatoms was calculated as the number of diatoms•μg^{-1} DM of host. The Shannon index was used as the diversity metric. This index is commonly used when there is little diversity and the system is dominated by one genus (Morris et al., 2014). The equation for the Shannon index is as follows: $H' = -\sum p_i \ln p_i$, where p_i is the proportion of species i.

The first and second questions were addressed in one model. A repeated measures 3-way factorial ANOVA was used to test for differences in diatom density and diversity between reaches and habitats (i.e., near-shore and mid-channel). The reaches were split into September and October sampling periods. A 2-way factorial ANOVA was also run for diatom density and diversity. September and October data were combined for the 2-way factorial ANOVA. The assumption of normality was not met for raw or log-transformed density ($W = 0.77$, $p = 1.2 \times 10^{-11}$; $W = 0.97$, $p = 0.01$) or diversity ($W = 0.94$, $p = 9.6 \times 10^{-5}$; $W = 0.95$, $p = 9.9 \times 10^{-4}$) data according to the Shapiro-Wilk test of normality. Subsequently, a non-parametric approach via a resampling procedure and pairwise comparisons with 10,000 permutations was used. Main effects of the model were only interpreted if the interactions between main effects were not significant. Main effects were not interpreted if the interaction was significant because the two factors would be dependent upon each other in that circumstance, and interpreting them separately would be misleading. A simple linear regression was run to see if there was a relationship between water velocity and diatom density or diversity to help explain any differences between habitat types. A non-parametric resampling procedure with 10,000 permutations was used for density as a function of water velocity and diversity as a function of water velocity.

To compare the communities between hosts a two-way factorial ANOVA was used. There was only enough *Cladophora* present during the October sampling period in reach 4 so hosts were only compared from this one sampling location and period. The data were not normally distributed for diversity according to a Shapiro-Wilk test ($W = 0.93$, $p = 0.048$); however, they were normally distributed for diatom density ($W = 0.95$, $p= 0.157$). Despite the normally distributed density data a non-parametric resampling procedure with 10,000 permutations was used. A parametric procedure was not used even though the density data were normally distributed because the sample size was low ($n = 30$) and in previous Shapiro –Wilk tests on density with greater sample sizes ($n = 108$) the data were always highly skewed. The observed normality in this case may be a happenstance of small sample size.

Results

Percent Cover

There was a trend for a decrease in the percent cover of *P. ceratophyllum* going from upstream to downstream in 2013 (Figure 2). The percent cover of reach 1 typically ranged between 55.2 – 72.0 % (IQR) cover. This is in contrast to reach 4, which typically ranged between 6.4 – 16.7 % (IQR) cover. The longitudinal decrease in percent cover was significant stepwise going downstream except from reach 3 to 4 ($p = 0.693$) (Table 2). The same pattern was observed in 2014, but the data were more variable (Figure 2). For example, the 1st and 3rd quartiles in reach 4 both increased to 9.4 – 30.4 % cover. The longitudinal decrease in percent cover was again significant stepwise going downstream except from reach 3 to 4 ($p =0.710$) (Table 2).

The percent cover of *Cladophora* was not significantly different between reaches in both 2013 and 2014. The sample sizes for these analyses, however, were very low due to *Cladophora* being continually scoured during several high flow events (Figure 3).When *Cladophora* was scoured only the holdfasts remained attached to rocks. In September 2013, *Cladophora* was present along only one transect each in reaches 1 and 2, six of the reach 3 transects, and eight of the reach 4 transects. Similarly, in October 2013 *Cladophora* was also present only along one transect each in reaches 1 and 2, two of the reach 3 transects, yet all ten reach 4 transects. In September 2014 *Cladophora* was again present only along one transect in reaches 1 and 2, two in reach 3 transects, and one reach 4 transect. In October 2014 *Cladophora* was absent except for holdfasts on rocks. When *Cladophora* was present in reaches the percent cover was substantially lower than *P. ceratophyllum* percent cover (Figures 2 and 4).

Diatom Community

A total of 12 different genera were observed across the study reaches on *P. ceratophyllum* and *Cladophora* (Tables 3 and 4). *Cocconeis* was the dominant genus on *P. ceratophyllum* and *Cladophora*, comprising > 91% of the community in every reach. *Cladophora* had a greater proportion of *Cocconeis* in the community than *P. ceratophyllum* in the one reach that had enough data for comparison (i.e., reach 4 in October) (Table 4). Depending on the reach the next most abundant genus on *P. ceratophyllum* was either *Navicula* or *Achnanthes.* These genera made up 1.8–3.4% of the community. *Navicula* was more abundant in upstream reaches whereas *Achnanthes* was more abundant in downstream reaches. With the exception of *Gomphonema* in

downstream reaches, all other genera made up < 1% of the community on *P.ceratophyllum* in a given reach (Table 3).

Question 1

The 3-way repeated measures factorial ANOVA revealed that there was a significant interaction between reach and month for the diatom density ($p < 0.001$) and diversity ($p = 0.004$) models. The main effects of reach in the density model and reach plus month in the diversity model were significant, but were not interpreted because of significant interactions involving those variables (Table 5). There was an increase in density for September going downstream with the exception of reach 4, which was sampled on September 13th after a high flow event (Figure 5). This high flow event had a peak discharge of 162,834 l•s^{-1} (Figure 3). The short time between the high flow event and sampling (i.e., 8 days) did not give the community enough time to recover. In September density was at a low in reach 1 and a high in reach 3 (Figure 5). Even though the means increased stepwise the only significant increase occurred from reach 1 to 3 (Table 6). Reach 2 was not significantly different from either reach 1 or 3. The decrease in density (17.3 diatoms•μg^{-1} DM) from reach 3 to reach 4 was significant ($p = 0.029$).

The density in October increased going downstream (Figure 5). The stepwise increase in means was significant between reaches 1 and 3, 1 and 4, and 2 and 4 (Table 6). The interaction of month was significant because of the continued increase in density in reach 4 in October compared to decreased density in this same reach in September (Figure 4). The mean difference in reach 4 between months (32.4 diatoms•μg^{-1} DM) was the only significant monthly difference ($p < 0.001$).

There was also a significant interaction in the diatom density 3-way repeated measures and 2-way factorial ANOVA's for reach and habitat (Table 5 and 7). In both near-shore and mid-channel habitats there was a trend for an increase in diatom density going downstream. This relationship was much stronger for mid-channel habitats. In mid-channel habitats the two upstream reaches had a significantly less dense diatom community than the two downstream reaches (Figure 6 and Table 9). The only significant difference in means for near-shore habitats was between reaches 1 and 3 ($p = 0.034$).

In September the diversity increased from upstream to downstream (Figure 7). Both of the upstream reaches had similar means and had significantly lower diversity than both of the downstream reaches, which also had similar means ($p < 0.001$) (Table 8). In October the pattern was different because reach 2 had a significantly higher mean than every other reach (Table 6). This reach had a low sample size ($n = 6$) and was influenced by two large means that skewed the data. The interaction of month was significant for reach 1 and 2 (Table 6). Both reach 1 and 2 had greater mean diversity in October (Figure 7). When months were pooled a similar pattern was observed. Reach 1 had significantly less diversity than every other reach (Table 10). Reach 2 was significantly less diverse than reach 3. Reaches 3 and 4 were not statistically different.

Overall the community composition did change longitudinally along the upper Green River (Figure 8). Both diversity and density tended to increase going down stream. This does not support the hypothesis that areas with higher percent cover of *P. ceratophyllum* would have a greater diatom density and diversity. In this study the opposite pattern was observed indicating other factors are more important.

Question 2

The 3-way repeated measures factorial ANOVA and 2-way factorial ANOVA also revealed that there was a significant interaction between reach and habitat for density ($p = 0.005$) but not diversity (Tables 5 and 7). The only significant mean difference between mid-channel and near-shore habitats for density was within reach 4 (14.6 diatoms•μg^{-1} DM) ($p = 0.024$) (Figure 6 and Table 12). In reach 4 there was greater diatom density in the mid-channel habitats (Figure 6 and Table 11) It was hypothesized that near-shore habitats would have a slower water velocity than mid-channel habitats and would therefore have a greater diatom density and diversity. Water velocity, however, appeared to be independent of near-shore and mid-channel habitats (Table 12). The simple linear regressions run on diatom density and diversity as a function of water velocity revealed that density and water velocity were correlated ($R^2 = 0.10$, $F = 11.74$, $p = 0.001$). There was no relationship between diversity and water velocity ($R^2 < 0.01$, $F = 0.122$, $p = 0.726$).

Question 3

The two-way ANOVA's comparing diatom density and diversity on *P. ceratophyllum* and *Cladophora* were both significant for the main effect of host (Table 10). Habitat was significant for diversity, but was not interpreted because of the lack of replication and results from the previous 3-way repeated measures and 2-way factorial ANOVA's that showed no relationship between habitat and diversity (Tables 5 and 7). The density of diatoms on *Cladophora* was >2X on *P. ceratophyllum* ($p < 0.001$) with a maximum value exceeding 140 diatoms•μg^{-1} DM *Cladophora* (Figure 10) The diversity

of diatoms was significantly greater on *P. ceratophyllum* than on *Cladophora* ($p = 0.012$) (Figure 9).

Discussion

Percent Cover

Percent cover of *P. ceratophyllum* was influenced by high flow events. Summer and fall 2013 had several high flow events that appeared to bury *P. ceratophyllum* in the study reaches (Figure 2). The high discharge from August 29th – September 3rd, 2013 that peaked at 162,833 l•s^{-1} was one of the high flow events that contributed to the burial of *P. ceratophyllum.* The effects of high flow events were more evident in the two downstream reaches. In 2012 the percent cover of *P. ceratophyllum* downstream was about equal to upstream, but in 2013 downstream cover decreased by approximately 50% (Malloy, 2014; Penick, 2012). Sand and pebbles were deposited where *P. ceratophyllum* was observed attached to cobbles prior to the disturbance. The trend for less *P. ceratophyllum* in downstream reaches was probably due to it being a higher order stream and having a larger watershed to contribute to discharge. The upstream reaches are also closer to the Green River Lake (Table 1). The Green River Lake dam has controlled water release, which helps buffer some of the flashiness of upstream reaches relative to downstream reaches.

In 2014 there were not as many high flow events and they were not as intense (Figure 2). Percent cover was more variable in 2014 yet higher on average than in 2013. This suggests that the *P. ceratophyllum* was beginning to recover. The full recovery of *P. ceratophyllum* will likely take a few years due to slow seed production and low dispersal

ability (Argentina et al., 2010). Despite the high flow events *P. ceratophyllum* was more stable in the system than *Cladophora* and provided a more stable substrate for diatoms.

Cladophora was not common during the sampling periods in this study. At its highest it covered only 10% of a reach. This is in contrast to 2012 when *Cladophora* covered 40% of the same reach (Malloy, 2014). When *Cladophora* did become established in the river it was usually scoured soon after leaving behind only holdfasts. Ensminger et al. (2000) found that when water velocity exceeded 0.75 $m \bullet s^{-1}$, *Cladophora* and other macroalgae were scoured. The mean water velocity in this study exceeded 0.75 $m \bullet s^{-1}$ several times during sampling periods (Table 12). In addition, between sampling periods and when the river was generally too high to wade it was expected that velocities well exceeded 0.75 $m \bullet s^{-1}$.

Diatom Community

Cocconeis was the dominant genus present on *P. ceratophyllum* in every reach (Table 3). *Cocconeis* was also the dominant genus on *Cladophora* in reach 4 (Table 4). The two most common *Cocconeis* species observed were *C. pediculus* and *C. placentla*. These species are generally regarded as being epiphytic (Patrick and Reimer 1966), but can be found growing epilithically and epipelically (Kolayli et al, 1998). *Cocconeis* is a low profile taxa. The top valve of *Cocconeis* is raphiless and convex while the bottom valve has a raphe and is concave (Wehr and Sheath, 2003). This morphology allows them to fit closely to the substrate and withstand scouring flows and resist grazing (Zulkifly et al., 2013; Furey et al., 2012).

Cocconeis is commonly one of the most abundant genera in epiphytic diatom studies. In a study on communities in western Kentucky and Tennessee *Cocconeis* was one of the dominant taxa in carbonate-dominated streams (Hunt and Hendricks, 2008). Similarly in Florida's St. John's River, *Cocconeis* was one of the most commonly observed genera and was the only genus found at all sites on all sampling dates (Dunn et al., 2008). Malkin et al. (2008) showed that *Cocconeis* nearly excluded every other epiphyte on *Cladophora* by the end of the growing season in Lake Ontario.

The dominance of *Cocconeis* is likely due to a combination of scouring flow and grazing pressures. *Cocconeis* has been observed to be the most dominant taxon during medium and high velocity conditions in a 3rd-order California stream (Bergey et al., 1995). They found that *Cocconeis* comprised ca. 86% of the epiphytic community on *Cladophora* in medium (0.16-0.27 m•s^{-1}) and fast (0.40-0.93 m•s^{-1}) flowing water. In other reaches with slower water velocity other genera became more abundant and dominated the community (Bergey et al., 1995). Near-shore and mid-channel water velocities recorded in the upper Green River in this study fell within their classification of medium or fast flowing water (Table 1).

Grazing may have also been an important factor. Common scrapers/grazers found in the upper Green River are snails (e.g., *Leptoxis praerosa*), mayflies (e.g., *Maccaffertium mediopunctatum*), and water penny beetles (*Psephenus herricki*) (Malloy, 2014). Isotopic analyses of these grazers revealed that they were consuming biofilm on wood and rock. Since they were consuming biofilm and, seemingly, were also feeding on epiphytes then they may have contributed to the dominance of *Cocconeis*. In a study on thirteen common stream taxa, *Cocconeis* was the most common taxa at all grazing

intensities and even increased in abundance as grazing intensity increased due to its low profile and resistance to grazing (Lange et al., 2011). Even though *Cocconeis* is grazer resistant it can be grazed in some cases. *Cocconeis* has been found in the guts of midges in large numbers but it is largely believed to be consumed incidentally as more accessible diatoms are targeted (Furey et al., 2012). It could be that more high profile taxa growing on top of *Cocconeis* are able to pull off *Cocconeis* as they are grazed. When *Cocconeis* is the dominant genus midges consume *Cladophora* filaments with *Cocconeis* still attached (Furey et al., 2012). What remains unclear in this study is if it is grazing intensity or water velocity that is keeping many of the high profile taxa at lower abundances.

Question 1

Diatom density and diversity values revealed that there were longitudinal differences in diatom communities in the Upper Green River. It was hypothesized that reaches with greater percent cover would have greater density and diversity because of reduced flow within macrophytes (Dodds and Biggs, 2002), but the opposite pattern was observed. Mean water velocity per reach (Table 15) seemingly had no effect on diatom density except for reach 4 in September when flow was very high for the two weeks leading up to sampling (Figure 3). Water velocity within a small range will typically not alter the density of diatoms, but may shift the proportions of species (Bergey et al., 1995). The reaches in this study were all in the same river and the changes in flow patterns affect each reach to a similar magnitude. There also was not a pattern of increasing local-scale velocity going downstream (Table 15). For these reasons the general trend for an increase in diatom density going downstream is likely due to a factor other than discharge

or velocity. Grazing pressure was also not likely an important factor in longitudinal differences in diatom density because the dominant genus in all reaches was grazer-resistant *Cocconeis* (Bergey et al., 1995). Another factor that was seemingly not important was nutrient levels. A previous study reported increases in total nitrogen and total phosphates going downstream in the upper Green River, but the system overall was eutrophic and total nutrients were not limiting to periphyton communities (Penick et al., 2010).

One possible explanation is stream order and how it relates to the River Continuum Concept (Vannote et al., 1980). Increases in density may have been a factor of a more open canopy downstream and longer exposure to photosynthetically active radiation (PAR) (Yang and Flower, 2012). This would fall into the realm of the river continuum concept where primary production increases from low order to mid order streams where the canopy is more open (Vannote et al., 1980). Although the downstream reaches in this study were only a single stream order higher than the upstream reaches they were about twice as wide as the upstream reaches (Table 1). The differences in PAR exposure time may have contributed to the higher diatom density downstream.

The diversity of the downstream diatom community was greater than the community found upstream in September. In October there was not much change in diversity longitudinally except with reach 2. In reality the diversity of reach 2 in October was probably similar to the diversity of reach 1, but it was highly skewed due to a low sample size and two abnormally high diversity values.

The increase in diversity downstream for September may be due to there being more sources of potential colonizing diatoms due to a greater number of tributaries

(Molloy, 1992). Similar diversity patterns were reported for nearby streams. Molloy (1992) reported that in two of three tributaries of the Kentucky River there were downstream longitudinal increases in diversity. Grazing may have played a role in the low diversity in the upper Green River (Bergey et al., 1995), but this is unknown because it was not documented in this study. Temperature is another factor that can influence diversity (Smith and Manovlov, 2013), but in this study the reverse pattern was observed based on what might be expected from temperature alone. The downstream reaches are typically cooler in summer and fall months because they are more heavily influenced by cool groundwater. This temperature regime does not help explain the pattern of diversity in this study because typically diatom diversity increases with temperature (Smith and Manovlov, 2013).

Question 2

The only difference between near-shore and mid-channel habitats occurred in reach 4. Within channel differences may be attributable to differences in water velocity, but mid-channel and near-shore classifications are probably too encompassing. Mean water velocity in each habitat in the reaches does not help explain the observed patterns of diatom density. It would be expected that the greater mean differences in velocity would result in greater differences in diatom density (Biggs et al., 2002; Lamb and Lowe 1987), but the opposite was observed. The only significant mean difference in diatom density had the least mean difference in water velocity (Tables 12 and 15). When comparing the density of diatoms simply as a function of water velocity there was a positive linear relationship. Biggs et al. (1998) reported similar findings where increases

in water velocity beyond 0.2 $m \cdot s^{-1}$ scoured diatoms, resulting in lower density. Part of the reason for the decrease in diatoms with increasing flow is that it limits vertical growth (Lamb and Lowe, 1987). In slow water velocity (0.15 $m \cdot s^{-1}$) epiphytic diatoms communities can be three times denser than communities found in fast water velocity (0.40 $m \cdot s^{-1}$) due to vertical growth (Lamb and Lowe, 1987).

Question 3

Even though there was not enough *Cladophora* present to have replicate reaches it appears that there were differences in the communities present on *Cladophora* and *P. ceratophyllum*. The mean density of diatoms on *Cladophora* was more than double that found on *P. ceratophyllum*. Much of this probably had to do with how density was quantified in this study. Using the number of diatoms per dry mass gave higher density values for *Cladophora* because it has a greater surface area to volume ratio. The range in density of diatoms on *Cladophora* found in this study (38 – 145 diatoms$\cdot \mu g^{-1}$ DM) was similar to values reported in the Wylye River, England (maximum 48 diatoms$\cdot \mu g^{-1}$ DM) (Moore, 1977). *Cladophora* numbers reported in a lentic system were much higher (155-602 diatoms$\cdot \mu g^{-1}$ DM) (Malkin et al., 2009). More studies have reported on the diatom density of *Cladophora*, but there have been many different methods used making comparisons difficult (Table 14). The *P. ceratophyllum* in this current study ranged from 0.33 – 77 diatoms$\cdot \mu g^{-1}$ DM (Figure 10). There have not been any other studies that have quantified the community composition of diatoms on *P. ceratophyllum*.

The diatom diversity on *P. ceratophyllum* was greater than the diversity on *Cladophora*. The greater diatom diversity may have been a function of host complexity

and niche availability. *Podostemum ceratophyllum* contains various structures including leaves, flowers, stems, and root-like holdfasts that can function as substrates for diatoms (Philbrick and Novelo, 2004). Also, structures like stems and holdfasts can be rigid (Philbrick and Novelo, 2004) and offer protection from scouring flow on the downstream side. *Cladophora* is made up of flexible filaments (Zulkifly et al., 2013) and is less complex. The flexible filaments offer little protection from scouring flow (Zulkifly et al., 2013), especially in fast currents where filaments can be fragmented (Bergey et al., 1995). The more complex structure of *P. ceratophyllum* offers niches for diatoms that may not be able to withstand scouring flow (Bazzaz, 1975).

The stability of *P. ceratophyllum* relative to *Cladophora* in higher water velocity habitats also helps explain the higher diversity on *P. ceratophyllum*. *Podostemum ceratophyllum* uses holdfasts to attach securely to the substrate in fast flowing water (Philbrick and Novelo, 2004). *Cladophora* is easily scoured, but is well adapted to recolonizing (Zulkifly et al., 2013). In the upper Green River there was about half the cover of *Cladophora* in September 2013 relative to October 2013. This suggests that at least half of the *Cladophora* in October was new growth (Figure 4). *Podostemum* c*eratophyllum* is slower growing and relatively stable in the environment so there was probably little new growth in October. (Argentina et al., 2010; Philbrick and Novelo, 2004) This is important for diatom diversity because *Cocconeis* is an early epiphytic colonizer (Marks and Power, 2001). As time goes on *Cocconeis* becomes less abundant at the expense of other taxa (Marks and Power, 2001) so the lower diversity on *Cladophora* may be due in part to it being new growth when sampled.

Conclusions

Epiphytic diatom communities in Kentucky's upper Green River show an overall trend of increasing density and diversity in a downstream direction. Both *Cladophora* and *P. ceratophyllum* are important primary producers in this system that host dense communities of diatoms. It remains unclear if the diatom communities in this study are a significant food source for grazers. It also remains unclear if the abundance of *Cocconeis* and overall low diversity are due to selective grazing pressure or flow scouring other genera.

It is possible that grazers promote the dominance of *Cocconeis* by selectively feeding on other genera, which keeps them at lower densities. If this is the case then epiphytes are obviously an important food source for grazers. If flow is keeping other genera at low density then it is less obvious if the diatom community can support grazers. If flow is responsible then is it possible that non-grazer resistant taxa which make up a small proportion of the community are still abundant enough to support grazers because of high diatom density? If this is the case then it would be expected that downstream epiphyte communities can best support grazers because there is greater diatom density.

Future studies should aim to see if grazing or flow is having a larger influence on the diatom communities in the Green River. If flow is responsible, then a gut analysis of common grazers may reveal if the diatom communities can support grazers despite the low relative abundance of non-grazer resistant taxa. It would also be interesting to see if at different times of the year, or in a season with less high flow disturbances, if the communities are more diverse and have a greater abundance of non-grazer resistant taxa.

Figures and Tables

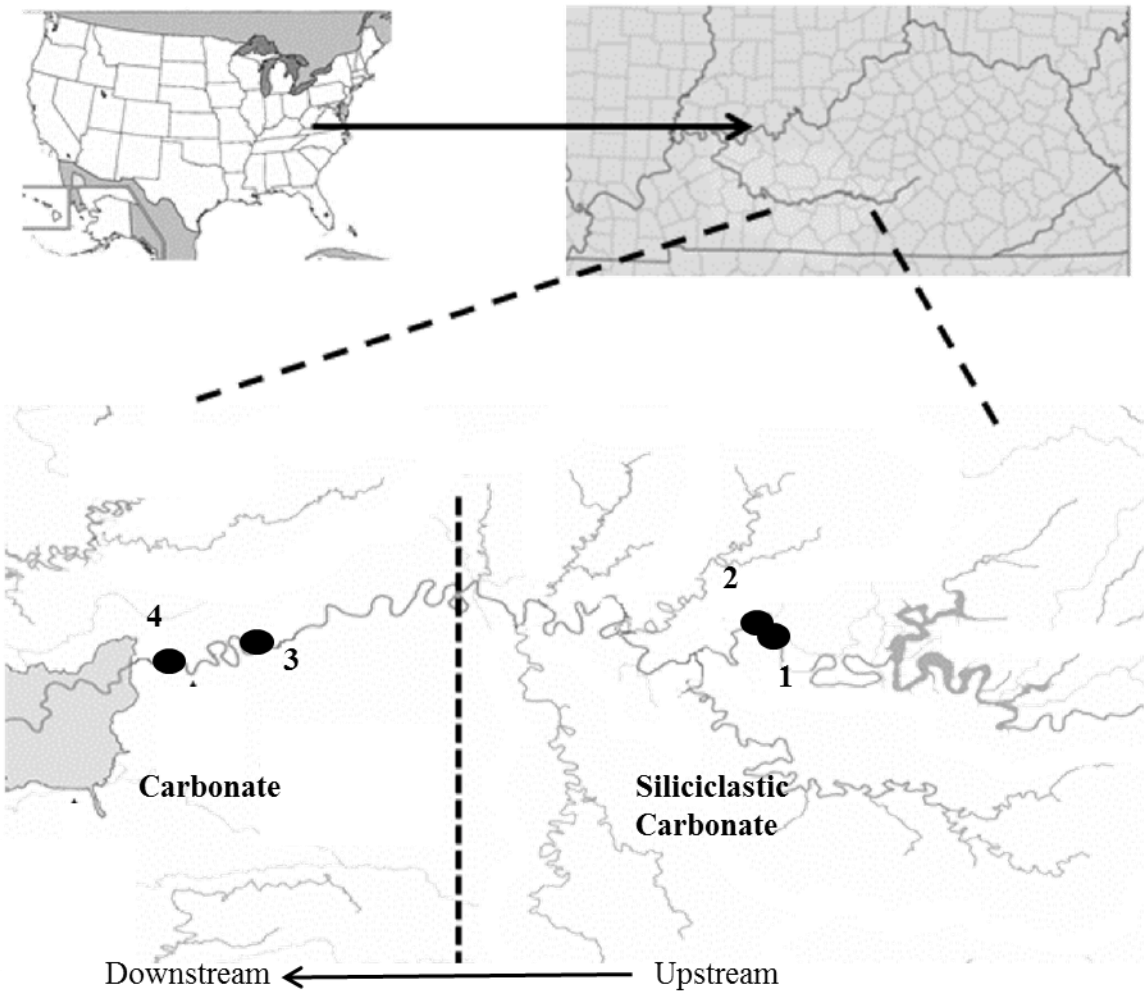

Figure 1: A map showing the 4 study reaches in the Green River.

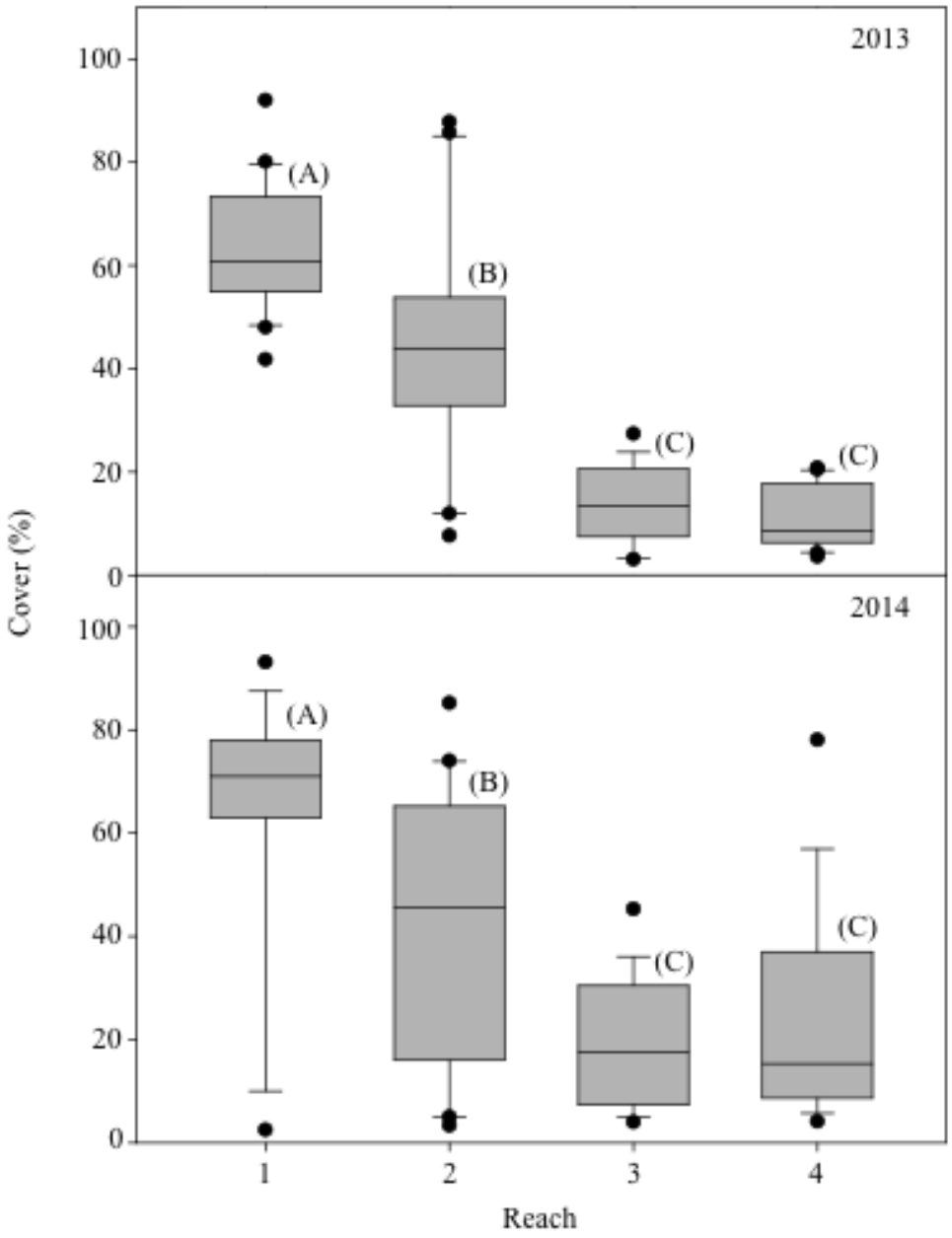

Figure 2: Boxplots of the percent cover of *P. ceratophyllum* for each reach in 2013 and 2014. September and October data were combined for both years. The boxes show the median and interquartile range. Whiskers are the 10th and 90th percentiles. Solid circles are outliers.

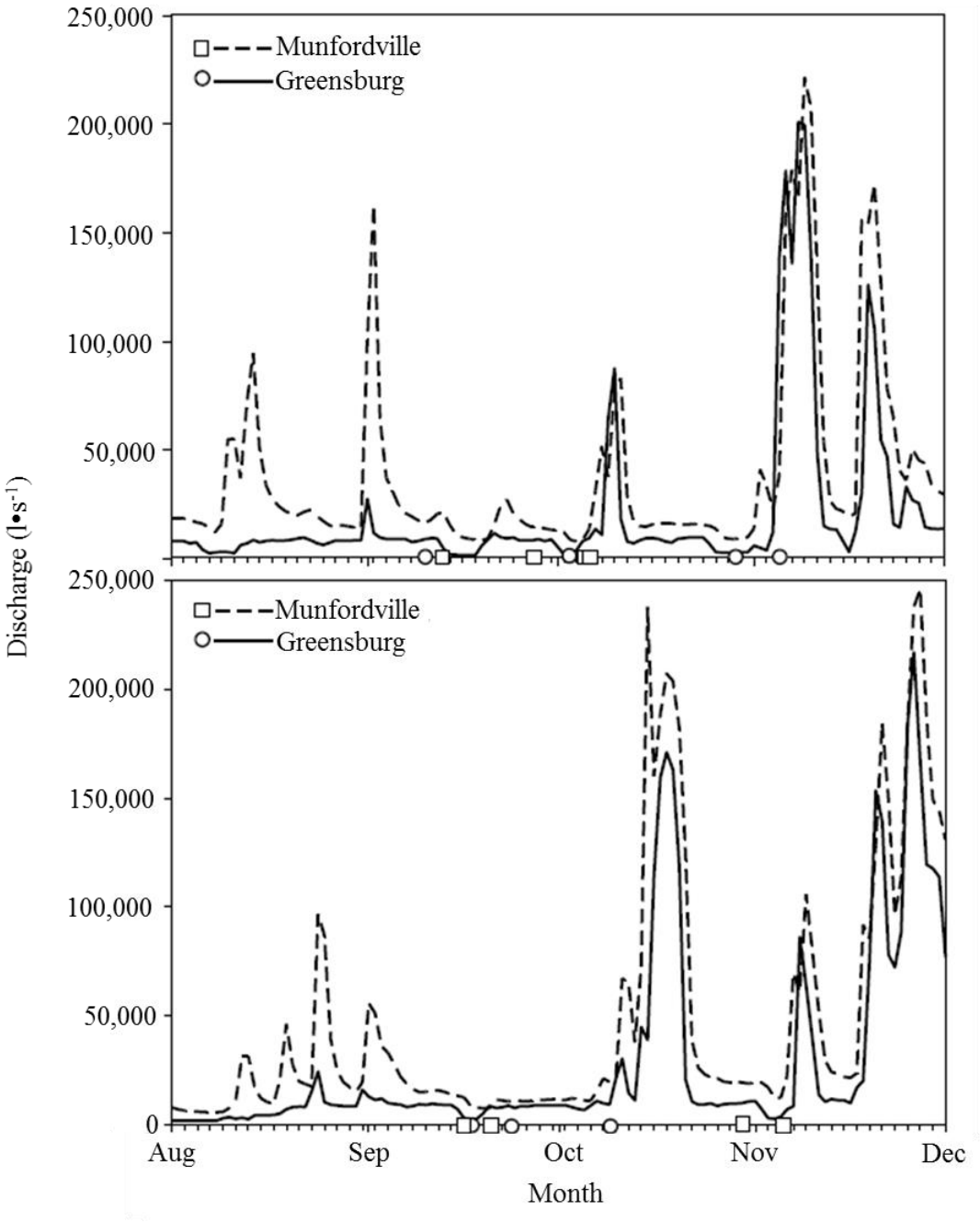

Figure 3: Discharge data from August through December of 2013 [top] and 2014 [bottom]. Reach 1 and 2 are represented by the Greensburg data and reach 3 and 4 are represented by the Munfordville data. The circles and squares on the x-axis indicate sampling days.

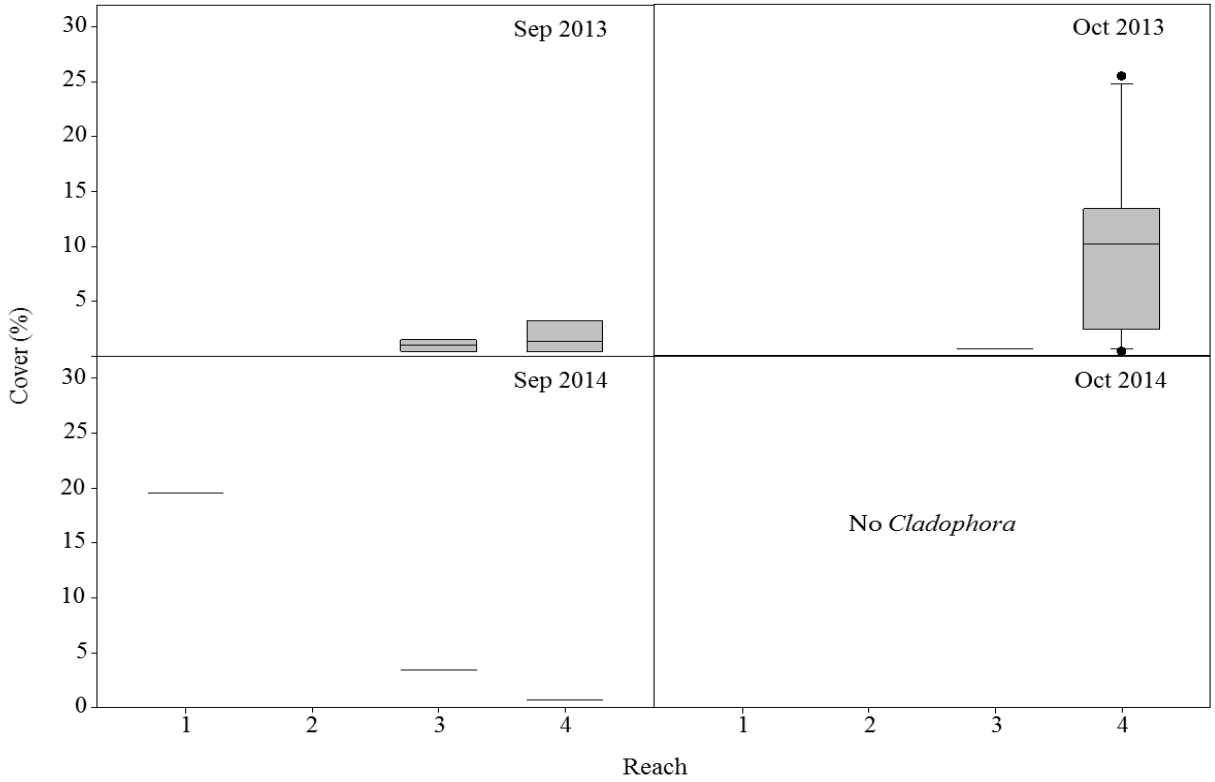

Figure 4: Boxplots of the percent cover of *Cladophora* for each reach in September and October of 2013 and September 2014. There was no *Cladophora* present in October 2014. The boxes show the median and interquartile range, the whiskers are the 10^{th} and 90^{th} percentiles. Solid circles are outliers. Flat lines mean there was only one value. Boxes without whiskers have a sample size less than 10. When there was no *Cladophora* there were still holdfasts present.

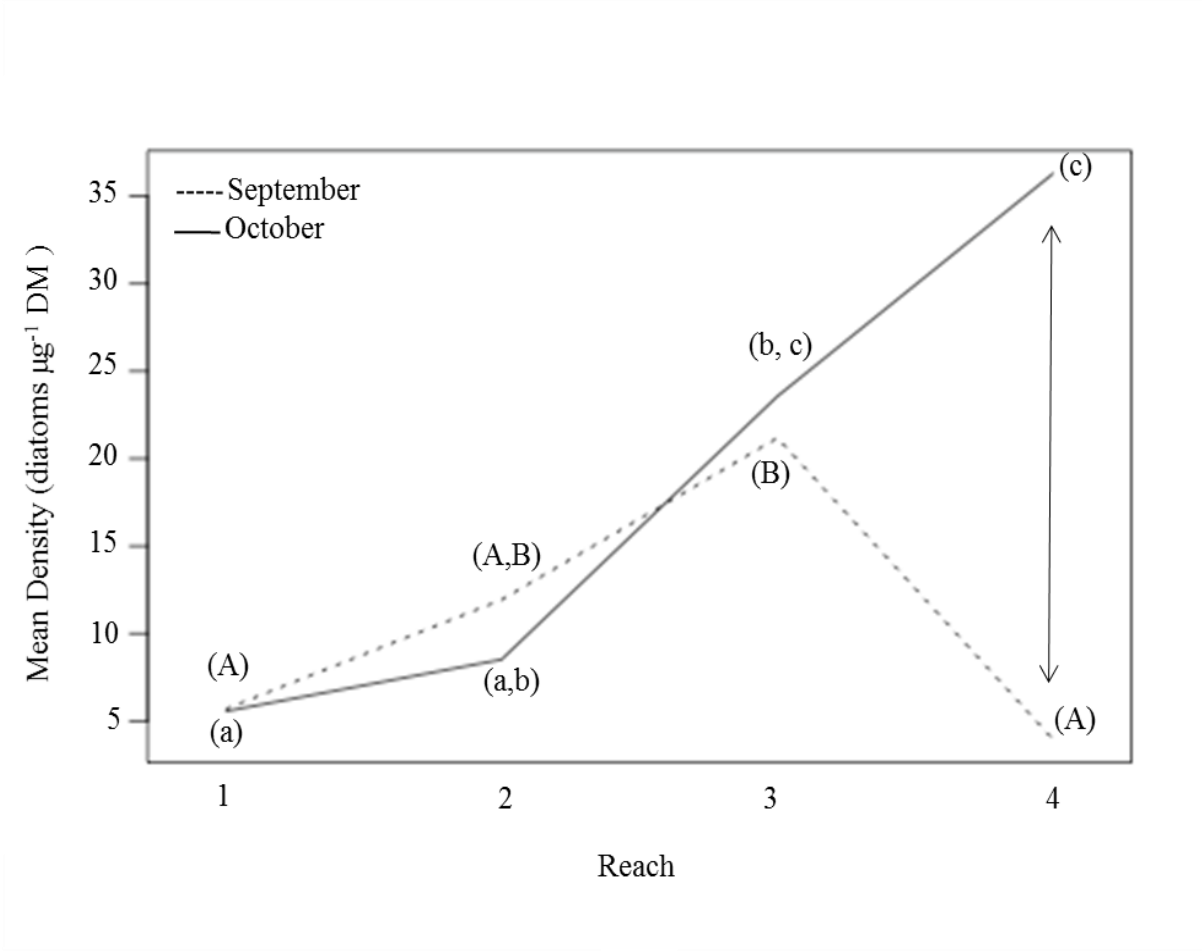

Figure 5: An interaction plot of the mean *P. ceratophyllum* diatom density in each reach for September and October. The mean density values were the same used in the resampling procedure. Letters indicate significance. Letters that are the same indicate no significant difference and letters that are different indicate significantly different. Capital letters correspond to September and lower case letters correspond to October. The arrow indicates significant differences between months.

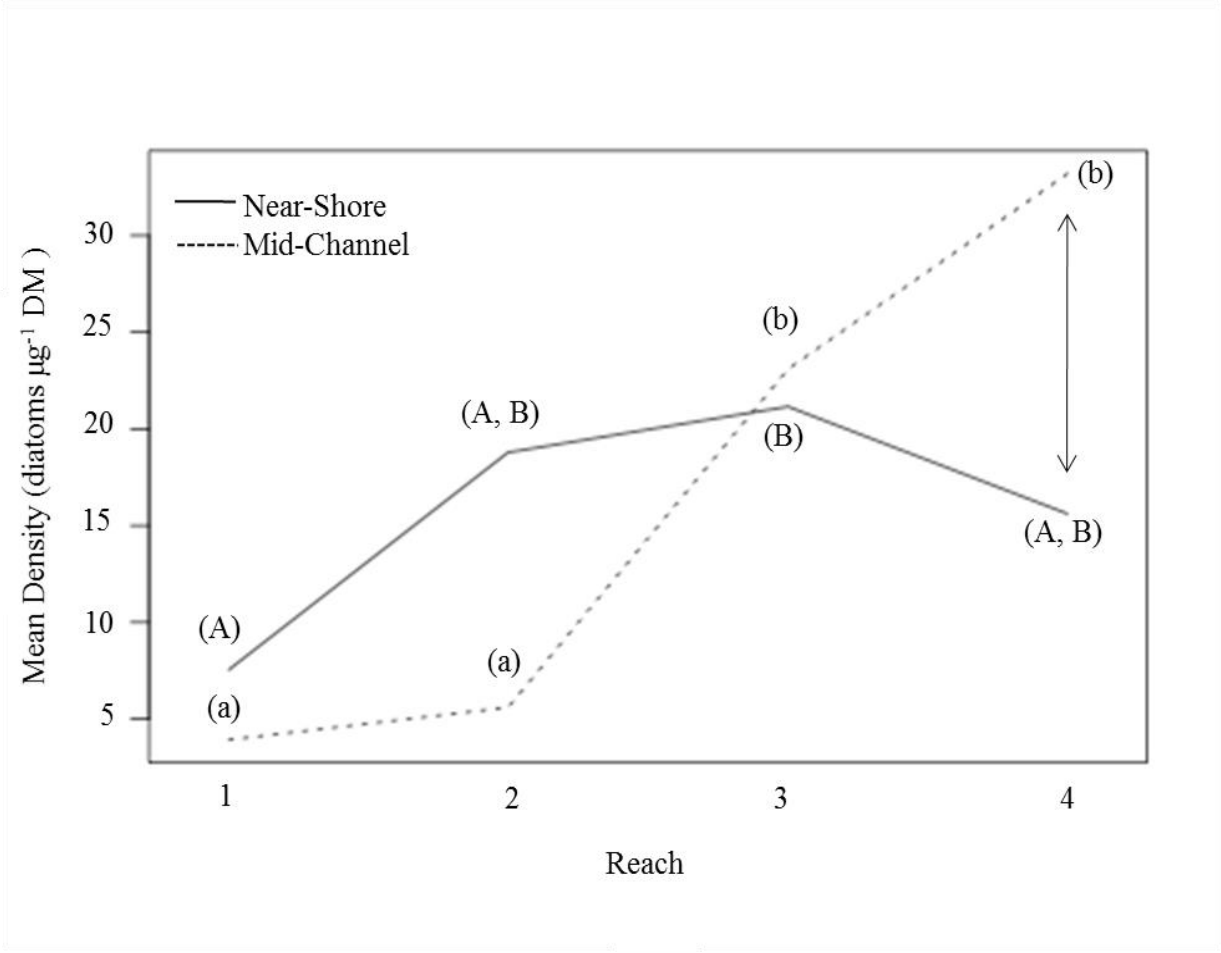

Figure 6: An interaction plot of the mean *P. ceratophyllum* diatom density in near-shore and mid-channel habitats for each reach. The mean density values were the same used in the resampling procedure. Letters indicate significance. Letters that are the same indicate no significant difference and letters that are different indicate significantly different. Capital letters correspond to near-shore and lower case letters correspond to mid-channel. The arrow indicates significant differences between habitat types.

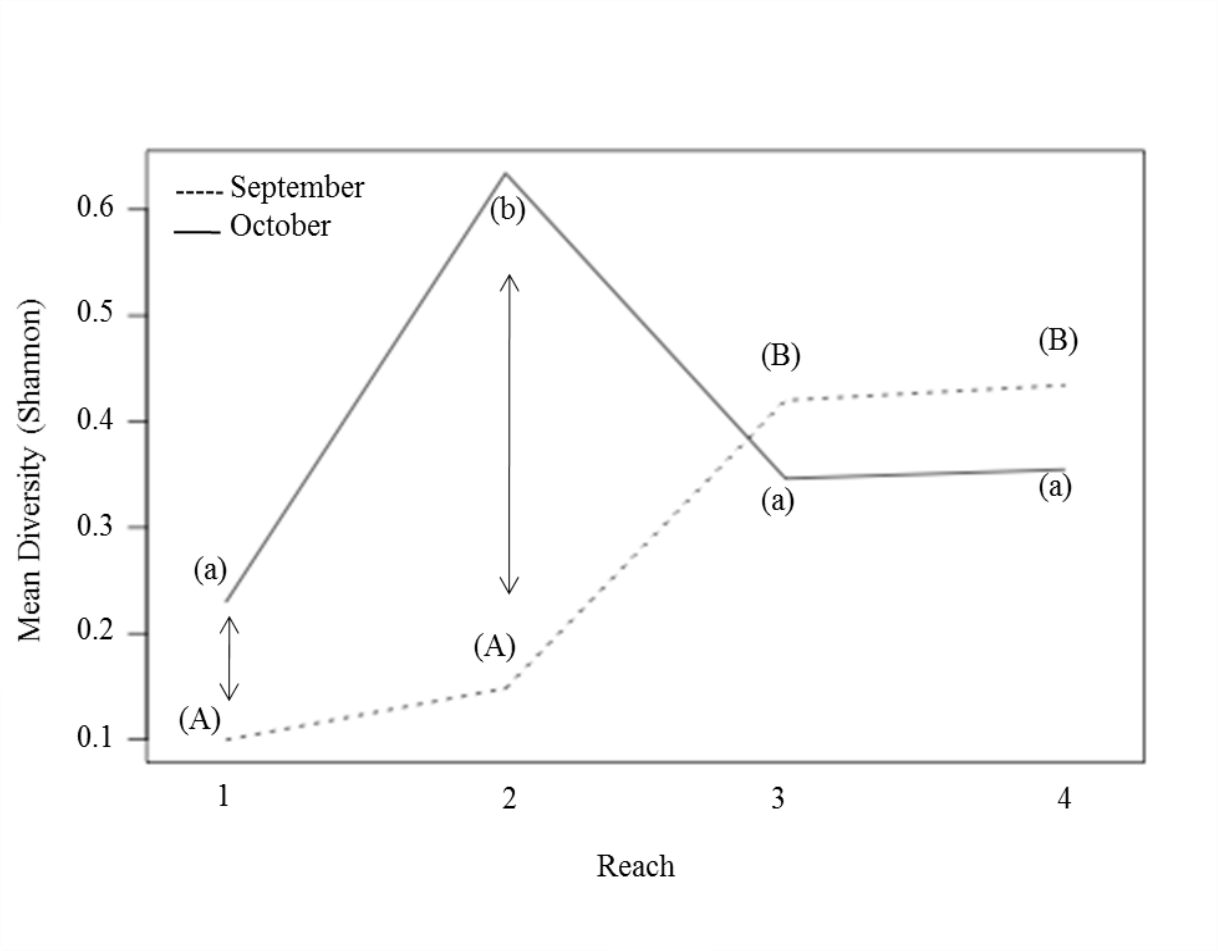

Figure 7: An interaction plot of the mean *P. ceratophyllum* diatom diversity in each reach for September and October. The mean diversity values were the same used in the resampling procedure. Letters indicate significance. Letters that are the same indicate no significant difference and letters that are different indicate significantly different. Capital letters correspond to September and lower case letters correspond to October. The arrows indicates significant differences between months.

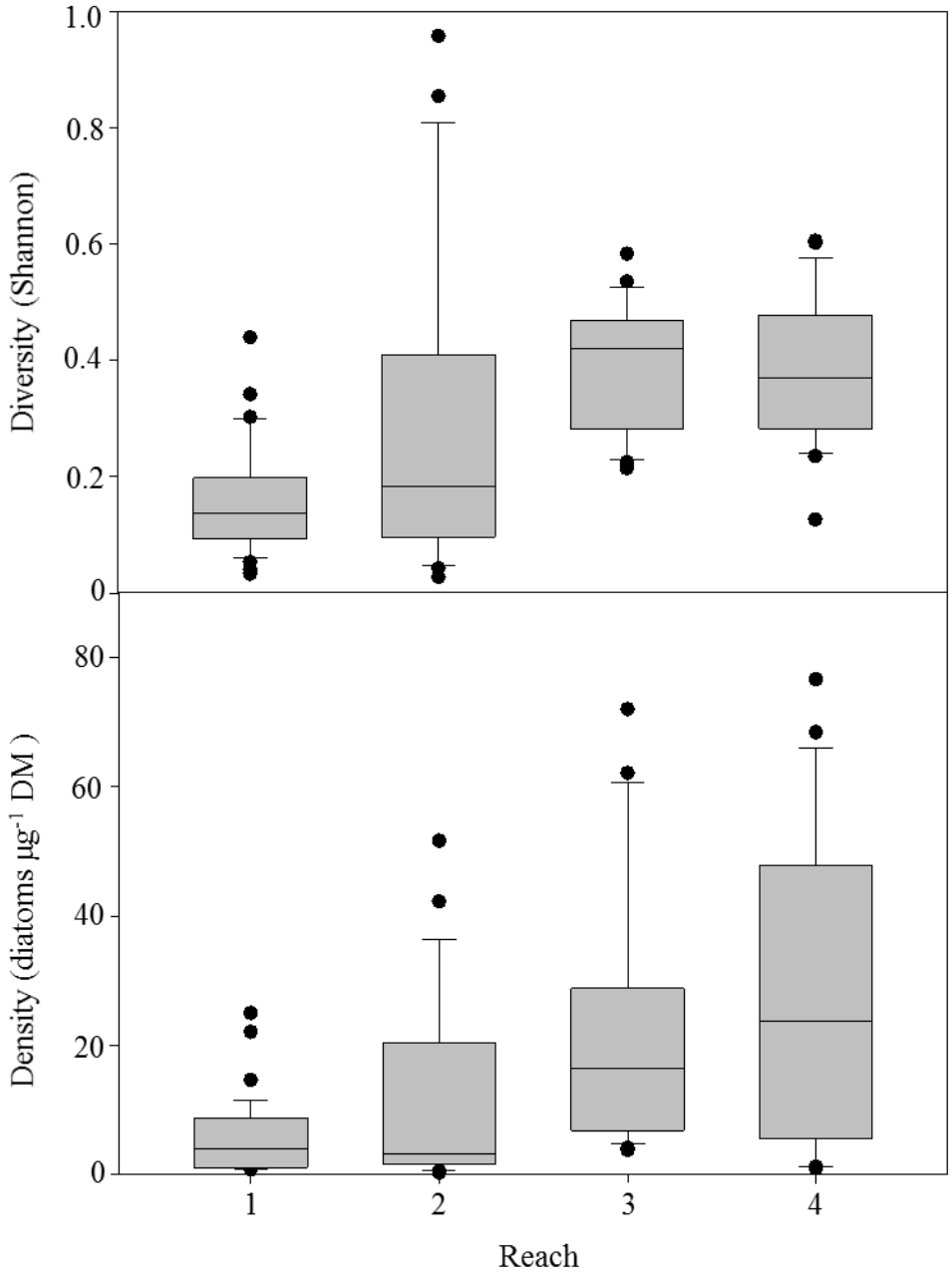

Figure 8: Boxplots of diatom diversity and density in each reach. The boxes show the median and interquartile range, the whiskers are the 10th and 90th percentiles. Solid circles are outliers. These data are showing month and habitat types combined.

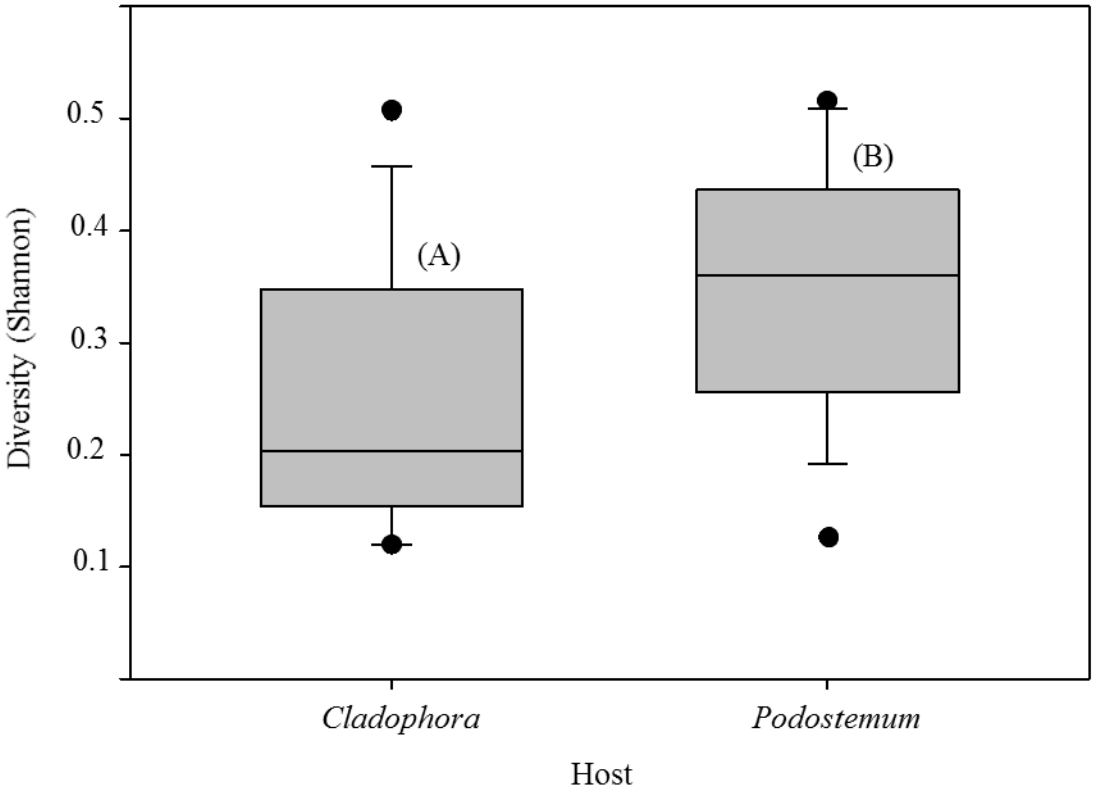

Figure 9: A boxplot of diatom density on *Cladophora* and *P. ceratophyllum.* The whiskers are the 10th and 90th percentiles. Solid circles are outliers. Letters indicate significance. Letters that are the same indicate no significant difference and letters that are different indicate significantly different.

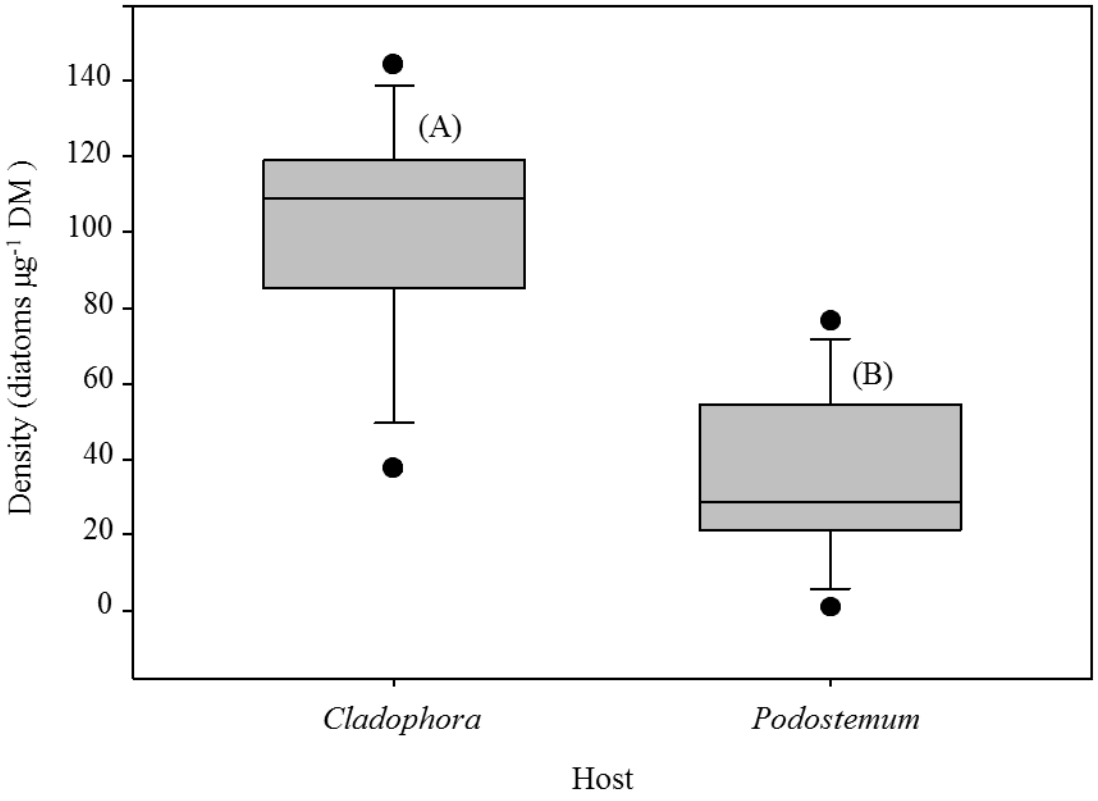

Figure 10: A boxplot of diatom diversity on *Cladophora* and *P. ceratophyllum.* The whiskers are the 10th and 90th percentiles. Solid circles are outliers. Letters indicate significance. Letters that are the same indicate no significant difference and letters that are different indicate significantly different.

Table 1: Locations and characteristic of the study reaches. Temperature and discharge were calculated as the annual mean of 2013 and 2014.

Reach	Location	GPS (Lat., Long.)	Distance from GRL (km)	Channel width (m)	Discharge ($l \bullet s^{-1}$)	Temperature (°C)
1	Greensburg, KY	37.25365, -85.50200	38	40.8	30,590 ± 48,907	20.7 ± 5.2
2	Greensburg, KY	37.25777, -85.50567	39	40.9	30,590 ± 48,907	20.7 ± 5.2
3	Munfordville, KY	37.27102, -85.88168	130	88.1	60,284 ± 86,749	19.7 ± 5.0
4	WKU GRP	37.24902, -86.01386	150	76.2	60,284 ± 86,749	19.7 ± 5.0

WKU = Western Kentucky University

GRP = Green River Preserve

Table 2: A pairwise comparison of the percent cover of *P. ceratophyllum* in each reach for 2013 and 2014. September and October data were combined in each year. The numbers below the diagonal are p-values and the numbers above the diagonal are the corresponding Euclidean distances.

	2013				2014			
	1	2	3	4	1	2	3	4
1	---	0.185*	0.488*	0.521*	---	0.195*	0.432*	0.398*
2	0.023*	---	0.304	0.336*	0.029*	---	0.237*	0.203*
3	< 0.001*	< 0.001*	---	0.033	< 0.001*	0.006*	---	0.034
4	< 0.001*	< 0.001*	0.693	---	< 0.001*	0.019*	0.710	---

*P < 0.05

Table 3: A list of each genus observed on *P. ceratophyllum* and the percentage of the community that they made up in each reach. The guild classifications are based on Passy (2007).

		Reach			
Genus	Guild	1	2	3	4
Cocconeis	Low Profile	96.7	94.3	91.6	93.1
Navicula	High Profile	1.8	2.8	1.6	1.7
Achnanthes	Low Profile	0.2	0.2	3.4	2.7
Gomphonema	High Profile	0.4	1.0	2.2	1.3
Rhoicosphenia	Low Profile	0.1	0.7	0.9	0.9
Synedra/*Fragilaria*	High Profile	0.5	0.5	0.1	0.1
Nitzschia	Motile	< 0.1	0.4	0.1	0.1
Cymbella	Low Profile	0.1	0.2	0.1	0.1
Diatoma	High Profile	0.1	0.0	< 0.1	< 0.1
Cyclotella	Low Profile	< 0.1	0.0	< 0.1	0.0
Gyrosigma	High Profile	0.0	< 0.1	< 0.1	< 0.1
Pleurosira	High profile	< 0.1	< 0.1	< 0.1	< 0.1

Table 4: A list of each genus observed on *P. ceratophyllum* and *Cladophora* and the percentage of the community that they made up. This table is showing data from reach 4 in October. The guild classifications are based on Passy (2007).

		Host	
Genus	Guild	*P. ceratophyllum*	*Cladophora*
Cocconeis	Low Profile	92.6	95.3
Navicula	High Profile	1.9	0.7
Achnanthes	Low Profile	2.8	1.7
Gomphonema	High Profile	1.4	1.4
Rhoicosphenia	Low Profile	0.9	0.8
Synedra/*Fragilaria*	High Profile	0.1	0.1
Nitzschia	Motile	0.1	0
Cymbella	Low Profile	0.1	< 0.1
Diatoma	High Profile	< 0.1	< 0.1
Gyrosigma	High Profile	< 0.1	0
Pleurosira	High profile	0	< 0.1

Table 5: Results of the 3-way repeated measures factorial ANOVA's for diversity and density of diatoms on *P. ceratophyllum*

Independent Variable	Diversity (Shannon Index)		Density (diatoms•μg^{-1} DM)	
	F	P	F	P
Reach	35.20	<0.001*	14.72	<0.001*
Month	22.41	0.007*	5.61	0.081
Habitat	10.60	0.068	0.32	0.674
Reach × Month	32.46	<0.001*	8.00	0.004*
Reach × Habitat	1.10	0.795	7.48	0.005*
Habitat × Month	1.06	0.556	0.27	0.711
Reach × Habitat ×Month	0.91	0.834	2.78	0.210

*P < 0.05

Table 6: A pairwise comparison of the significant interaction between month and reach for diatom density on *P. ceratophyllum.* The numbers below the diagonal are p-values and the numbers above the diagonal are the corresponding Euclidean distances. N/A indicates not of interest.

		September				October			
		1	2	3	4	1	2	3	4
September	1	----------	6.25	15.51*	1.76	0.13	N/A	N/A	N/A
	2	0.299	----------	9.26	8.01	N/A	3.42	N/A	N/A
	3	0.008*	0.130	----------	17.27*	N/A	N/A	2.38	N/A
	4	0.829	0.317	0.029*	----------	N/A	N/A	N/A	32.41*
October	1	0.983	N/A	N/A	N/A	----------	2.96	18.02*	30.78*
	2	N/A	0.694	N/A	N/A	0.730	----------	15.06	27.81*
	3	N/A	N/A	0.724	N/A	0.008*	0.092	----------	12.75
	4	N/A	N/A	N/A	<0.001*	<0.001*	0.003*	0.068	----------

*P < 0.05

Table 7: Results of the 2-way repeated measures factorial ANOVA's for diatom diversity and density on *P. ceratophyllum*

Independent Variable	Diversity (Shannon Index)		Density (diatoms•μg^{-1} DM)	
	F	(P)	F	(P)
Reach	15.82	< 0.001*	10.73	< 0.001*
Habitat	2.43	0.187	0.01	0.957
Reach * Habitat	0.23	0.926	3.83	0.030*

*P < 0.05

Table 8: Mean (± 1 S.D.) of diversity and density of diatoms on *P. ceratophyllum* for each reach in September and October.

Month	Reach	Diversity (Shannon)	Density (diatoms•μg^{-1} DM)
September	1	0.10 ± 0.04	5.67 ± 5.65
	2	0.15 ± 0.09	11.92 ± 13.60
	3	0.42 ± 0.10	21.19 ± 18.68
	4	0.43 ± 0.14	3.91 ± 2.27
October	1	0.23 ± 0.09	5.55 ± 5.98
	2	0.63 ± 0.25	8.51 ± 16.56
	3	0.35 ± 0.11	23.57 ± 18.61
	4	0.35 ± 0.10	36.32 ± 22.17

Table 9: A pairwise comparison of the significant interaction between month and reach for diatom diversity on *P. ceratophyllum.* The numbers below the diagonal are p-values and the numbers above the diagonal are the corresponding Euclidean distances. N/A indicates not of interest.

		September				October			
		1	2	3	4	1	2	3	4
September	1	----------	0.049	0.320*	0.334*	0.130*	N/A	N/A	N/A
	2	0.426	----------	0.272*	0.286*	N/A	0.486*	N/A	N/A
	3	<0.001*	<0.001*	----------	0.014	N/A	N/A	0.074	N/A
	4	<0.001*	<0.001*	0.855	----------	N/A	N/A	N/A	0.080
October	1	0.029*	N/A	N/A	N/A	----------	0.405*	0.117	0.125
	2	N/A	<0.001*	N/A	N/A	<0.001*	----------	0.288*	0.279*
	3	N/A	N/A	0.290	N/A	0.099	0.002*	----------	0.008
	4	N/A	N/A	N/A	0.343	0.052	0.002*	0.910	----------

*P < 0.05

Table 10: A pairwise comparison of diatom diversity in each reach. The numbers below the diagonal are p-values and the numbers above the diagonal are the corresponding Euclidean distances

		Reach			
		1	2	3	4
Reach	1	----------	0.123	0.234	0.223
	2	0.012*	----------	0.110	0.099
	3	< 0.001*	0.034*	----------	0.011
	4	< 0.001*	0.075	0.824	----------

*P < 0.05

Table 11: Mean (± 1 S.D.) for diversity and density of diatoms on *P. ceratophyllum* in near-shore and mid-channel habitats. These values are for September and October combined.

Reach	Habitat	Diversity (Shannon)	Density (diatoms•μg^{-1} DM)
1	Near Shore	0.17 ± 0.10	6.83 ± 7.05
	Mid Channel	0.15 ± 0.08	4.94 ± 5.85
2	Near Shore	0.31 ± 0.19	19.03 ± 18.15
	Mid Channel	0.24 ± 0.27	5.61 ± 6.82
3	Near Shore	0.39 ± 0.12	17.99 ± 16.52
	Mid Channel	0.38 ± 0.11	19.04 ± 10.32
4	Near Shore	0.40 ± 0.10	18.64 ± 16.43
	Mid Channel	0.36 ± 0.14	33.21 ± 28.00

Table 12: A pairwise comparison of the significant interaction between habitat and reach for diatom density on *P. ceratophyllum.* The numbers below the diagonal are p-values and the numbers above the diagonal are the corresponding Euclidean distances. N/A indicates not of interest.

		Near				Mid			
		1	2	3	4	1	2	3	4
Near	1	----------	11.27	13.64*	8.08	3.59	N/A	N/A	N/A
	2	0.125	----------	2.37	3.19	N/A	13.19	N/A	N/A
	3	0.034*	0.756	----------	5.55	N/A	N/A	1.94	N/A
	4	0.274	0.697	0.467	----------	N/A	N/A	N/A	17.62*
Mid	1	0.544	N/A	N/A	N/A	----------	1.68	19.17*	29.30*
	2	N/A	0.084	N/A	N/A	0.802	----------	17.50*	27.62*
	3	N/A	N/A	0.773	N/A	0.002*	0.009*	----------	10.12
	4	N/A	N/A	N/A	0.024*	<0.001*	<0.001*	0.146	----------

*P < 0.05

Table 13: Results of the 2-way factorial ANOVA for diversity and density of diatoms on *P. ceratophyllum* and *Cladophora*. The comparison indicates which host had a greater density or diversity value for significant effects.

Independent variable	Diversity (Shannon Index) F	P	Density (diatoms•μg^{-1} DM) F	(P)
Host	9.09	0.012*	74.15	<0.001*
Habitat	8.17	0.018*	15.33	0.053
Host × Habitat	0.03	0.883		0.19

*P < 0.05

Table 14: A list of other studies that quantified the density of diatoms on *Cladophora glomerata*.

Location	Habitat	Density	Citation
Ontario, CAN	Lake	Diatoms•μg^{-1} dry mass *C. glomerata*	Malkin et al. (2009)
California, U.S.A.	Stream	Epiphytes•*C. glomerata* $cell^{-1}$	Marks and Power (2001)
Arizona, U.S.A.	Stream	Cells•g^{-1} AFDM of *C. glomerata*	Benenati (1998)
California, U.S.A.	Stream	Cells•mm^{-1} of *C. glomerata*	Bergey et al. (1995)
Arizona, U.S.A.	Stream	Cells•g^{-1} wet mass of *C. glomerata*	Shannon et al., (1994)
California, U.S.A.	Stream	Cells•mm^{-2} of *C. glomerata*	Dudley (1992)
Arizona, U.S.A.	Stream	Cells•cm^{-2} of *C. glomerata* basal attachment	Hardwick et al. (1992)
Longbridge Deverill, ENG	Stream	Diatoms•μg^{-1} dry mass *C. glomerata*	Moore (1977)
Stockholm, SWE	Marine	Cells•mm^{-1} of *C. glomerata*	Jansson (1967)

Table 15: Mean (± 1 S.D.) discharge and water velocity for each reach in September and October. Discharge was recorded for the two upstream reaches combined and for the two downstream reaches combined. Water velocity was recorded on sampling days and is presented as mean per reach and habitat.

Month	Reach	Discharge	Velocity ($m \cdot s^{-1}$)	Habitat	Velocity ($m \cdot s^{-1}$)
September	1	8,355 ± 4,703	0.94 ± 0.21	Near	0.81 ± 0.16
				Mid	1.06 ± 0.19
	2		0.32 ± 0.15	Near	0.38 ± 0.14
				Mid	0.27 ± 0.15
	3	26,845 ± 31,591	0.75 ± 0.16	Near	0.71 ± 0.19
				Mid	0.78 ± 0.12
	4		0.86 ± 0.11	Near	0.82 ± 0.12
				Mid	0.90 ± 0.09
October	1	11,854 ± 17,805	0.49 ± 0.21	Near	0.36 ± 0.14
				Mid	0.62 ± 0.18
	2		0.46 ± 0.16	Near	0.53 ± 0.13
				Mid	0.39 ± 0.15
	3	20,709 ± 18,384	0.37 ± 0.22	Near	0.26 ± 0.21
				Mid	0.48 ± 0.18
	4		0.49 ± 0.20	Near	0.58 ± 0.20
				Mid	0.40 ± 0.16

Literature Cited

Acker F. (2002). Protocol P-13-50: preparation of USGS NAWQA program algal samples for analysis using Palmer-Maloney cells. *Protocols for the analysis of algal samples collected as part of the US Geological Survey National Water-Quality Assessment Program*, 02-06.

Angradi T.R. (1994). Trophic linkages in the lower Colorado River: multiple stable isotope evidence. *Journal of the North American Benthological Society*, 13, 479-495.

Argentina J.E., Freeman M.C. & Freeman B.J. (2010). Predictors of occurrence of the aquatic macrophyte *Podostemum ceratophyllum* in a Southern Appalachian River. *Southeastern Naturalist*, 9, 465-476.

Al-Handal A.Y. & Wulff A. (2008). Marine epiphytic diatoms from the shallow sublittoral zone in Potter Cove, King George Island, Antarctica. *Botanica Marina*, 51, 411-435.

Bazzaz F.A. (1975). Plant species diversity in old-field successional ecosystems in southern Illinois. *Ecology*, 56, 485-488.

Benenati P.L., Shannon J.P. & Blinn D.W. (1998). Desiccation and recolonization of phytobenthos in a regulated desert river: Colorado River at Lees Ferry, Arizona, USA. *Regulated Rivers: Research & Management*, 14, 519-532.

Bergey E.A., Boettiger C.A. & Resh V.H. (1995). Effects of water velocity on the architecture and epiphytes of *Cladophora glomerata* (Chlorophyta). *Journal of Phycology*, 31, 264-271.

Biggs B.J.F., Goring D.G. & Nikora V.I. (1998). Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. *Journal of Phycology*, 34, 598-607.

Blinn D.W., Truitt R. & Pickart A. (1989). Response of epiphytic diatom communities from the tailwaters of Glen Canyon Dam, Arizona, to elevated water temperature. *Regulated Rivers: Research & Management*, 4, 91-96.

Brown J.W. (1975). Land evaluation studies in the mid-wear lowlands of County Durham. Unpublished Doctoral Dissertation, Durham University.

Cattaneo A. & Kalff J. (1980). The relative contribution of aquatic macrophytes and their epiphytes to the production of macrophyte beds. *Limnology and Oceanography*, 25, 280-289.

Collyer M.L., Sekora D.J. & Adams D.C. (2015). A method for analysis of phenotypic change for phenotypes described by high-dimensional data. *Heredity*, in press.

Dodds W.K. (1991). Community interactions between the filamentous alga *Cladophora glomerata* (L.) Kuetzing, its epiphytes, and epiphyte grazers. *Oecologia*, 85, 572-580.

Dodds W.K. & Gudder D.A. (1992). The ecology of *Cladophora. Journal of Phycology*, 28, 415–427.

Dudley T.L. (1992). Beneficial effects of herbivores on stream macroalgae via epiphyte removal. *Oikos*, 65, 121-127.

Dunn A.E., Dobberfuhl D.R. & Casamatta D.A. (2008). A survey of algal epiphytes from *Vallisneria americana* Michx. (Hydrocharitaceae) in the Lower St. Johns River, Florida. *Southeastern Naturalist*, 7, 229-244.

Ensminger I., Hagen C. & Braune W. (2000). Strategies providing success in a variable habitat: I. Relationships of environmental factors and dominance of *Cladophora glomerata*. *Plant, Cell & Environment*, 23, 1119-1128.

Falkowski P.G., Barber R.T. & Smetacek V. (1999). Biogeochemical controls and feedbacks on ocean primary production. *Science*, 281, 200-206.

Francoeur S.N. & Biggs B.J.F. (2006). Short-term effects of elevated velocity and sediment abrasion on benthic algal communities. Developments in Hydrobiology, 185, 59-69.

Frankovich T.A., Gaiser E.E., Zieman J.C. & Wachnicka A.H. (2006). Spatial and temporal distributions of epiphytic diatoms growing on *Thalassia testudinum* Banks ex Konig: relationships to water quality. *Hydrobiologia*, 569, 259-271.

Ferreiro N., Giorgi A. & Feijoo C. (2013). Effects of macrophyte architecture and leaf shape complexity on structural parameters of the epiphytic algal community in a Pampean stream. *Aquatic Ecology*, 47, 389-401.

Fiala A., Garman S.L. & Gray A.N. (2006). Comparison of five canopy cover estimation techniques in the western Oregon Cascades. *Forest Ecology and Management*, 232, 188-197.

Finlay J.C., Power M.E. & Cabana G. (1999). Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. *Limnology and Oceanography*, 44, 1198-1203.

Furey P.C., Lowe R.L., Power M.E. & Campbell-Craven A.M. (2012). Midges, *Cladophora*, and epiphytes: shifting interactions through succession. *Freshwater Science*, 31, 93-107.

Gerbersdorf A.U., Bittner R., Lubarsky H., Manz W. & Paterson D.M. (2009). Microbial assemblages as ecosystem engineers of sediment stability. *Journal of Soils and Sediments*, 9, 640-652.

Hardwick G.G., Blinn D.W. & Usher H. D. (1992). Epiphytic diatoms on *Cladophora glomerata* in the Colorado River, Arizona: longitudinal and vertical distribution in a regulated river. *The Southwestern Naturalist*, 37, 148-156.

Hecky R. E. & Kilham P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: a review of recent evidence on the effects of enrichment. *Limnology and Oceanography*, 33, 196-822.

Hill B.H. & Webster J.R. (1984). Productivity of *Podostemum ceratophyllun* in the New River, Virginia. *American Journal of Botany*, 71, 130-136.

Hunt C.C. & Hendricks, S.P. (2008). Diatom species composition and environmental conditions at four perennial springs in western Kentucky and Tennessee. *Journal of the Kentucky Academy of Science*, 69, 141-151.

Hutchens J.J., Wallace J.B. & Romaniszyn E.D. (2004). Role of *Podostemum ceratophyllum* Michx. in structuring benthic macroinvertebrate assemblages in a southern Appalachian river. *Journal of the North American Benthological Society*, 23, 713-727.

Jansson A.M. (1967). The food-web of the *Cladophora*-belt fauna. *Helgoländer Wissenschaftliche Meeresuntersuchungen*, 15, 574-588.

Kaplan D., Maymon M., Agapakis C.M., Lee A., Wang A., Prigge B.A., Volkogon M. & Hirsch A.M. (2013). A survey of the microbial community in the rhizosphere of two dominant shrubs of the Negev Desert Highlands, Zygophyllum dumosum

(Zygophyllaceae) and *Atriplex halimus* (Amaranthaceae), using cultivation dependent and cultivation-independent methods. *American Journal of Botany*, 100, 1713-1725.

Kentucky Geologic Survey. 2015. [Interactive Kentucky geologic map]. Kentucky Geologic Map Information Service. Retrieved from http://kgs.uky.edu/kgsmap/kgsgeoserver/viewer.asp#.

Knapp J.M. & Lowe R.L. (2009) Spatial distribution of epiphytic diatoms on lotic bryophytes. *Southeastern Naturalist*, 8, 305-316.

Kricher J. (2011) Tropical Ecology. Princeton, NJ: Princeton University Press.

Kolayli S., Baysal A. & Sahin B. (1998). A study on the epipelic and epilithic algae of Sana River (Trabzon/Turkey). *Turkish Journal of Biology*, 22, 163-170.

Kooistra W.H. & Medlin L.K. (1996). Evolution of the diatoms (Bacillariophyta). IV. A reconstruction of their age from small subunit rRNA coding regions and the fossil record. *Molecular Phylogenetic Evolution*, 6, 391-407.

Lamb M. A. & Lowe R.L. (1987). Effects of current velocity on the physical structuring of diatom (Bacillariophyceae) communities. *Ohio Journal of Science*, 87, 72-78.

Lange K., Leiss A., Piggott J.J., Townsend C.R. & Matthaie C.D. (2011). Light, nutrients and grazing interact to determine stream diatom community composition and functional group structure. *Freshwater Biology*, 56, 264–278.

Madsen J.D. (1999). Point intercept and line intercept methods for aquatic plant management (No. WES-MI-02). Army Engineer Waterways Experiment Station. Vicksburg, MS.

Malkin S.Y., Sorichetti R.J., Wiklund J.A. & Hecky R.E. (2009). Seasonal abundance, community composition, and silica content of diatoms epiphytic on *Cladophora glomerata. Journal of Great Lakes Research,* 35, 199–205.

Malloy E. (2014). Trophic dynamic interactions in a temperate karst river. Master's Thesis, Western Kentucky University, Bowling Green, Kentucky.

Marcarelli A.M. & Wurtsbaugh W.A. (2009). Nitrogen fixation varies spatially and seasonally in linked stream-lake ecosystems. *Biogeochemistry*, 94, 95-110.

Marks J.C. & Power M.E. (2001). Nutrient induced changes in the species composition of epiphytes on *Cladophora glomerat*a Kutz. (Chlorophyta). *Hydrobiologia*, 450, 187-196.

Mayer M.S. & Likens G.E. (1987). The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). *Journal of the North American Benthological Society*, 6, 262-269.

Meijer W. (1976). A note on *Podostemum ceratophyllum* Michx., as an indicator of clean streams in and around the Appalachian Mountains. *Castanea*, 41, 319–324.

Millner G.C. & Sweeney R.A. (1982). Lake Erie *Cladophora* in perspective. *Journal of Great Lakes Research,* 8, 27–29.

Miralto A., Barone G., Romano G., Poulet S.A., Ianora A., Russo G.L. & Buttino I. (1999). The insidious effect of diatoms on copepod reproduction. *Nature*, 402, 173-176.

Molloy J.M. (1992). Diatom communities along stream longitudinal gradients. *Freshwater Biology*, 28, 59-69.

Montagnes D.J.S. & Franklin D.J. (2001). Effect of temperature on diatom volume, growth rate and carbon and nitrogen content: reconsidering some paradigms. *Limnology and Oceanography,* 46, 2008-2018.

Moore J.W. (1977). Seasonal succession of algae in a eutrophic stream in southern England. *Hydrobiologia, 53*, 181-192.

Mpawenayo B. & Mathooko J.M. (2005). The structure of diatom assemblages associated with *Cladophora* and sediments in a highland stream in Kenya. *Hydrobiologia*, 544, 55-67.

Palmer T.C. & Keeley F.J. (1900). The structure of the diatom girdle. *Academy of Natural Sciences Philadelphia,* 52, 465-479.

Passy S.I. (2007). Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquatic Botany*, 86, 171-178.

Patrick R. & Reimer C.W. (1966). Diatoms of the United States. Volume I. Monograph 13, Academy of Natural Sciences of Philadelphia, 688 pp.

Penick M.D., Grubbs S.A. & Meier A.J. (2012). Algal biomass accrual in relation to nutrient availability and limitation along a longitudinal gradient of a karst riverine system. *International Aquatic Research*, 4, 1-13.

Philbrick C.T. & Novelo A. (2004). Monograph of *Podostemum* (Podostemaceae). *Systematic Botany Monographs*, 1-106.

Power M.E., Lowe R., Furey P., Welter J., Limm M., Finlay J., Bode C., Chang S., Goodrich M. & Sculley J. (2009). Algal mats and insect emergence in rivers

under Mediterranean climates: towards photogrammetric surveillance. *Freshwater Biology*, 54, 2101–2115.

Saunders L.L., Kilham S.S., Fairchild W.G. & Verb, R. (2012). Effects of small- scale environmental variation on metaphyton condition and community composition. *Freshwater Biology*, *57*, 1884-1895.

Scala S. & Bowler C. (2001). Molecular insights into the novel aspects of diatom biology. *Cellular and Molecular Life Sciences*, 58, 1666-1673.

Shannon J.P., Blinn D.W. & Stevens L.E. (1994). Trophic interactions and benthic animal community structure in the Colorado River, Arizona, USA. *Freshwater Biology*, 31, 213-220.

Sims P.A., Mann D.G. & Medlin L.K. (2006). Evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia*, 45, 361–402.

Smetacek V. (1985). Role of sinking in diatom life history cycles: ecological, evolutionary, and geologic significance. *Marine Biology*, 84, 239-251.

Smith M.E. & Manoylov K.M. (2013). Changes in Diatom Biodiversity in Lake Sinclair, Baldwin County, Georgia, USA. *Journal of Water Resource and Protection*, 5, 732-742.

Snyder E.B., Robinson C.T., Minshall G.W. & Rushforth S.R. (2002). Regional patterns in periphyton accrual and diatom assemblage structure in a heterogeneous nutrient landscape. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, 564–577.

Soininen J. & Eloranta P. (2004). Seasonal persistence and stability of diatom communities in rivers: are there habitat specific differences? *European Journal of Phycology*, 39, 153-160.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980). The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, 37, 130-137.

Webster W.J., Benjield E.F., Golladay S.W., Hill B.H., Hornick L.E., Kazmierczak R.F. & Perry W.B. (1987). Experimental studies of physical factors affecting seston transport in streams. *Limnology and Oceanography*, 32, 848-863.

Wehr J.D. & Sheath R.G. (2002). Freshwater Algae of North America: Ecology And Classification. Academic Press, San Diego, 918 pp.

Wetzel, R.G. & Likens, G.E. 1991. Limnological Analyses, 2nd ed. Springer-Verlag, New York, 391 pp.

Yang H. & Flower R. J. (2012). Effects of light and substrate on the benthic diatoms in an oligotrophic lake: a comparison between natural and artificial substrates. *Journal of Phycology*, 48, 1166-1177.

Zotz G. (2005). Vascular epiphytes in the temperate zones - a review. *Plant Ecology*, 176, 173-183.

Zulkifly S.B., Graham J.M., Young E.B., Mayer R.J., Piotrowski M.J., Smith I. & Graham, L.E. (2013). The genus *Cladophora* Kützing (Ulvophyceae) as a globally distributed ecological engineer. *Journal of Phycology*, 49, 1-17.