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Relationships between nutrients, periphyton abundance, and benthic macroinvertebrate scrapers in Ozark Highland streams

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Abstract

We collected samples from seven Arkansas streams during the months of August 2014 and February 2015 with the objective of determining possible relationships between nutrients, periphyton abundance and benthic macroinvertebrates. We hypothesized that periphyton would be positively associated with nutrients, and that increases in periphyton biomass would increase the abundance and diversity of benthic scraper families. Samples were collected from three transects at each site. The periphyton was analyzed for carbon, nitrogen, phosphorus, and ash free dry mass and Chl-a content. Macroinvertebrates families belonging to the feeding group of grazers/scrapers were compared with water column and periphyton nutrient concentrations, as well as Chl-a and ash free dry mass of periphyton from each site using correlation techniques. Negative linear correlations were observed between particulate phosphorus within the periphyton and Simpson (1-D) diversity ($r=-0.87$; $p<0.0025$), Shannon (H) diversity ($r=-0.82$; $p<0.0025$) and equitability (J) ($r=-0.88$; $p<0.0025$). The percentage of scrapers from the family Heptageniidae was positively correlated with particulate phosphorus in the periphyton ($r_s=0.92$; $p<0.0025$). Contrary to our hypothesis, these results suggest that that Heptageniidae may come to dominate in areas with P-rich periphyton, thereby decreasing benthic scraper diversity.

Introduction

The preservation of water quality in freshwater streams and rivers has become an issue of growing interest in recent decades. According to the United States Environmental Protection Agency, around 40% of wadeable streams did not meet designated use criteria or were not suitable for uses such as drinking, swimming, or fishing (USEPA, 2002; USEPA, 2006). Wadeable streams compose approximately 90% of all United States perennial streams and rivers, thus the condition of United States wadeable streams can significantly influence the quality of water downstream in larger rivers or other bodies of water (USEPA, 2006; Alexander et al., 2008; Dodds and Oakes, 2008). By and large, the decline of water quality in United States freshwater streams has highlighted a need for the development of reliable water quality assessment techniques. Improving our understanding of freshwater ecosystems will refine our ability to assess water quality and to ensure that freshwater streams are properly maintained.

Biomonitoring, which consists of sampling stream communities to assess water quality or ecosystem health, relies on the principle that organisms living in the water represent the quality of the water during their life in the system in question. In other words, the community structure can relate to the quality of the water in which organisms live (Byl *et al.* 1994). It has been well documented that observation of benthic macroinvertebrate community structure is an effective means of assessing ecosystem health in freshwater streams (Hilsenhoff, 1987; Washington, 1984; Smith et al., 2007; Justus et al, 2010). Benthic macroinvertebrates are organisms less than or equal to 1 mm in size during some point in their life cycle that live among stones, logs, sediments and aquatic plants on the bottoms of streams, rivers and lakes. These organisms primarily include insects, molluscs and crustaceans in temperate freshwaters. Benthic macroinvertebrates are useful as bioindicators because they have diverse community structure, are visible to the naked eye, and are relatively easy to collect. The high diversity among benthic macroinvertebrates makes them ideal for studies involving stream water integrity because they have varying sensitivities to changes in environmental quality. Assessment of benthic community structure may reveal the absence of sensitive taxa, dominance of particular taxa, low taxa richness, or other measureable changes in community structure. When compared with some reference condition, such as the presence or absence of a given stressor, results can provide a quantified measure of stream integrity (Plafkin *et al*., 1989; Lazorchak *et al*., 1998; Barbour *et al*., 1999; USEPA, 2006).

While macroinvertebrate communities as a whole are well established as bioindicators, many aspects of the interactions between different trophic levels within benthic communities and their environment remain poorly characterized. It is difficult to characterize the specific food types that macroinvertebrates consume because benthic food resources are heavily intermingled (Steinman, 1996). For this reason, they have often been grouped in terms of their method of acquiring food rather than by the food resources they actually consume. These categories, initially developed by Cummins (1973), are known as functional feeding groups (FFGs). The Environmental Protection Agency currently classifies benthic macroinvertebrates into the following functional feeding groups: parasites, predators, omnivores, gatherer-collectors, filterer-collectors, scrapers, shredders, and piercers (Barbour *et al*., 1999). Of these feeding groups, scrapers, piercers and shredders represent categories of particular importance to researchers because they are more specialized than the other groups. Specialist feeders are often more sensitive to pollutants and are expected to be better represented in healthy streams (Barbour *et al*., 1999). Scrapers in particular represent a unique group because they feed primarily on the periphyton, which is a biofilm matrix of algae and bacteria growing on inorganic benthic surfaces. Periphytons utilize dissolved nutrients as a resource and can respond positively to increasing nutrient concentrations. Scrapers that are reliant on periphyton as a food resource may therefore respond positively to this change in food resource availability.

Many studies have demonstrated a close relationship between algal growth and herbivory by benthic scrapers. The relationship is complicated, however, because it is not unidirectional. That is to say, benthic invertebrate scrapers both are affected by and have an effect on algae in aquatic ecosystems (Gregory, 1983; Lamberti and Moore, 1984; Wallace, 1996). In this study, we hypothesize that increases in dissolved nutrient concentrations will have an overall positive effect on periphyton biomass. Additionally, increases in periphyton biomass may have a positive effect on benthic scraper community as a result of increased food availability. If periphyton does respond positively to increases in dissolved nutrients, then we expect abundance and diversity of scrapers reliant on periphyton as a food resource to respond positively as well. The indirect effect of nutrients on benthic scrapers may not be immediately detectable, however, because of the time required for benthic scrapers to colonize areas with increased periphyton growth. Thus benthic scraper responses to dissolved nutrient concentrations are not expected to be as significant as their responses to increased periphyton biomass.

Previous studies have shown algal biomass generally decreases in the presence of herbivory (Gregory, 1983; Steinman, 1996). These trends, however, are highly dependent on the alga and the herbivore involved. In certain circumstances, herbivory on overstory algae may increase the abundance of understory algae in periphyton as a result of increased habitat availability (Steinman, 1996). The extent to which herbivory may stimulate algal growth to the point that it exceeds the removal rate, however, is not well characterized. For the samples collected in this study, we expected benthic scrapers to exert an overall negative effect on periphyton biomass.

Study Area

This study took place within the Ozark Highlands ecoregion of northwestern Arkansas. The Ozark Highlands is characterized by karst topography, which has a basement of igneous rocks that is covered by a layer of sedimentary rock as thick as 1,524 meters. This sedimentary rock is largely composed of dolomite, sandstone and limestone, along with some shale. The climate is temperate, with four defined seasons; fall, winter, spring and summer. Average annual precipitation ranges from around 97 to 122 cm (Peterson, 1995). Before settlement by Europeans, the ecosystem in this area was composed of deciduous oak-hickory forests and bluestem prairie. Much of the primary growth forests from this time no longer exist due to intensive logging, and the forests that exist currently are composed of secondary or tertiary growth (Peterson, 1995). Land use in the study area for the most part takes the form of forest and pastureland. Pastureland is predominately used to grow fescue and Kentucky blue grass. In terms of livestock, poultry, cattle and swine are the most typical, and since the 1930s poultry has been a major industry in the northern regions of Arkansas and Oklahoma (Peterson, 1995).

These land use changes may have stressed water quality throughout the region through a variety of mechanisms, including but not limited to increases in nutrients, sedimentation or pesticides. For the purposes of this study, we focused on the potential impacts of changing nutrient concentrations on the benthic scraper community.

At this point in time, few studies have addressed macroinvertebrate responses to water quality within the Ozark Highlands ecoregion, and even fewer specifically focus on how benthic scrapers may be affected by changes in nutrient concentrations. In light of this deficit, the aim of this study was to explore possible relationships between benthic macroinvertebrate scrapers, periphyton abundance, and nutrients concentrations within several Ozark Highlands streams in a summer (August) and a winter (February) season. In general, we expected abundance and diversity of benthic scrapers to be higher in the summer than in the winter. We hope to gain a better understanding of to what extent scraper communities may be affected by or have an effect on periphyton conditions. This research will provide information on how changing nutrient conditions may relate to scraper populations and communities and could provide information relevant for managing Ozark Highland wadeable freshwater ecosystems.

Materials and Methods

We sampled seven stretches of freshwater streams within the Ozark Highlands ecoregion. Sampling locations are included in Table 1. Drainage area and land use percentages were compiled using USGS Streamstats. All sampling during a sampling event was performed within the shortest time frame possible, within three to four weeks, in order to reduce variability within seasons. Samples were collected during the months of August 2014 and February 2015. As much as possible, all samples were collected together and during base flow conditions, at minimum two weeks after any major rain so that floodwater conditions had time to recede.

At each sampling site, water column samples were collected from the stream. These samples were filtered through 25 mm Whatman glass fiber filter within 48 hours and analyzed for sestonic chl-a. They were then analyzed for soluble reactive phosphorus (SRP), dissolved organic carbon (DOC), total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), ammonium (NH_4^+), nitrate plus nitrite (NO_3+NO_2), total nitrogen (TN), and total phosphorus (TP) on a Skalar San Plus Segmented Flow Analyzer at the Arkansas Water Resource Center's Certified Lab, Fayetteville, AR.

After collection of water column samples, three transects were established along different riffles at the site. Riffles were characterized as shallow sections of the stream with a relatively rapid flow and a surface that is broken up by cobble, gravel or boulders. These were regarded as the highest quality habitat in lotic ecosystems and typically have the highest ecological diversity (Barbour *et al*., 1999). Along each transect we established five equidistant points. At each of these points, a rock was selected that was roughly representative of the size of rocks in the surrounding area. The top surfaces of these five rocks were then scrubbed into a bucket to remove all periphyton and then the surface area of each rock was measured. The periphyton was collected and placed on ice for transport back to the University of Arkansas. Once back in the laboratory, the samples were homogenized with a handheld blender and the total slurry volume was recorded for each sample. Subsamples of the slurry were then filtered onto a 25 mm Whatman glass fiber filter and analyzed for benthic chl-a, particulate phosphorus (PP), carbon (C) and nitrogen

(N) within the periphyton. Periphyton Chl-a was analyzed using a Turner Trilogy Fluorometer. Periphyton P content was analyzed using ammonium molybdate method following persulfate digestion (APHA 2005) and the periphyton C and N were measured using a Thermo Scientific Flash 2000 organic elemental analyzer. The ash free dry mass (AFDM) was also estimated after the sample was dried and ashed at 450 degrees Celsius (APHA 2005)

Macroinvertebrate samples were also collected from each of the five equidistant points along transects within each riffle. These five samples were then combined to form a composite of each transect. All samples were collected using a Hess Sampler fitted with a 500mm mesh net. Each composite sample was preserved in 70% ethanol and transported to the University of Arkansas for processing. In the lab, the composite samples were subsampled by divided the composite into fourths and randomly selecting one of the four. The randomly selected subsamples were then sorted under 10X-40X magnification compound microscope and all macroinvertebrates were counted. A minimum of 300 individuals were counted and identified from each subsample. If the first subsample did not contain 300 individuals, additional subsamples were counted until the count exceeded 300 individuals. The abundance counts from the subsamples were then standardized by the factor corresponded to the number of subsamples counted, so that the final abundance data was representative of the full sample. Macroinvertebrates belonging to the scraper functional feeding group (Barbour *et al*., 1999) were identified to the family level. Family identifications were assigned using the dichotomous keys found in Merritt and Cummins (1996).

Each of the variables observed in this study was examined in order to determine if there were differences between the seasons August of 2014 and February of 2015. The data collected was subjected to statistical analysis using the Paleontological Statistics Software Package (PAST) (Hammer, 2001). T-tests were performed using an alpha value of p=<0.05 on each of the variables observed during August 2014 and February 2015 to determine if significant differences were present between the summer and winter months. The predictive variables of PP, N, C, Chl-a, and AFDM within the periphyton were compared with response variables of TN, TDN, TP, TDP, SRP, NH_4^+, NO_3+NO_2, scraper abundance, percentage of scraper abundance, percent Gastropoda, percent Ephemeroptera, percent Trichoptera, percent Coleoptera, Shannon (H), Simpson (1-D) and equitability (J) of macroinvertebrate scrapers. TN, TDN, TP, TDP, SRP, NH_4^+ and NO_3+NO_2 were also used as predictive variables and were compared to the response variables of scraper abundance, percentage of scraper abundance, percent Gastropoda, percent Ephemeroptera, percent Trichoptera, percent Coleoptera, Shannon (H), Simpson (1-D) and equitability (J) of macroinvertebrate scrapers. These relationships were analyzed using Pearson and Spearman correlation coefficients, and because of the large number of tests run, a Bonferonni corrected alpha value of p=<0.0025 was used in order to reveal significant relationships.

Results

A raw total of 25,882 macroinvertebrate individuals were collected and counted during the course of this study. Of those, 2,017 individuals belonged to FFG of scraper. After the results were standardized based on subsampling, the percentage of scraper individuals constituted about 8% of the total number of individuals collected. Average temperature water temperature was significantly higher in August 2014 than in February 2015 ($t=16.6$; $p=1.2x10^{-9}$). Average abundance of scrapers, however, tended to be higher in August than in February (Figure 1) but did not differ statistically ($t=1.93$; $p=0.07$).

By far the most abundant scraper taxa observed was the family Heptageniidae, which was present in every single sample and the only scraper present in the samples collected from Little Buffalo River during February. The second most abundant observed scraper family was Psephenidae, which was present in all but one of the samples. The family Glossosomatidae, though not present in any of the samples from August, was observed in the majority of samples taken in February (Table 2). At the level family, average Simpson (1-D) diversity ($t=3.24$; $p=7.1x10^{-3}$), Shannon (H) diversity ($t=3.09$; $p=9.4x10^{-3}$) and equitability ($t=3.09$; $p=9.3x10^{-3}$) of scrapers were found to be higher in August 2014 than in February 2015 (Table 3). Richness did not vary significantly between seasons.

The average amount of scrapers belonging to the order Ephemeroptera tended to be higher in February than in August, but did not vary statistically ($t=0.95$; $p=0.36$). Average percentage of scrapers from the order also Trichoptera tended to be higher in February but did not differ statistically between the seasons ($t=-1.14$; $p=0.28$). Coleoptera constituted a significantly higher average percentage of the scraper population in August than in February ($t=3.05$; $p=0.01$) and Gastropoda abundance tended to be higher during August than in February ($t=2.19$; $p=0.05$) (Figure 2).

When the data sets from both August and February were combined, numerous relationships were observed. Positive linear correlations were observed between sestonic

Chl-a and TD ($r=0.62$; $p=0.02$) and TDP ($r=0.57$; $p=0.03$). A positive linear correlation was also observed between sestonic Chl-a and SRP ($r=0.70$; $p=0.01$). Negative linear correlations were observed between PP within the periphyton and Simpson (1-D) diversity ($r=-0.87$;$p=4.2x10^{-5}$), Shannon (H) diversity ($r=-0.82$; $p=2.9x10^{-4}$) and equitability (J) ($r=-0.88$;$p=3.4x10^{-5}$). The percentage of scrapers belonging to the order Ephemeroptera, however, revealed a positive correlation with both PP ($r=0.86$; $p=8.9x10^{-5}$) and Chl-a ($r=0.78$; $p=1.0x10^{-3}$) within the periphyton. Additionally, the percentage of Gastropoda as a proportion of total abundance had a positive correlation ($r=0.77$; $p=1.2x10^{-3}$) with sestonic Chl-a content.

Spearman correlation revealed a significant negative relationship between periphyton Ch-a and Simpson diversity ($r_s=-0.75$; $p=1.8x10^{-3}$). Percent Ephemeroptera abundance showed a positive relationship between periphyton Chl-a ($r_s=0.81$; $p=4.3x10^{-4}$). Significant relationships were also observed between percent Ephemeroptera and periphyton N ($r_s=0.76$; $p=1.7x10^{-3}$), periphyton C ($r_s=0.76$; $p=1.5x10^{-3}$), periphyton PP ($r_s=0.92$; $p=4.1x10^{-6}$), and periphyton AFDM ($r_s=0.76$; $p=1.7x10^{-3}$). Simpson diversity ($r_s=-0.83$; $p=2.0x10^{-4}$), Shannon diversity ($r_s=-0.75$; $p=2.1x10^{-3}$), and equitability ($r_s=-0.86$; $p=7.6x10^{-5}$) showed significant negative correlations with periphyton PP.

Discussion

There was a significant amount of observable variation between the samples collected during August 2014 when compared to those from February 2015. In terms of scraper abundance, it makes sense that there would be higher abundance in August rather than in February. The summer months have more sunlight than winter and early spring, so algae is more likely to be abundant. Since scrapers are largely herbivorous on algae, increased algal abundance may increase scraper abundance as well. In addition to having higher abundance, scraper communities also had higher diversity metrics in August than in February. This indicates that in addition to having higher abundance in August, the scraper community may have higher richness or evenness. Because of the fact that richness does not increase dramatically between August and February, we conclude that the increase in diversity metrics is actually due to an increased level of evenness in the scraper community during August.

In terms of the relative composition of the scraper community, the clear dominant throughout the study was Heptageniidae (Ephemeroptera). The fact that this family was present in every single sample collected and that it was the most abundant family indicates that it is highly competitive, and that it may be able to live within a wider variety of habitats or environmental conditions than other more sensitive scraper taxa. Due to this, Heptageniidae may be a less useful measure of water quality than other scraper taxa.

Our hypothesis that increases in dissolved nutrient concentrations would have an overall positive effect on periphyton biomass was partially supported by our results. Sestonic Chl-a did increased significantly in response to TP, TDP, and SRP, but did not increase in response to the other nutrients examined in this study. The significant relationships observed between sestonic Chl-a and TP, TDP, and SRP indicate that phosphorus may be a more significant factor affecting the streams in this study than other nutrients.

The hypothesis that the scraper community would respond positively to increased periphyton biomass was not supported. In fact, in the sites with the higher levels of Chl-a, a significant decrease in the Simpson diversity index (1-D) of the scraper community was observed (Figure 7). In terms of PP, this trend is even more pronounced, with significant linear declines in Simpson diversity, Shannon diversity and equitability (Figure 3). These results add further support to the argument that phosphorus as a limiting nutrient may have a significant role in the ecosystems in this study. Previous studies have demonstrated that increases in phosphorus rich food sources may play a role in decreasing biodiversity within shredder and collector-gatherer macroinvertebrate feeding groups (Evans-White 2009). This decrease in biodiversity may be the result of macroinvertebrate taxa with high body phosphorus demands outcompeting other groups in the presence of foods rich in phosphorus.

This may be the case for the results observed in this study, because the decreases in scraper diversity are all the result of a marked increase in one taxonomic group: Heptageniidae (Ephemeroptera). The positive relationships between Heptageniidae and benthic Chl-a (Figure 4), C, N, PP and AFDM (Figure 5) suggest that Heptageniidae may become a competitive dominant in areas with higher periphyton biomass. Moreover, because Simpson diversity, Shannon diversity and equitability only showed significant correlations with PP, it seems likely that Heptageniidae may come to dominate in areas with P-rich periphyton. This is further supported by the fact that Heptageniidae showed the strongest positive relationship with PP out of any of the other factors observed (Figure 6).

Though we expected the presence of organisms that consume periphyton to be inversely related to the amount of periphyton observed, we did not find any evidence to support this. However, correlational studies are not the best way to determine whether scrapers are affecting periphyton. The best way to determine if grazers affect periphyton in this system would be to manipulate the presence, absence, or abundance of grazers and look at the response of periphyton to that manipulation. Future studies may want to examine the potential effect of Heptageniidae in particular.

The results of this study suggest that benthic scrapers respond to changing seasonal, nutrient and periphyton conditions in Ozark Highland streams. It is clear that seasonal variability played a role in scraper community structure, so future studies should take this variability into account and collect samples representing this seasonal variation. Additionally, the results of this study suggest that phosphorus enrichment may have significant implications on benthic scrapers in Ozark Highland streams. Increased levels of soluble reactive phosphorus in the water column have been positively related to sestonic Chl-a content across a broader 2-year study (Ashley Rodman, unpublished) and increased Chl-a in the present study was associated with lower scraper diversity due to increased Heptageniidae dominance. Particulate phosphorus within the periphyton was also shown to increase the dominance of Heptageniidae. The main factor contributing to declining Simpson diversity, Shannon diversity and equitability may be the propensity of Heptageniidae to become a competitive dominant or they are a tolerant species of enriched nutrient conditions. Phosphorus enrichment should therefore be considered a potentially significant factor affecting stream ecosystem health in Ozark Highland wadeable streams.

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Figures

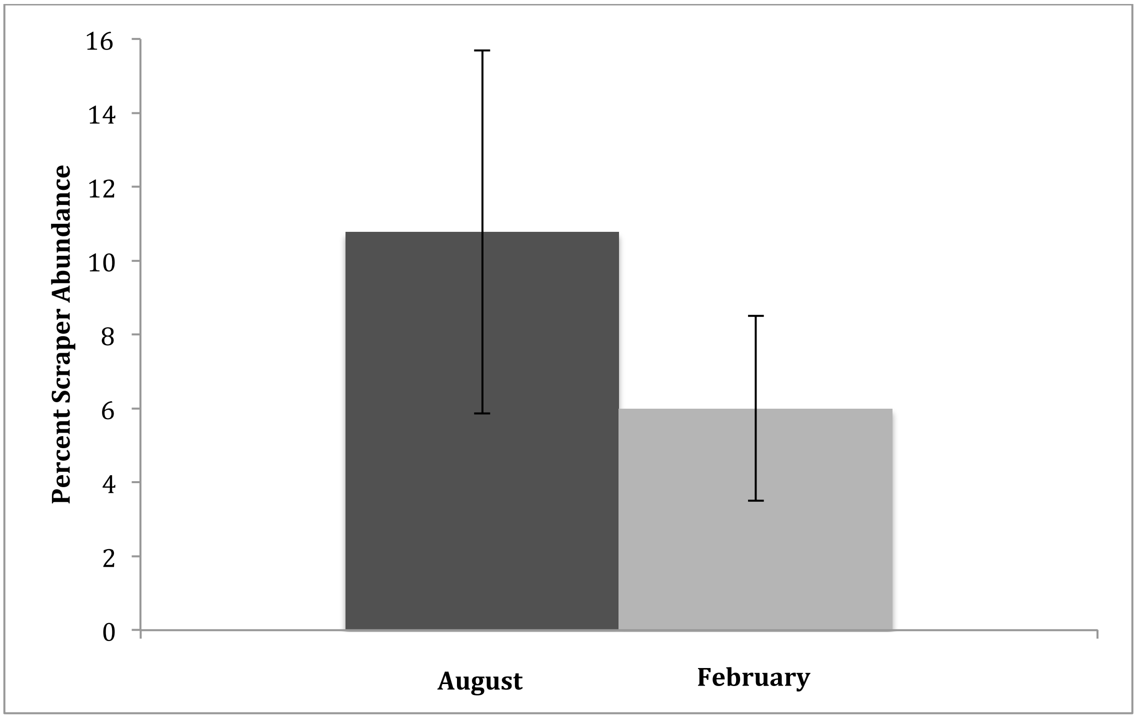

Figure 1. Average percent abundance of scrapers as a proportion of total abundance during August 2014 and February 2015. Error bars represent standard deviation.

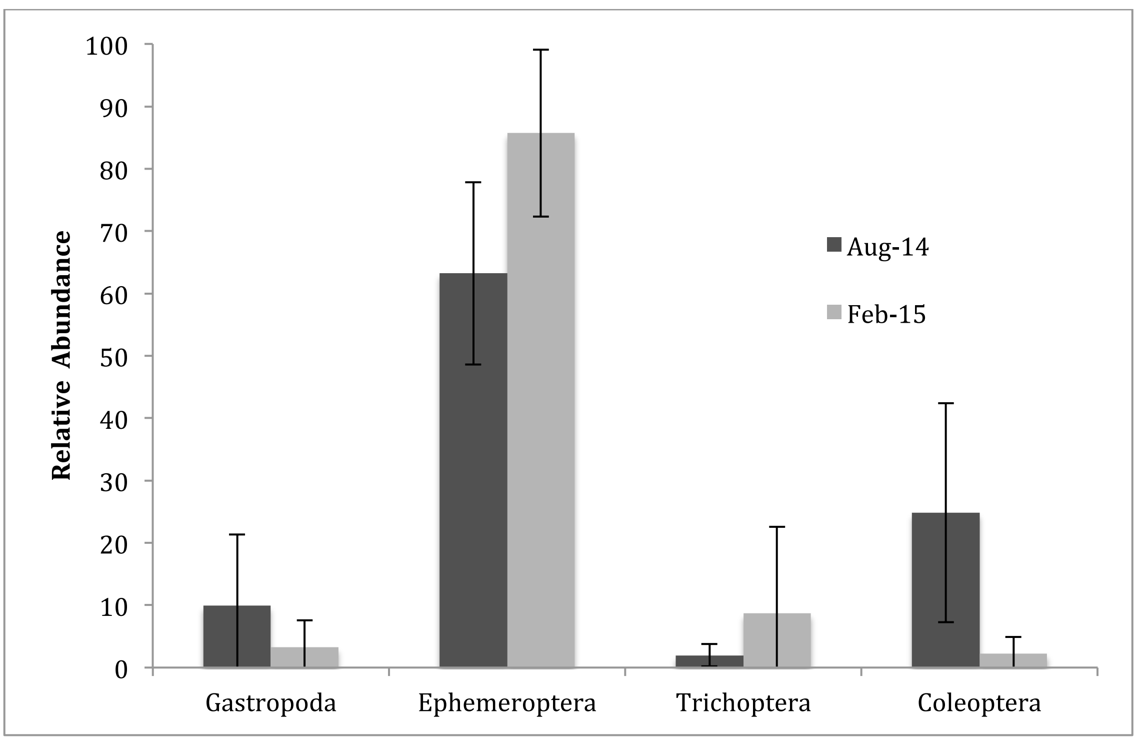

Figure 2. Average percent abundance of scraper orders as a proportion of overall scraper abundance during the months of August 2014 and February 2015. Error bars represent standard deviation.

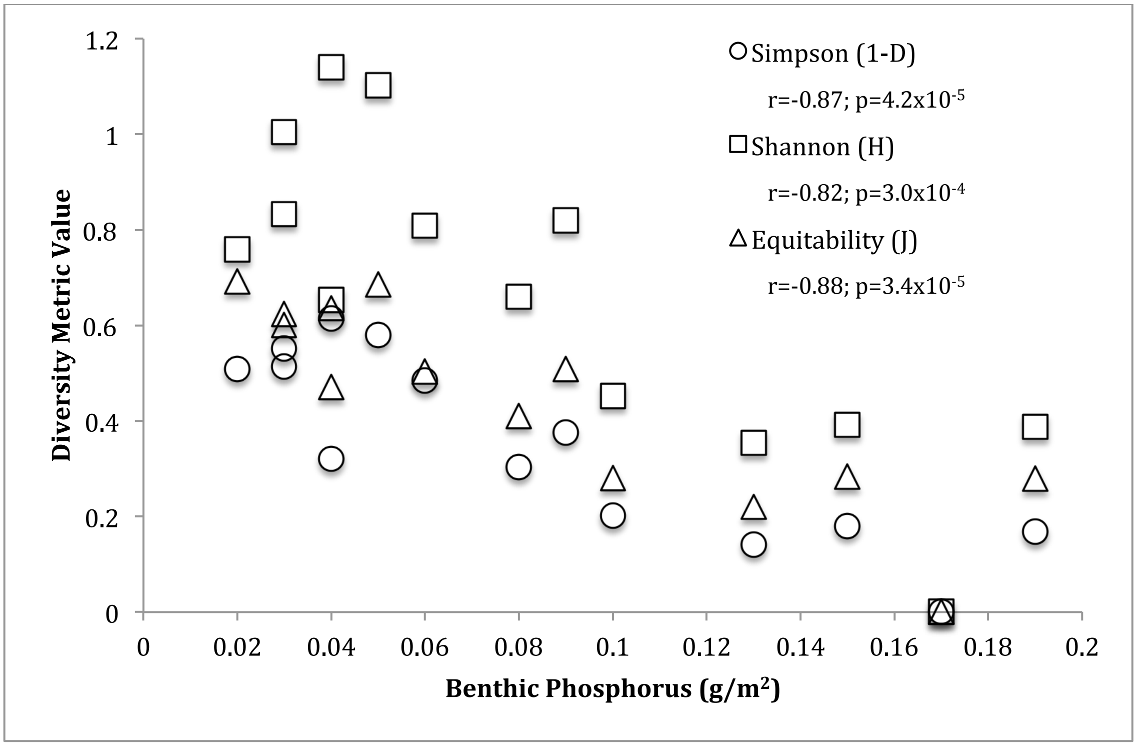

Figure 3. Simpson diversity, Shannon diversity and equitability as a function of average particulate phosphorus (g/m^2) during the months of August 2014 and February 2015. Pearson correlation coefficients (r) and p-values (p) are included.

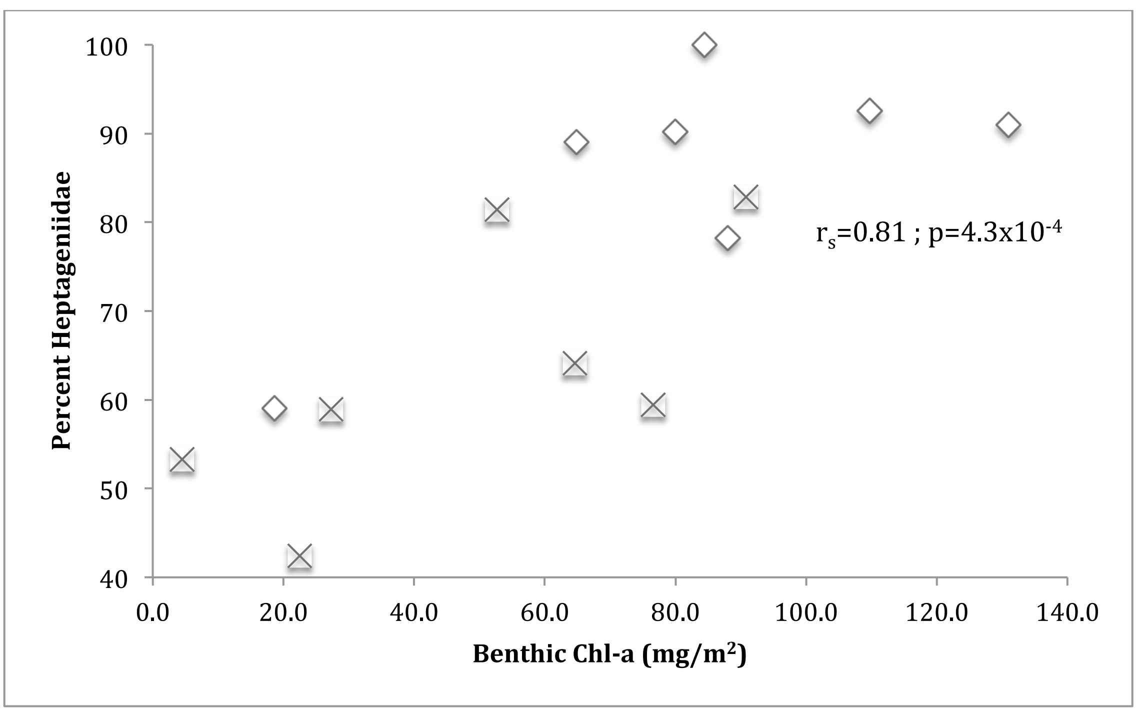

Figure 4. Average percent Heptageniidae out of total scraper abundance as a function of periphyton Chl-a (mg/m^2) from samples collected during August 2014 (represented with an X) and February 2015 (represented with a diamond). The Spearman correlation coefficient (r_s) and p-value (p) are included.

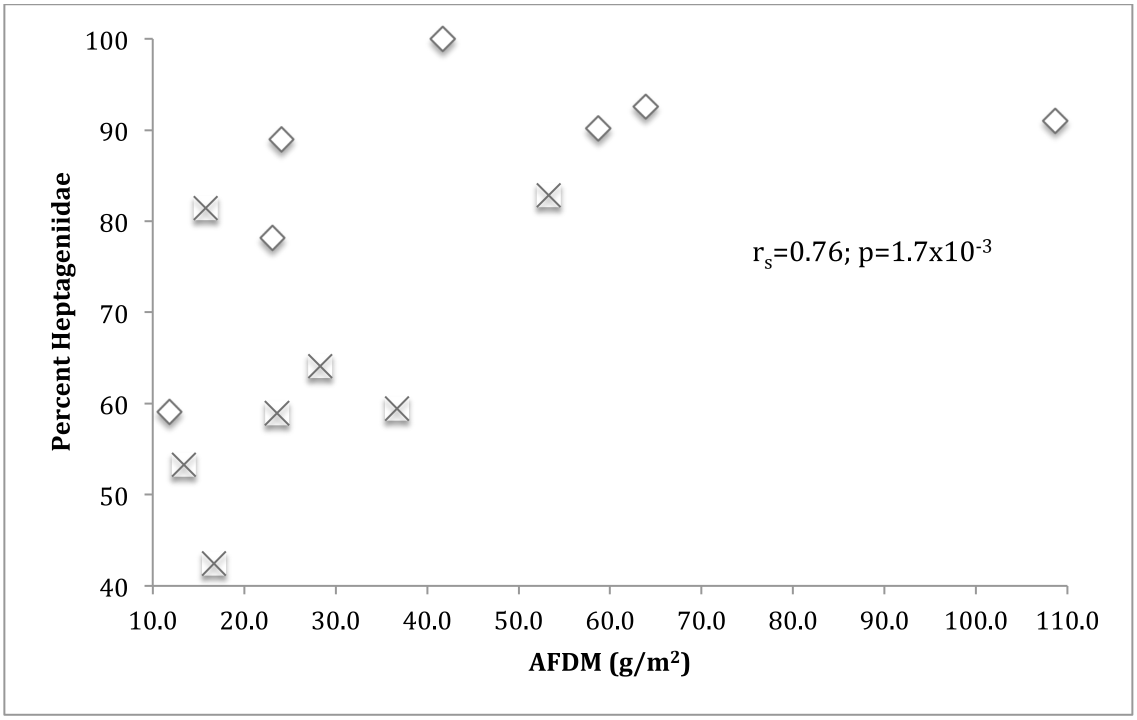

Figure 5. Average percent Heptageniidae as a function of ash free dry mass (g/m^2) found in the periphyton during August 2014 (represented by an X) and February 2015 (represented by a diamond). The Spearman correlation coefficient (r_s) and p-value (p) are included.

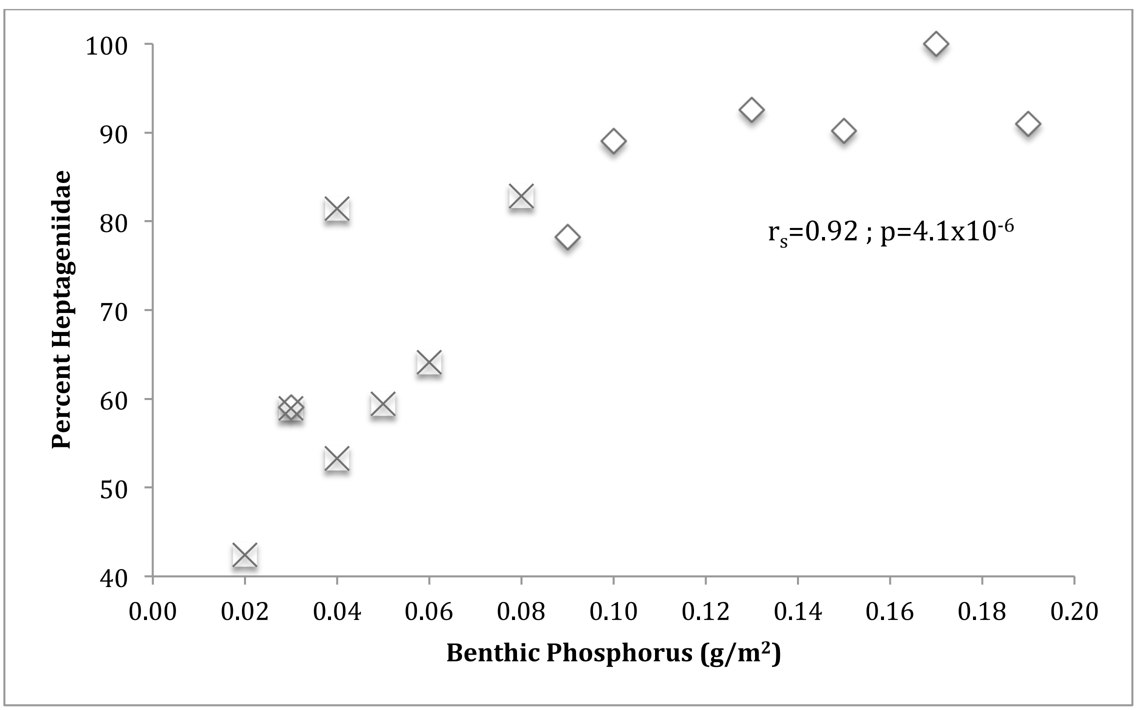

Figure 6. Average percent Heptageniidae as a function of particulate benthic phosphorus (g/m^2) found in the periphyton during August 2014 (represented by an X) and February 2015 (represented by a diamond). The Spearman correlation coefficient (r_s) and p-value (p) are included.

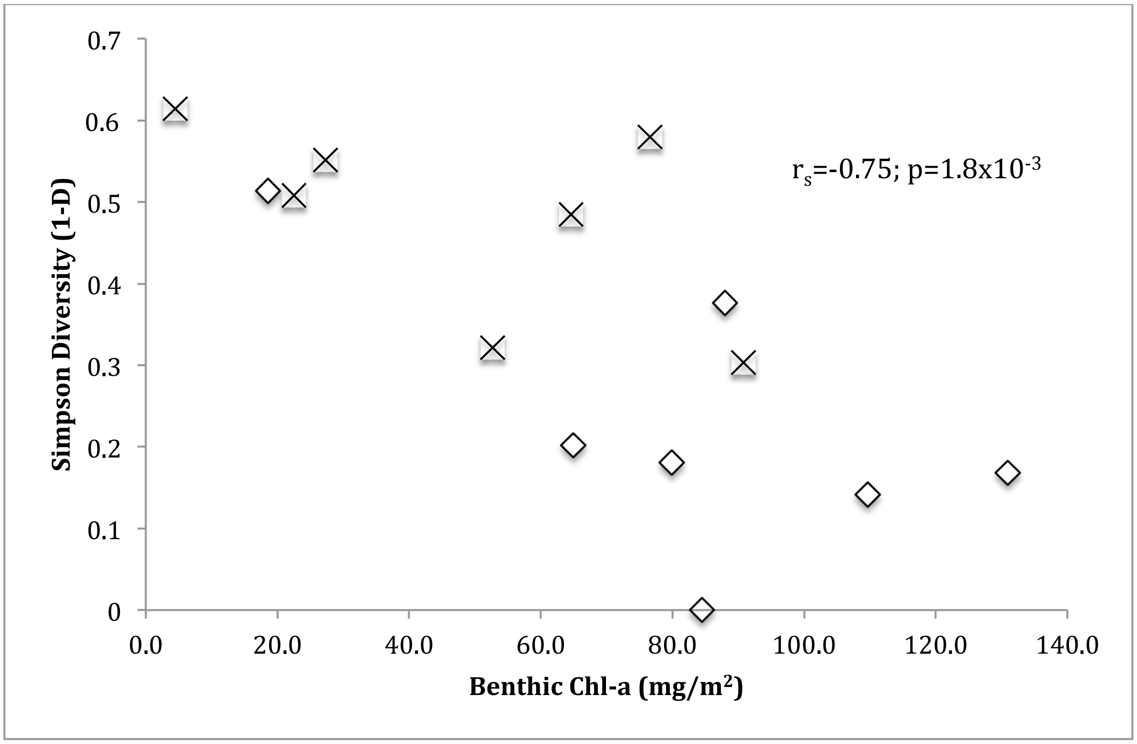

Figure 7. Average Simpson diversity (1-D) as a function of Chl-a (mg/m^2) found in the periphyton during August 2014 (represented by an X) and February 2015 (represented by a diamond). The Spearman correlation coefficient (r_s) and p-value (p) are included.

Tables

Table 1. GPS coordinates, drainage area and land use of sampling sites used in this study.

Site	Drainage Area (km^2)	% Pasture	% Forest	% Urban	Latitude	Longitude
Bear	237.76	21.4	75.4	2.9	35.9929°	-92.7015°
Big	232.84	10.3	88.8	0.5	35.9775°	-93.0434°
Cave	130.53	21.4	77.5	0.8	35.9679°	-92.9636°
Kings	165.76	6.5	91.8	1.3	36.0184°	-93.5393°
Little Buffalo	367.78	10.7	88.5	0.4	36.033682	-93.120823°
War Eagle	271.95	12.8	86.1	0.8	36.0420°	-93.7041°
White River	232.32	4.4	94.3	1.1	35.8279°	-93.8318°

Table 2. Average abundances of scraper families collected during August 2014 and February 2015

August 2014							
	Bear	Big	Cave	Kings	Little Buffalo	War Eagle	White
Ancylidae	0	0	0	0	0	2.67	0
Lymnaeidae	33	6.67	6.67	8	8.67	29.33	0
Physidae	0	0	0	0	0	1.33	0
Valvatidae	12.67	6	2.67	0	2	4	0
Heptageniidae	85	268	88	257.33	116	108	48.67
Glossosomatidae	0	0	0	0	0	0	0
Helicopsychidae	1.33	2.67	5.33	16	2.67	0	2
Psephenidae	11	134.67	46.67	34.67	10.67	57.33	64
February 2015							
	Bear	Big	Cave	Kings	Little Buffalo	War Eagle	White
Ancylidae	0	0	0	1.33	0	5.33	0
Lymnaeidae	0.67	5.33	1	0	0	5.33	0
Physidae	4	0	0	0	0	0	0
Valvatidae	0	0	0	0	0	0	0
Heptageniidae	49.11	182.67	98	194.67	42.67	81.33	50
Glossosomatidae	0	5.33	4.67	16	0	4	31.33
Helicopsychidae	0	3.33	0	4	0	0	2
Psephenidae	0.67	0.67	4	2.67	0	8	1.33

Table 3. Diversity metrics by sample site during August 2014 and February 2015. All metrics were calculated using average abundance values for each sample site

August 2014					
	Abundance	Richness	Simpson (1-D)	Shannon (H)	Equitability (J)
Bear	143	5	0.58	1.10	0.69
Big	418	5	0.48	0.81	0.50
Cave	149.33	5	0.55	1.01	0.62
Kings	316	4	0.32	0.65	0.47
Little Buffalo	140	5	0.30	0.66	0.41
War Eagle	202.67	6	0.61	1.14	0.64
White	114.67	3	0.51	0.76	0.69

February 2015					
	Abundance	Richness	Simpson (1-D)	Shannon (H)	Equitability (J)
Bear	54.44	4	0.18	0.3926	0.28
Big	197.33	5	0.14	0.3548	0.22
Cave	107.67	4	0.17	0.3875	0.28
Kings	218.67	5	0.20	0.4529	0.28
Little Buffalo	42.67	1	0	0	0
War Eagle	104	5	0.38	0.8195	0.51
White	84.67	4	0.51	0.8328	0.60

Table 4. Average values of diversity metrics by sample site, calculated from average of August 2014 and February 2015 diversity metrics.

	Abundance	**Richness**	**Simpson (1-D)**	**Shannon (H)**	**Equitability (J)**
Bear	98.72	4.5	0.38	0.75	0.48
Big	307.67	5	0.31	0.58	0.36
Cave	128.5	4.5	0.36	0.70	0.45
Kings	267.34	4.5	0.26	0.55	0.38
Little Buffalo	91.34	3	0.15	0.33	0.21
War Eagle	153.34	5.5	0.50	0.98	0.57
White	99.69	3.5	0.51	0.80	0.65