The Upper Main Stem of the Susquehanna River: Biological Assessment Using Phytoplankton and Periphyton Together With Comparisons between Passive and Active Methods for Estimating Algal Communities

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ABSTRACT

Five sites were monitored between Sunbury and Selinsgrove on a transect near Shady Nook, Byers Island on the Susquehanna River for diatom periphyton and seven sites for phytoplankton from June through October, 2012. The purpose of this study was to tests methods, both passive and active, for use in monitoring the upper main stem in the Susquehanna River at the Byers Island transect by multimetric analysis. Furthermore sites on that transect were compared between one another and with collections made during the summer and fall of 2012. A total of 82 species of algae were identified in the phytoplankton communities taken from whole water samples. Artificial substrates made of glass microscope slides (periphytometer) were placed in the river for a total of 3 weeks and 40 diatom taxa were identified. Additionally, during the fall study, native substrate were sampled along a 500m reach at each of two sites: at the west shore with water from the West Branch and on the shore of Byers Island with water from the North Branch. These samples were taken from cobble, stone, plant, and sediment, which were calculated proportionally according to each site. Together, a total of 136 species of algae were identified within the transect (both diatom periphyton and phytoplankton). Metrics such as the Pollution Tolerance Index (PTI), Shannon Weaver diversity index and Bray-Curtis similarity index were used to determine community composition, species distribution, site similarities, and to compare active methods against passive methods. Data analyzed from the

summer months showed Shannon Weaver values for phytoplankton that ranged from 1.53 to 2.90 and 0.31 to 1.91 for diatom-periphyton. Shannon Weaver values for environmental samples ranged from 0.26 to 2.46, which were compared to data from periphytometers at the same sites. PTI values based on the diatoms collected from the periphytometers ranged from 2.7 to 2.99 during the summer months and 2.91 to 3.3 during autumn. PTI values calculated from the environmental samples, however, ranged from 1.72 to 2.76. Active methods provided higher values than passive methods for most metrics.

INTRODUCTION

Systems, such as large rivers, have not been well studied (Sedell et al. 1989) unlike lakes and small streams, which have different ecological and biological characteristics and management problems. Simply put, the reason large rivers are problematic is due mainly to the logistics of working on large bodies of moving water. Furthermore, methods for sampling biota that are standard in small wadeable streams do not translate easily to large lotic environments. Likewise, Vannote et al. (1980) treats the river continuum as a means to describe a river as a continuously integrated series of physical changes and resource gradients along which the biota are constantly in flux such that biota in the lower reaches does not resemble that of the headwater streams.

One of the main differences in the lower reaches of a large river is the relative importance of autochthonous carbon fixation (Vannote et al. 1980). Whereas the upper reaches rely mainly on the influx of organics from the surrounding terrestrial environment, lower reaches of large rivers rely on photosynthetic carbon fixation by algae and aquatic plants. The algae are

components of two major photosynthetic communities the periphyton, attached algae, and a suspended assemblage that includes true phytoplankton (Bellinger and Sigee 2010).

Stevenson and Smol (2003) state that algae can be used to study aquatic systems because they are the foundation of most aquatic food chains, and they are important in biogeochemical cycling. Furthermore, they serve as habitats for many organisms in aquatic environments (Stevenson and Smol, 2003). Likewise, algae have short life cycles and as a result respond to biogeochemical changes quickly (Stevenson and Smol, 2003), which enables relatively rapid assessment of many aquatic systems. Moreover, diatom periphyton (Bacillariophyta) communities have been used world-wide to diagnose biological stressors, such as inorganic and organic contaminates, as well as lake and river acidification, climate change, and nutrient concentrations (Stevenson et al. 2008). According to Stevenson et al. (2008), diatom periphyton indicators can be more precise than a one-time measurement of water chemistry because they integrate impacts of stressors over time. Likewise, diatom periphyton, since they form biofilms and are benthic organisms, are important food sources for macroinvertebrates (Patil and Anil 2005) and, according to Flotemersch et al. (2006), are used to indicate biogeochemical conditions within the benthic zone. Phytoplankton, like periphyton, also plays a pivotal role in the assessment of large rivers. More specifically, the phytoplankton community serves as a set of bio-indictors to study large lakes and rivers (Flotemersch et al. 2006). Similarly, according to Barbour et al. (1999), artificial substrates (periphytometers) should be used to collect diatom-periphyton samples as a means to compare one location with another location and in the event that a periphytometer cannot be used or cannot be retrieved or is damaged, a rock scraping should be taken in its place.

The methods for sampling algal communities in wadable (Barbour et al. 1999) and non-wadable (Flotemersch et al. 2006) have been established. However, the usefulness of passive versus active methods have not been determined and perhaps are site or reach specific. The collection of phytoplankton or suspended algae is necessarily active, but its disadvantage is that it could be a sample of a community that is caused by an ephemeral upstream event. Periphyton collection can be active or passive. The advantages of active periphyton methods (e.g. rock scrapings) are that they require a single visit and are relatively easy to collect; however, communities taken from native substrates may have been established for different periods of time due to high water, sedimentation events, or other disturbances. Passive methods, primarily periphytometers, allow for the development of periphyton communities for a known length of time on the same substrates so that direct comparisons from one site to another can be made. Disadvantages of passive methods are that they have to deployed, monitored, and then collected thus requiring more time in the field.

This study tests methods, both passive and active, for use in monitoring the upper main stem in the Susquehanna River at the Byers Island transect by multimetric analysis. Furthermore sites on that transect are compared between one another and with collections made during the summer and fall of 2012.

SITE DESCRIPTION

The Byers Island Transect (Figure 1) straddles Byers Island 7km south of the confluence of the West branch (WB) (sites 1, 1.3, and 5) and North branch (NB) (sites 1.6, 2, 3, 3.5, and 4) branches of the Susquehanna River. Sites 1-4 are below the low head dams at the Sunbury Generation plant. Site 5 is above the low head dam on the west side of the river. Periphyton was sampled at sites 1-5 (which are denoted with stars). Phytoplankton was sampled at sites 1-4 and

between them at 100m intervals (blue dots). Additionally, a 500m sampled at 100m intervals reach (orange dots) was established 50m above sites 1 and 2.

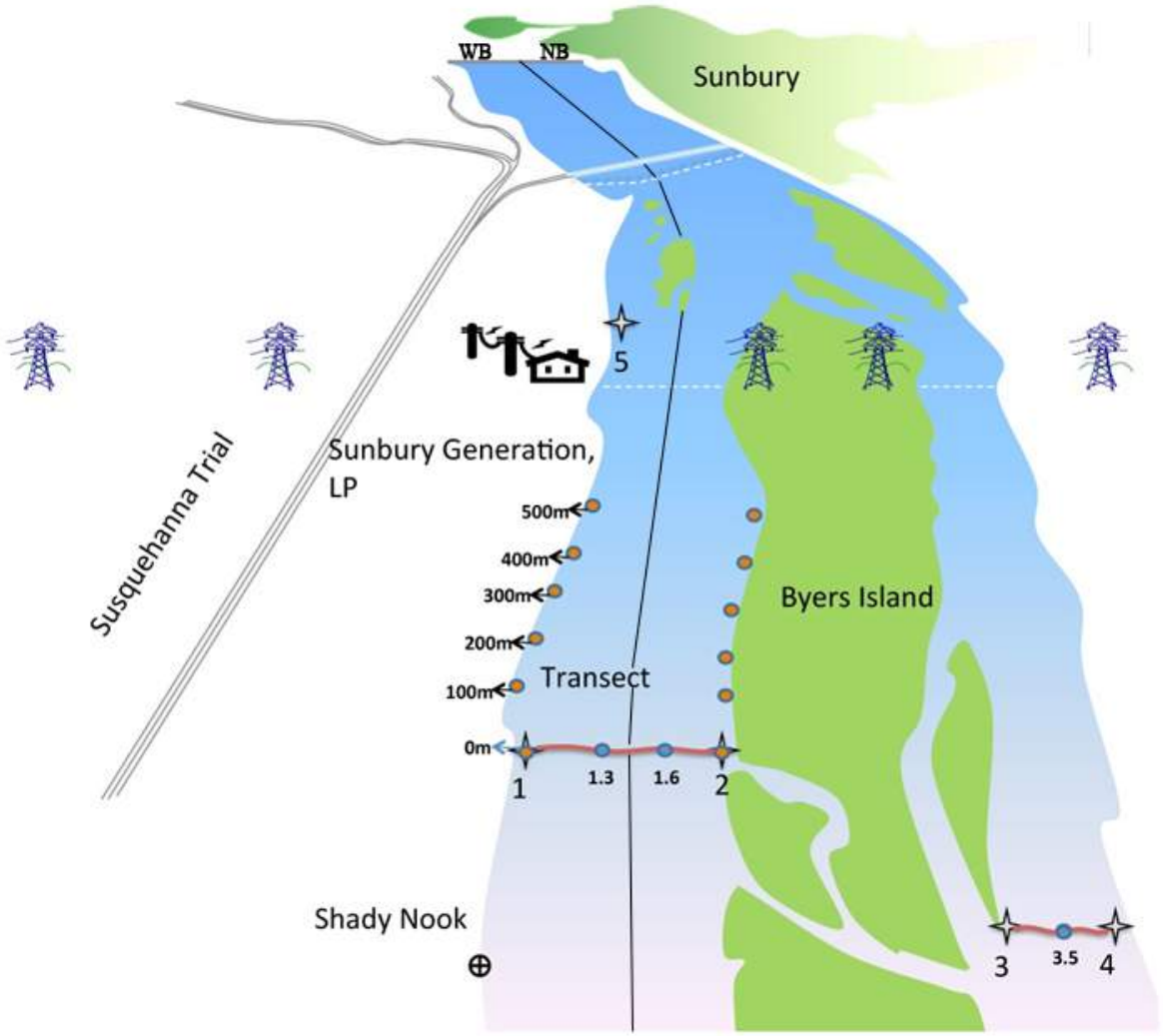

Figure 1: Map of the sites studied. Sites 1, 1.3, 1.6, 3, 4, and 5 were examined in the between June 4 through July 19 and whole water phytoplankton samples were taken at each of those site. Periphytometers were placed for 3 weeks, during the during that time period, at sites 1, 2, 3, 4, 5. During September 17 through October 24 sites 1, 1.3, 1.6, and 2 were examined for phytoplankton and periphytometers were placed at sites 1 and 2. Also during the fall months a 500 m reach established on either bank of the river and natural substrate were sampled for diatom-periphyton.

METHODS

In general, the methods were those of Barbour et al. (1999) as modified by Wargo *and Holt* (1998). We deployed periphytometers (prepared Carolina blue boxes) each with 5 slides at sites 1, 2, 3, 4, and 5 during the summer and at sites 1 and 2 in the fall of 2012 (Figure 1). Periphytometers were allowed to incubate *in situ* for 3 weeks after Richardson et al. (1996). Slides from the periphytometer were fixed with Carosafe and mounted with Karo syrup. A Minimum of 300 individual periphyton cells were counted from each slide or until 25 fields had been examined (1500 cells per periphytometer) at 1000x magnification. Identifications of diatom-periphyton were confirmed by use of a JEOL 5970LV SEM. The Pollution Tolerance Index (PTI) was calculated according to Stephenson et al. (2008) and Barbour et al. (1999) using calibrations from Kentucky Division of Water (KDOW 2008). The Bray-Curtis Proportional Similarity index was created according to Bloom (1981). A total of 7 whole water samples were taken in the summer weekly (June 4-July 19) and 5 samples were taken in the fall (September 17- October 24). Phytoplankton samples were preserved with Lugols iodine. Phytoplankton taxa were identified and counted (300 cell minimum) or until 25 fields had been reached using a Nikon eclipse E200 light microscope with a Palmer-Maloney slide at 400x magnification. Environmental samples were collected from native substrates (plants, cobble, large stone, bark, and sediment) along a 500m reach (Figure 1). Samples were given a relative proportion value according to habitat frequency within each location on the reach. The identification of algal taxa was determined by Prescott (1973), Wehr and Sheath (2003), Whitford and Schumacher (1984), Hustedt (1985), Round et al. (1990), Bellinger and Sigee (2010), Starmach (1985), Krammer and Lange-Bertalot (1986, 1988, 1991a, and 1991b), and Popovskỳ and Pfiester (1990). Current taxonomic list was made using KDOW (2008).

RESULTS

The suspended assemblage (phytoplankton) is dominated by Bacillariophyta (68%) and Chlorophyta 19%, (Figure 2 and Table 2). Figure 3 indicates that plankton diversity does not change very much between summer and fall. The periphyton diversity (Figure 4 and Table 1) is generally higher on native substrates than it is on the artificial glass slides of the periphytometers. The diversity, based on Shannon-Weaver and total taxa of periphyton varies from site to site (Figure 5) in periphytometers, mainly based on the relative importance of *Cocconeis***.**

PTI values (0-4) indicate levels of inorganic and organic pollutants with low values meaning high levels of pollutants. PTI values (Table 3) were generated from assemblages taken from all samples in the fall (Figure 4). The PTI values from the periphytometers ranged from 2.91-3.3, while the PTI values for the environmental samples were lower and ranged from 1.72-2.76. These ranges differ significantly (Table 3). The high PTI values on the periphytometers were largely due to the dominance of *Cocconeis* (Table1), which has a PTI value of 3. Table 4A-D represents how related each site is to each other, in terms of the types of taxa and frequency of said taxa where Table4A-B demonstrate similarities between sites, comparing the occurrence of phytoplankton. Table4C-D, compare sites bases on periphyton collects, which was either passive or active.

Table 4A demonstrates that, with respect to the phytoplankton in the summer, all sites are moderate to moderately high in similarity. Likewise, phytoplankton samples taken in the fall (Table 2) show moderately high to very high similarity. Periphyton analysis in the summer (Table 1) indicates that there is a degree of both dissimilar and high similarity, with ranges from moderately low and very high similarity. A Bray-Curtis similarity for sites 1 and 2 during the fall was 39%.

DISCUSSION

The 500m reach (Figure 2) above of sites 1 and 2 allowed for a direct comparison to be made between substrates that naturally occur in the Susquehanna River and artificial substrates, made of glass microscope slides, namely the periphytometers. Thus we were able to compare the results of passive and active methods.

Table 4D illustrates that no single native substrate integrates periphyton such that it is representative of diatom-periphyton communities in the Susquehanna River. As a result, we infer that individual microhabitats cannot replace each other. Both periphytometers and rock scrapings show very low overlap with other substrates and are not particularly representative of local communities as a whole (Table 4D). According to Kireta et al. (2011) there are strong relationships between diatom-periphyton, their environments, and the quality of water. Both Stevenson and Smol (2003) and Flotemersch et al. (2006) suggest that substrate types have distinct effects on periphyton colonization. This is to say that factors relates to substrate play a major role on the communities of periphyton that colonize differing substrates. For example, Figure 4 demonstrates that species richness does increase when multiple assemblages are sampled. Allen et al. (1998) state that periphyton communities are influenced to a greater extent by non-anthropogenic factors, which is indicative of the differences that exist in microhabitats. As a result, data provided solely through periphytometers or rock scrapings do not represent the complexity of periphyton communities in rivers.

We agree with Flotemersch et al. (2006) that periphytometers should only be used when it is not possible to sample multiple assemblages in a variety of local microhabitats. Likewise, periphytometers should only be used to compare aquatic environments that are not within the vicinity of one another with the understanding that periphytometers and rock scraping are only useful as a means to reduce variability between un-localized sites and to crudely and quickly

examine rivers. Furthermore, periphytometers in the upper main stem of the Susquehanna River overestimate the PTI values as assigned by KDOW (2008) because of the dominance of *Cocconeis* species (Table 3) in the communities that develop on glass slides; thus, native collections that take into account the relative importances of microhabitats from a location should be used. We suspect that the dominance of *Cocconeis* on periphytometer slides creates an artificially high similarity between our sites on the Byers Island transect (Table 4C-D).

Phytoplankton collection, while an active method, provides little discrimination between sites because of the homogeneity between sites (Figure 3 and Table4A-B). As a result, active, whole water sampling for phytoplankton is only useful in describing the stability of the river with regard to the continuity of rivers in general. Likewise, whole water sampling does not allow for direct comparisons between sites, like other active methods such as, native substrate sampling.

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Figures and Tables

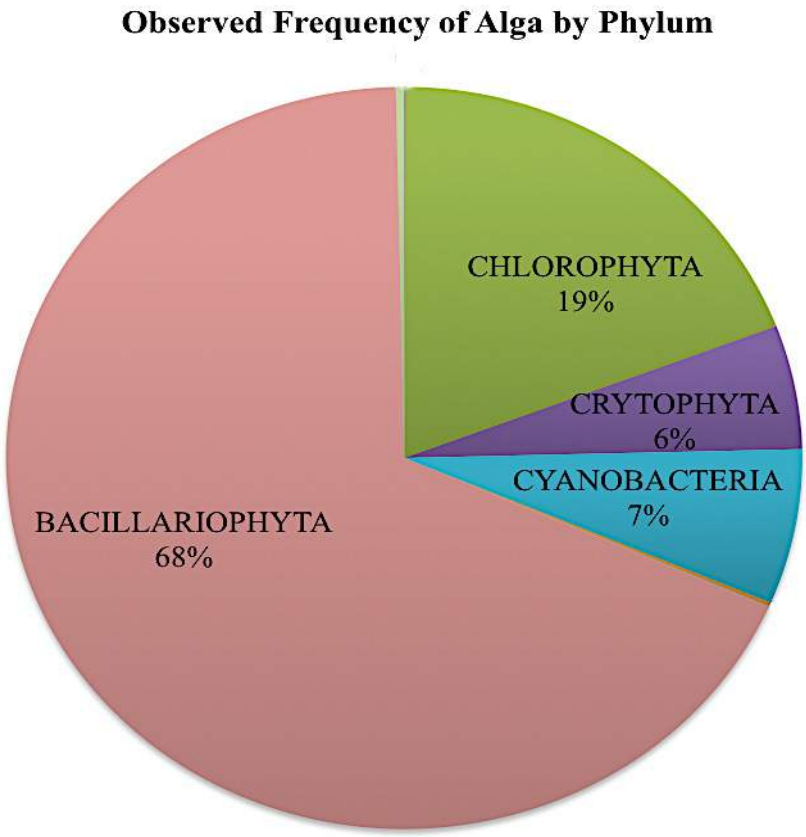

Figure 2: The occurrence of different phyla in the Susquehanna River from the months of June through October. Phyla such as Chrysophyta, Cercozoa, Charophyta, Euglenophyta, and Haptophyta are present, however their respected frequency values are below 1%.

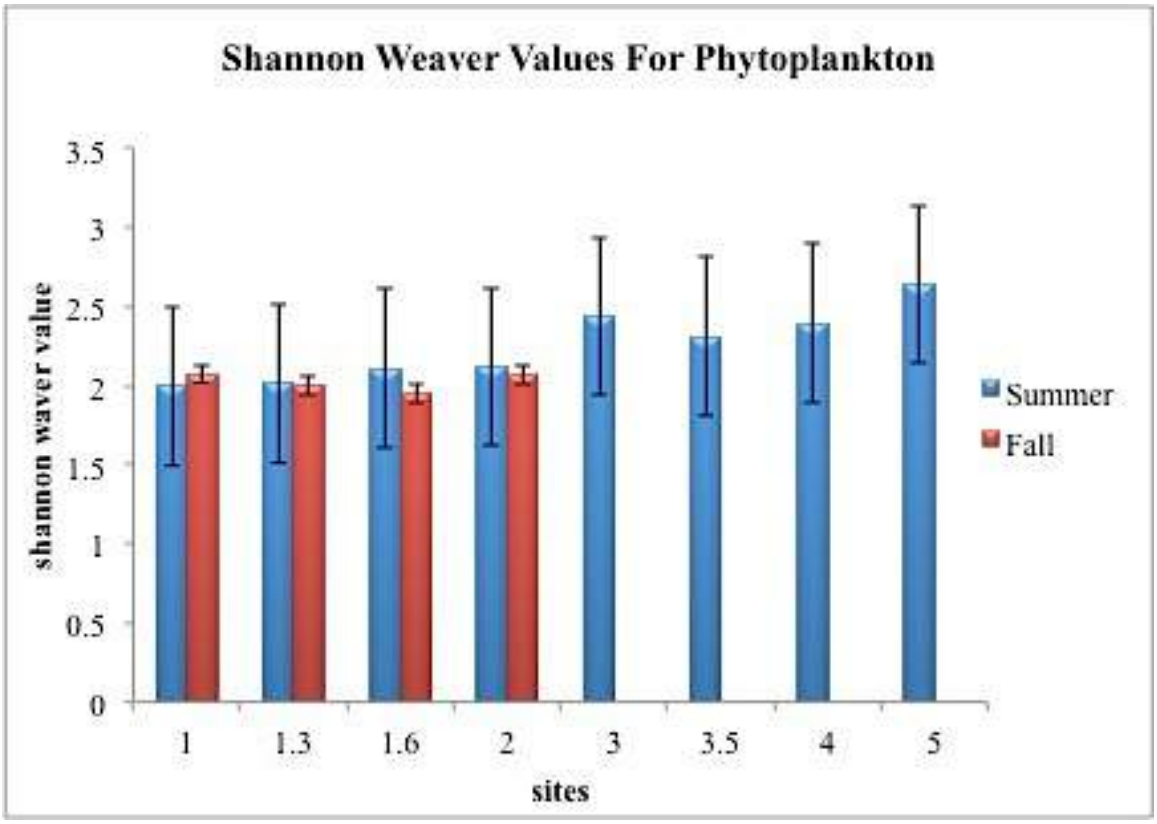

Figure 3: Shannon Weaver values for summer months and autumn months, where studied autumn sites were not significantly different from the summer months, which suggest that species richness remains constant throughout the summer and fall.

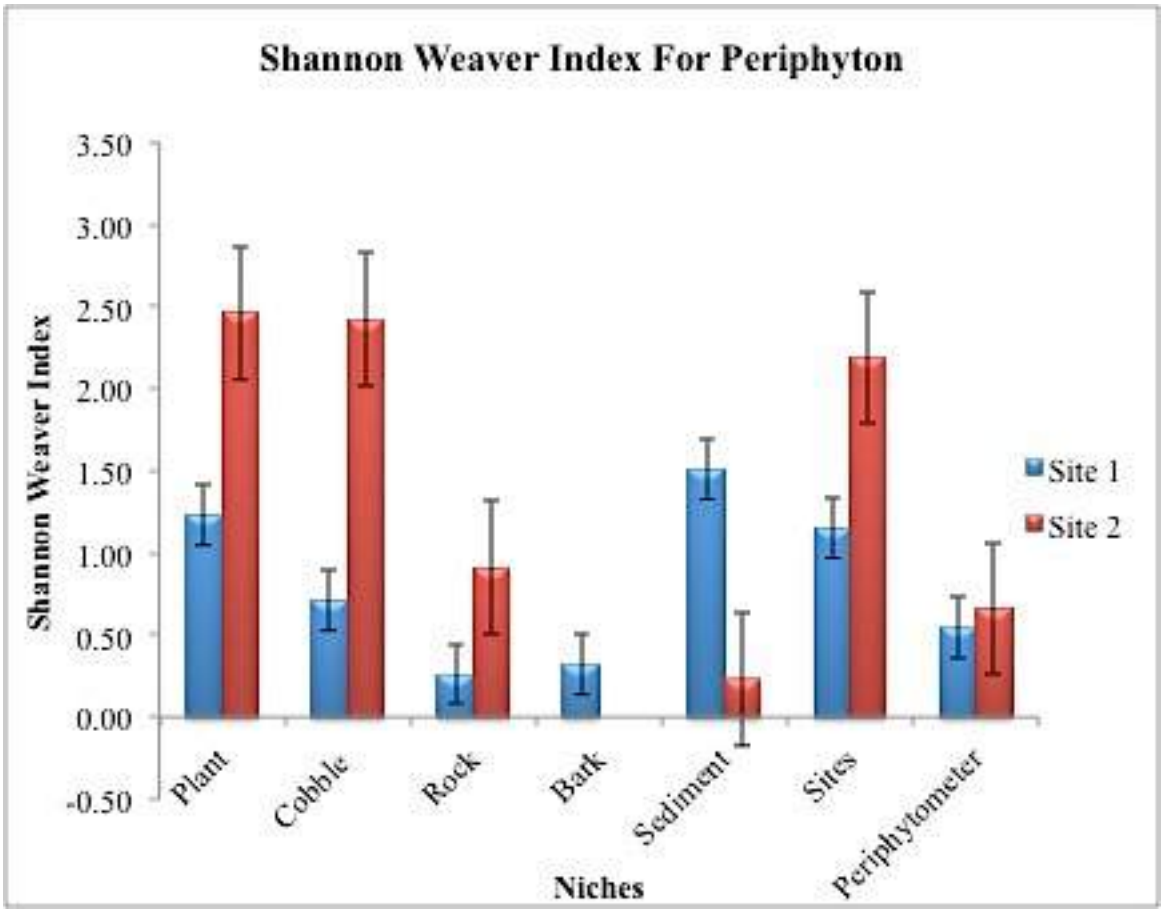

Figure 4: Illustrates the proportional diversity values for different substrate along the 500m reach as well as a comparison between Shannon Weaver values calculated in natural substrate versus artificial substrate (periphytometers). Periphytometer shows one of the lowest species richness along with rock (scrapings) and bark. There was no bark sample present at site 2.

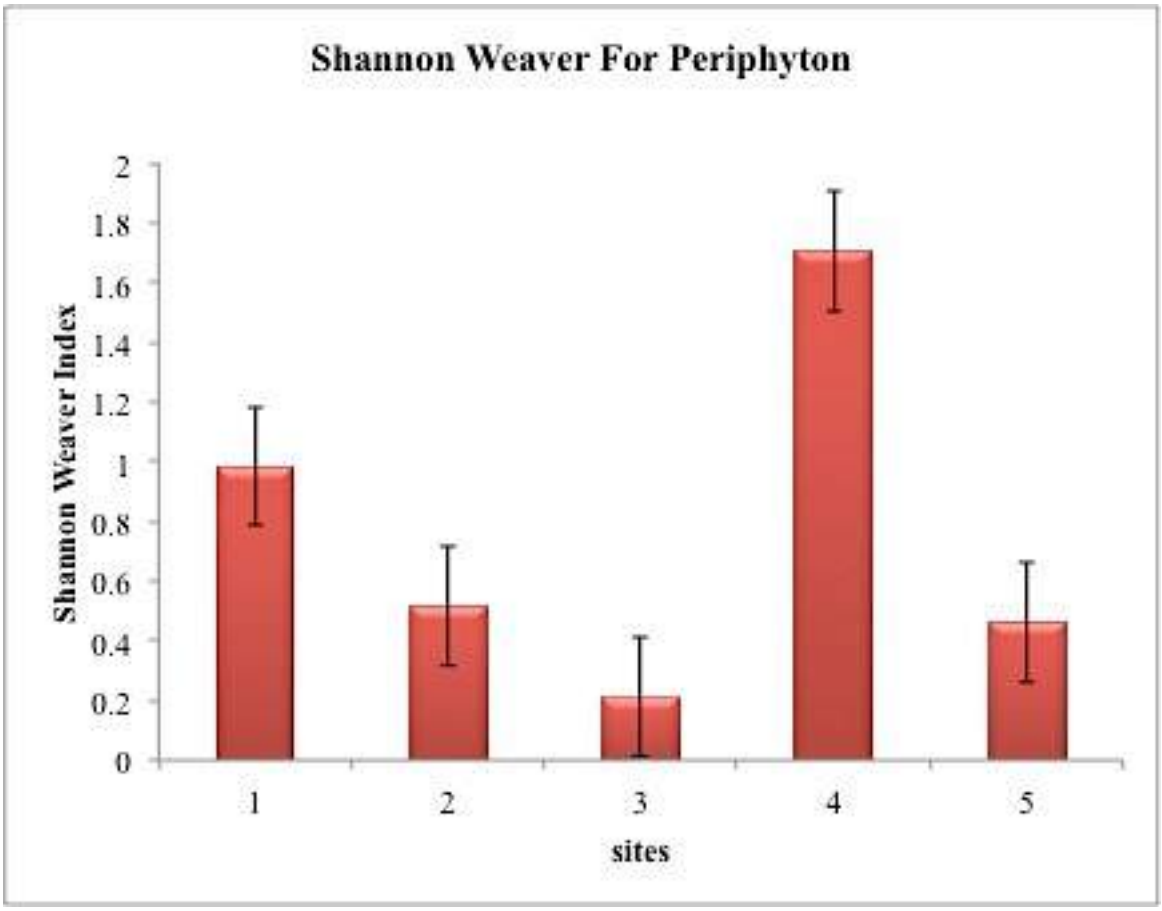

Figure 5: Shannon Weaver values from counted periphyton found on periphytometers for the summer months are shown.

Table 1: frequency of periphyton at site 1 and 2. It also compares the periphyton frequencies seen in the environmental samples with frequencies found in the periphytometers. Likewise, white spaces mean that the taxa did not occur for that particular site. Environmental samples prove to be more diverse. Red is >10% , Purple is 1-10%, and Mustard is >1%.

	Environmental		Periphytometer	
AlGAE	**Site 1**	**Site2**	**Site 1**	**Site 2**
Achanthidium lanceolata	0.0089	0.0049		
Achnanthidium minutissimum	0.1930	0.1109	0.0507	0.5556
Achnanthes lanceolata	0.0528	0.0114		
Cocconeis pediculus	0.0505	0.0311		0.0715
Cocconeis placentula	0.3171	0.0690	0.8629	0.3020
Cyclotella meneghiniana	0.0098	0.0017	0.0013	
Cymbella minuta	0.0298	0.0043	0.0012	
Cymbella naviculiformis	0.0698	0.0423		
Cymbella prostrata	0.0166	0.0049		
Cymbella tumidula	0.0112	0.0010	0.0007	
Cymbella minuta	0.0056	0.0078		
Diatoma vulgare	0.0500	0.0162	0.0006	
Eunotia bilunaris	0.0059			
Eunotia exigua	0.0095	0.0063		
Eunotia minor	0.0095	0.0056		
Fragilaria capucina	0.1222	0.0843	0.0364	0.0334
Fragilaria crotonensis	0.0110	0.0048	0.0165	
Frustalia rhomboides	0.0073	0.0004		
Gomphonema angustatum	0.0256	0.0004		
Gomphoneis herculeana	0.0076	0.0009		
Gyrosigma spencerii	0.0056			
Melosira ambigua	0.0470			
Melosira varians	0.0354	0.0192	0.0044	0.0364
Navicula atomus	0.0092	0.0039	0.0006	
Navicula cryptocephala	0.0400	0.0453	0.0151	
Navicula lanceolata	0.1687	0.0974	0.0019	
Navicula minisculus	0.0435	0.0174		
Navicula pusilla	0.0001	0.0076		
Navicula rhynchocephala	0.0182	0.0131	0.0032	
Nitzschia palea	0.0432	0.0275	0.0012	
Nitzschia recta	0.0181	0.0209		
Rhoicosphenia curvata	0.0188	0.0113		
Stephanodiscus hantzchii	0.0120	0.0046		
Surirella angusta	0.0507	0.0327		
Surirella minuta		0.0054		
Surirella patella	0.0005	0.0073		
Synedra ulna	0.0237	0.0091		

Table 2: Relative frequency of algae from June through October, where taxa highlighted are true plankters.

	FREQUENCY	HAPTOPHYTA	
CERCOZOA		*Chrysochromulina parva*	0.0004
Cercomonas euglypha	0.0004	**BACILLARIOPHYTA**	
CHAROPHYTA		*Achnanthidium lanceolata*	0.0760
Cosmarium sp.	0.0003	*Achnanthidium subatomus*	0.0038
CHLOROPHYTA		*Achnanthidium minutissimum*	0.0964
Actinastrum hantzschii	0.0074	*Amphora sp.*	0.0070
Ankistrodesmus falcatus	0.0123	*Cocconeis placentula*	0.0735
Carteria sp.	0.00003	*Cyclotella meneghiniana*	0.0043
Coelastrum microporum	0.0130	*Cymatopleura elliptica*	0.0011
Chlamydomonas globosa	0.0330	*Cymbella affinis*	0.0074
Echinosphaerella limnetica	0.0002	*Cymbella cistula*	0.0038
Golenkenia	0.0455	*Cymbella gracilis*	0.0026
Kirchneriella obesa	0.0050	*Cymbella silesiaca*	0.0002
Lagerheimia quadriseta	0.0001	*Cymbella minuta*	0.0160
Micractinium pusillum	0.0010	*Cymbella helvetica*	0.0004
Tetrastrum elegans	0.0001	*Diatoma vulgare*	0.0198
Tetradesmus heteracanthum	0.0006	*Eunotia bilunaris*	0.0003
Scenedesmus abundans	0.0007	*Frustalia rhomboides*	0.0014
Scenedesmus acuminatus	0.0024	*Fragilaria capucina*	0.1223
Scenedesmus armatus	0.0019	*Fragilaria crotonensis*	0.0349
Scenedesmus bijuga	0.0052	*Gomphonema olivaceum*	0.0005
Scenedesmus dimorphus	0.0001	*Gompheoneis*	0.0002
Scenedesmus opoliensis	0.0025	*Gyrosigma exilis*	0.0002
Scenedesmus quadricauda	0.0282	*Gyrosigma acuminatum*	0.0004
Selenastrum gracile	0.0034	*Melosira ambigua*	0.0166
Oedogonium longatium	0.0024	*Melosira varians*	0.0511
Oocystis burgei	0.0010	*Navicula capitata*	0.0003
Oocystis elliptica	0.0028	*Navicula cryptocephala*	0.0316
Oocystis soliteria	0.0102	*Navicula lanceolata*	0.0145
Pediastrum boryanum	0.0007	*Navicula meniscus*	0.0163
Pediastrum tetras	0.0079	*Navicula pusilla*	0.0003
Pyramimonas tetrarhynchus	0.0040	*Navicula rhynchocephala*	0.0020
CRYTOPHYTA		*Navicula veridula*	0.0001
Cryptomonas erosa	0.0466	*Nitzschia dissipata*	0.0002
Cryptomonas ovata	0.0044	*Nitzschia lanceolata*	0.0003
Cryptomonas pusillum	0.0030	*Nitzchia minuta*	0.0003
CYANOBACTERIA		*Nitzschia palea*	0.0276
Anabaena flos-aquae	0.00003	*Rhoicoshenia curvata*	0.0007
Chroococcus dispersus	0.0580	*Stephanodiscus hantzchii*	0.0029
Chroococcus varius	0.0072	*Surirella minuta*	0.0007
Gloeothece rupestris	0.0016	*Synedra ulna*	0.0105
Spirulina sp.	0.0008	*Tabellaria sp.*	0.0316
EUGLENOPHYTA		**CHRYSOPHYTA**	
Euglena sp.	0.0005	*Dinobryon sociale*	0.0028
Trachelomonas volvocina	0.0011	*Nephrochloris sp.*	0.0006

Table 3: PTI values generated using both the environmental samples and periphytometers. The signal tailed T-test generated P-scores that indicate PTI values are statistically different for both periphytometer and Environmental samples.

PTI	Site 1	Site 2
Periphytometer	2.91	3.3
Environmental	1.72	2.76
		P>.05
	T-test	
	Site1	**Site 2**
T Value	0.68	0.21
P Value Single Tail	0.25	0.42

Table 4A-D: Table 4A-D are proportional Bray-Curtis tables. Table 4A shows phytoplankton similarity from the summer months, where there is moderate similarity among most sites. The phytoplankton data for Table B, as seen below, only covers sites 1-2, however these are for the autumn months. Again there is moderate similarity between sites. Table 4C is data generated from the summer periphytometers. Data for autumn periphytometers were generated and indicated that sites 1 and 2 were 39% similar; a Bray-Curtis table was not need to compare two sites. Table 4D is a comparison of samples at site 1. The importance this table is that it compares the proportional similarities between the different samples (substrates) analyzed.

A

1							
1.3	0.68						
1.6	0.62	0.65					
2	0.59	0.62	0.80				
3	0.58	0.57	0.71	0.69			
3.5	0.52	0.73	0.63	0.72	0.72		
4	0.52	0.59	0.74	0.75	0.72	0.79	
5	0.71	0.58	0.65	0.65	0.61	0.53	0.57
	1	**1.3**	**1.6**	**2**	**3**	**3.5**	**4**

0-20
21-40
41-60
61-80
81-100

B

1				
1.3	0.83			
1.6	0.78	0.63		
2	0.65	0.64	0.66	
	1	**1.3**	**1.6**	**2**

C

1					
2	0.37				
3	0.29	0.89			
4	0.60	0.44	0.36		
5	0.39	0.96	0.90	0.46	
	1	**2**	**3**	**4**	**5**

D

Plant						
Cobble	0.20					
Rock	0.11	0.10				
Bark	0.05	0.05	0.04			
Sediment	0.24	0.19	0.11	0.05		
Periphytometer	0.17	0.12	0.06	0.03	0.11	
	Plant	**Cobble**	**Rock**	**Bark**	**Sediment**	**Periphytometer**