Short-Term Abiotic Controls of Stream Benthic Algae in Two Northwestern Pennsylvania Streams

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

Alexis L. Jarvie

Pledge

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ABSTRACT

Benthic algae are commonly the most important primary producers in streams. Abiotic factors (i.e. discharge, light) and their interactions with nutrients strongly influence algal biomass and production. Previous studies have found that algae should be high where nutrient concentrations are high, unless other, externally regulated factors, inhibit growth. Other findings indicate that faster current velocities can increase nutrient availability, but high current velocities can also decrease algal biomass. This research sought to find the temporal dynamics of physical and chemical parameter influence on benthic algae in two streams in Northwestern PA. I measured benthic algae and eight abiotic parameters at Little Sugar Creek (LSC) and Woodcock Creek (WC) over a 12-week period during the spring season of 2021. Discharge was positively related to chlorophyll levels at Woodcock Creek ($R^2 = 0.163$, $p=0.09$) while NO_3 ($p=0.03$) and pH ($p=0.039$) were negatively correlated with chlorophyll levels at Little Sugar Creek. Overall, benthic algae biomass was approximately 7.3x higher at WC (average 656.64 ± 106.40 units) than LSC (average 89.92 ± 12.56 units). This difference is probably due to the higher amount of light at WC as a result of a larger stream width (averages: WC = 14.04m vs LSC = 2.71m) providing a larger canopy opening for light. My results show that abiotic control of benthic algae can be context dependent and that stream size and light availability seem to be two of the stronger factors determining algal biomass.

INTRODUCTION

Ecosystems are shaped by interactions between physical disturbance and biological processes. In ecological systems, disturbance varies spatially among habitat patches (Ledger et al., 2008). A number of physical, biological, and chemical factors can interact to control spatial variation in nutrient uptake rates in streams, which is a central, necessary ecosystem function of streams (Coble et al., 2019). Understanding controls on the uptake, transformations, and export of nutrients is essential because excess nutrient loading in aquatic ecosystems, including streams, can cause harmful algal blooms and dissolved oxygen depletion (Coble et al., 2019).

Disturbance in a stream ecosystem can impact essential processes, including nutrient uptake and acidity levels, which then in turn influence food webs and ecosystem composition. Storm events and snowmelt cause episodic acidification in Pennsylvania mountain streams, where there is a short-term drop in acid-neutralizing capacity (ANC) (MacDougall et al., 2008). Snowmelt delivers pulses of organic matter in the spring (Coble et al., 2019) and the timing and rate of this melt can influence solute concentrations in the water (Peterson et al., 2001). The presence or absence of ecological disturbances can impact the livelihood of entire stream ecosystems from producers (algae, phytoplankton) to higher level consumers (invertebrates, fish).

Algae are a highly diverse group of aquatic, photoautotrophic, nonvascular, plantlike organisms with chlorophyll-α and unicellular reproductive structures (Stevenson, 2011). Types of algae are found in almost all aquatic systems and account for around 23% of the world's total primary production (Adams et al., 2013). They play a key role in aquatic food web structure because their cell size can influence the flow of energy to higher trophic levels, as well as influencing an ecosystem's carbon cycle (Adams et al., 2013). Due to their rapid response to

environmental change and the fact that they can be manipulated easily in the confines of stream mesocosms, algae are used often to study stream ecosystems (Ledger et al. 2008).

Benthic algae live on submerged substrates and are critical to freshwater ecosystems, since these can regulate secondary production and nutrient and energy flow pathways in benthic food webs, as well as provide habitats for many organisms within the stream ecosystem (Stewart & Lowe, 2008). They are common acidification indicators and play an important role in aquatic food webs as well as biogeochemical cycling (MacDougall et al., 2008). Benthic algae are commonly the most important primary producers in streams, because they transform light energy into biomass that fuels secondary production. When the growth of algae is limited by nutrients, its nutritional quality becomes limited, meaning that it then limits secondary production of consumers (Beck et al., 2021). Physical and chemical parameters can affect the composition and distribution of benthic algae in streams (Connor et al., 2018).

Environmental factors and their interactions with nutrients strongly influence algal primary production and nutrient limitation (Beck et al., 2021). Inorganic dissolved nutrients and deposited fine sediment are common stressors in streams that can be expected to influence benthic algae, among other things in the stream ecosystem (Wagenhoff et al., 2013). Nutrients, light, temperature, and flow velocity are more factors that have been studied in regards to how they influence algal growth. The amounts and ratios of nutrients (N and P) are important in determining biodiversity and composition of a community (Budnick et al., 2021). If streams have excessive amounts of nutrients such as nitrogen and phosphorus, this can result in a boom of algal growth, especially if the stream has little canopy coverage (Porter et al., 2008). If streams have low nitrogen or phosphorus levels, they typically exhibit inhibited algal growth, meaning that algae in this case is limited by the presence or absence of N or P (Schmidt et al., 2019).

Algal growth rates in streams should be high where nutrient concentrations are high, unless other, externally regulated, factors inhibit growth. Light, for instance, has been found to be a primary, externally regulated limiting resource for algal growth (Hepinstall & Fuller, 1994). The effects of nutrients and light on algal communities has been found to be interdependent (Rosemond et al., 2000). The increase in light or nutrients that is a result of seasonal change, may only have positive effects on algal communities that are not constrained by either of those factors (Rosemond et al., 2000). In regards to discharge, faster current velocities have been found to increase nutrient availability, but high current velocities have also been found to decrease algal growth rates when they are nutrient limited already, due to physiological stress (Beck et al., 2021).

Variations in abiotic factors present in stream ecosystems lead to alterations in the composition of benthic algae. These alterations need to be monitored closely under short- and long-term periods in order to better understand how they impact the ecosystem. I aim to find the physical and chemical parameter relationships that may influence benthic algae biomass (measured as chlorophyll-α). I will be researching how the short-term variability of abiotic factors (i.e. nutrients, discharge) influences the biomass of benthic algae in two streams in Northwestern, PA. Previous studies have shown that algal growth should be high where nutrient levels are high (Hepinstall & Fuller, 1994), and high discharge can decrease algal biomass (Beck et al., 2021). I will be sampling these streams weekly, allowing me to capture any short-term shifts that may impact algal abundance. I expect that high discharge events will decrease algal biomass, which will result in a decrease of chlorophyll-α concentrations, at both of my study sites. I also expect nutrient levels and algal biomass to have a positive relationship at both study

sites. Lastly, I expect the two streams to follow similar trends, at different scales due to their size difference, because they are within the same region during the same seasonal time period.

MATERIAL AND METHODS

Study Sites

Benthic algae assemblages are studied at two different control stream sites. Both sites are located in Crawford County, in Northwestern Pennsylvania, and empty into French Creek. The first site is Little Sugar Creek (41°35'52.7"N 80°02'41.1"W), located in Allegheny's Bousson Environmental Research Reserve. This stream is a low order stream that has a lot of canopy coverage guarding it from a large amount of sunlight. When I first started sampling, I measured the width of this stream to be about 2 feet wide. The second site is Woodcock Creek (41°41'26.8"N 80°02'52.0"W), located upstream from the Woodcock Creek Dam, coincidentally at a pre-existing United States Geological Survey (USGS) continuous monitoring site. This is a medium sized (fourth order) stream that has barely, if any, canopy coverage, allowing plenty of sunlight to penetrate the water's surface. I measured this stream to be about 13.5 meters in width at the beginning of my research. The same physical and chemical parameters were measured at each site so that a comparison could be made (Table 1).

Algae Sampling

Benthic algae were sampled by collecting periphyton in an approximately 50 meter stretch of each of the streams, once a week in the spring, starting in the middle of March, and ending at the beginning of June (12 weeks). A loeb sampler was used to collect algae from rocks at each site. A loeb sampler resembles a syringe with a brush instead of a tip at the bottom of the

plunger. The brush is placed on a rock, twisted to pick up substrate, and then the plunger is pulled upwards to collect the water and algae sample. Three samples of 4 composite loeb sub-samples were taken weekly at each site (one at one end of the 50-meter section, one in the middle area, and one at the other end). A total of three periphyton sample bottles were collected at each stream site, each bottle consisting of four loeb samples each. Water quality measurements, including pH, turbidity, conductivity, and temperature, were taken weekly at each site using a YSI multi parameter probe. Two water samples for chemical analysis were collected at each site and filtered using a nitrocellulose membrane filter (0.45 microns). The collected water samples were frozen until ready for analysis. They were all thawed and analyzed at the same time for NH_4, PO_4, and NO_3.

Width, depth, and velocity were measured at each site using a measuring tape and a streamflow meter (HACH FH950). All of these measurements from Little Sugar Creek were used to calculate discharge for the site. I used the online published data from the USGS continuous monitoring site for the discharge measurements at Woodcock Creek.

Chlorophyll Extractions

Collected periphyton from the composite loeb samples were vacuum filtrated using glass fiber filter (GFF, 0.7 microns) and frozen until further analysis within 2 weeks of collection. Chlorophyll-α was extracted from the frozen filters, following the chlorophyll-α extraction protocol for fluorometry (Steinman et al., 2007). Chlorophyll samples were added to tubes of ethanol and put in a 78ºC water bath for five minutes. They were then frozen for about nine hours, centrifuged, and then processed. After extraction, the total chlorophyll-α concentrations for each sample at each site was determined by using a Turner Fluorometer. The Turner

Fluorometer was calibrated using algal chlorophyll standards to create a standard curve between raw fluorescence and chlorophyll concentration. Chlorophyll concentrations from the fluorometer were converted to benthic algae biomass per area (units) using the area sampled from the loebs.

Statistical Analyses

I used multiple linear regression analyses to assess the effect of abiotic variables on benthic chlorophyll levels. Chlorophyll-α values were regressed against discharge, temperature, pH, turbidity (NTU), conductivity, NH_4, PO_4, and NO_3 measurements at each site. To compare the difference in algal biomass between LSC and WC, I used a t-test assessing the differences in average chlorophyll at each site for the entire 12-week period. Additionally, I calculated the sum of discharge for my entire sampling period of twelve weeks using the USGS continuous monitoring site data located at Woodcock Creek. The instantaneous, 2-day, 3-day, and 5-day sums were tabulated for discharge and I used these data to help determine whether discharge had a significant effect on chlorophyll levels at this site using additional linear regression analyses between chlorophyll and these discharge values for my entire sampling period.

RESULTS

Abiotic parameters and chlorophyll levels between study sites

Average environmental variables fell within normal values for the region for both study streams (Table 1). Woodcock Creek's average discharge is significantly higher than that of Little Sugar Creek, due to the fact that it is a wider, higher order stream, receiving significantly larger amounts of sunlight.

Table 1. Measured abiotic parameter Between Woodcock Creek and Little Sugar Creek. Values represent the 12-week average (03/16/21 - 06/01/21) +/- 1SE

Parameter:	Little Sugar Creek	Woodcock Creek
Discharge (liters/sec)	89.92 ± 12.56	656.64 ± 106.40
Temperature (C)	12 ± 1.04	12.47 ± 1.23
pH	8.41 ± 0.13	8.28 ± 0.060
Turbidity (NTU)	5.90 ± 3.40	3.95 ± 0.48
Conductivity (µS/cm)	123.26 ± 5.47	137.75 ± 8.86
NO_3 (mg/L)	0.47 ± 0.027	0.27 ± 0.016
NH_4 (mg/L)	0.059 ± 0.0074	0.052 ± 0.0069
PO_4 (mg/L)	0.056 ± 0.012	0.050 ± 0.012

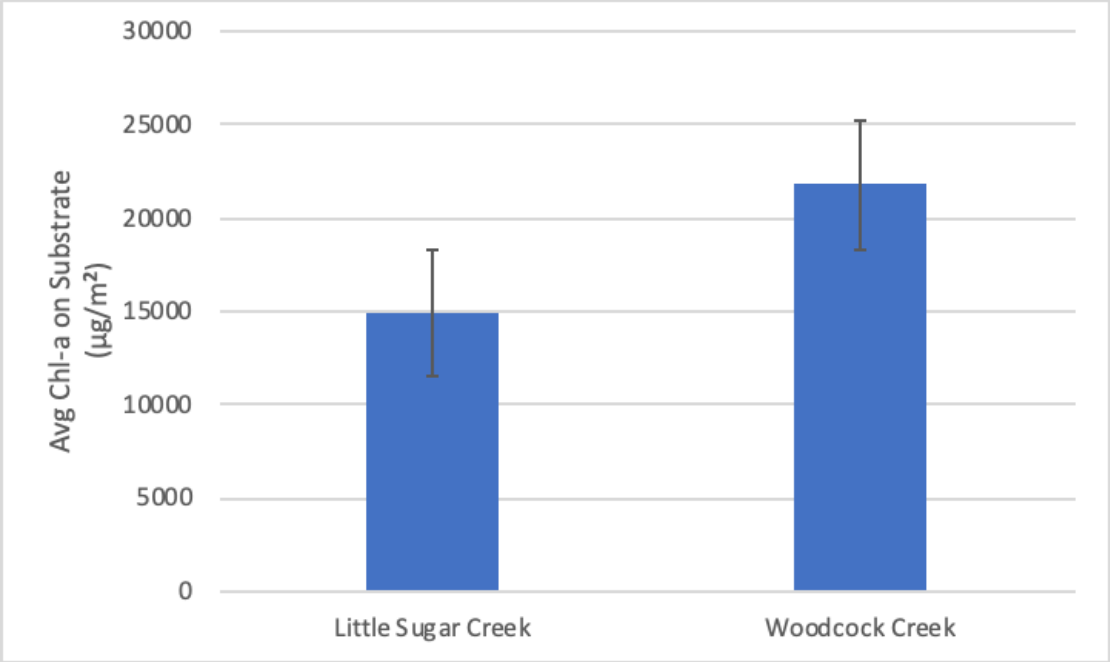


Figure 2. Average Chlorophyll-a on substrate (µg/m^2) at Little Sugar Creek and Woodcock Creek. N=12 weekly samples between 03/16/21 and 06/01/21.

Weekly variation of chlorophyll concentration at each study site

The regression model including: discharge, temperature, pH, turbidity (NTU), conductivity, NO_3, NH_4, and PO_4 did not explain the weekly chlorophyll variation at Woodcock ($p=0.2820$). The one thing to note here is that discharge had a somewhat positive effect on chlorophyll-α ($p=0.09$). Since I had the USGS continuous monitoring site data readily available for use, I compared the discharge that was measured on the day that I sampled, the two day sum (starting when I measured on day one), three day sum, and five day sum in an additional model to assess any effect of discharge variation on chlorophyll-α levels. I analyzed these numbers using single linear regressions but no significant results or relationships between discharge and chlorophyll were seen (Table 2). As the length of the discharge sum increased, the model actually got worse.

Table 2. Significance of additional linear regressions assessing discharge effects on Chlorophyll concentrations at Woodcock Creek.

Sums of Measured Discharge:	**P-Value:**	**R^2 Values:**
Instantaneous	0.513	0.163
2-Day	0.624	0.110
3-Day	0.668	0.0472
5-Day	0.723	0.0165

The same parameters were used here as in the Woodcock Creek analyses to test the effects of the measured abiotic factors on chlorophyll-α at Little Sugar Creek. The multiple regression model with all of the parameters was not significant ($p=0.1325$), however, within this

regression, NO_3 and pH were found to have a significant, negative effect on chlorophyll levels (Table 3). When more NO_3 was present, there was less chlorophyll, and high pH levels also were associated with less algae.

Table 3. Parameter estimates from the Multiple linear regressions results of measured abiotic parameters against benthic chlorophyll concentrations at Little Sugar Creek N= 12 weekly samples between 03/16/21 - 06/01/21.

Parameter:	**Parameter Estimates:**	**P-Value:**
Discharge (units)	-47.8	0.652
Temperature (Fahrenheit)	4230	0.107
pH	-25,600	0.0394*
Turbidity (NTU)	-561	0.0578
Conductivity	-829	0.153
NO_3	-108,000	0.0320*
NH_4	403,000	0.0879
PO_4	-231,000	0.0511

DISCUSSION

My goal for this research was to study the physical and chemical parameter relationships that may influence benthic short-term variation of algae biomass (measured as chlorophyll-α) in two streams in Northwestern, PA. I hypothesized that both streams (WC and LSC) would follow similar trends, those trends being a negative relationship between discharge and algal biomass, and a positive relationship between nutrient levels and algal biomass.

Woodcock Creek's model regression analysis revealed normal results for the region, for a majority of the parameters, but did however show a slight positive relationship between discharge and algal biomass. This suggests that at WC in spring, high discharge cleans sediment and brings lots of nutrients downstream, leaving room on substrate for algae to grow and nutrients for them to consume. The highest measured discharge had the highest chlorophyll-α concentrations, which leads me to believe that this stream is physically controlled by variables including discharge and sunlight. This creek is about five times wider than LSC, is a higher order stream, and is lower in the watershed, which means that this stream gets plenty of internally regulated resources, and because of this, the physical environment has a bigger impact on the system than chemical parameters. My results suggest that algae will mainly be regulated by light availability and discharge in this stream system. A number of externally regulated factors, including light, can regulate algal biomass accumulation in streams (Hepinstall & Fuller, 1994).

My measured parameters at Little Sugar Creek revealed mostly normal values, compared to those of the region, aside from NO_3 and pH. I am able to deduce from the result that NO_3 and pH have somewhat of a negative relationship with chlorophyll levels, that the chemical environment will regulate this system, as opposed to the physical environment that was seen at WC. This creek is a lower order stream that is higher in the watershed, so its nutrient levels are more limiting than its physicality. My results suggest that algae will be limited by chemical parameters (including NO_3 and pH) in this stream system. Other studies have also noted this negative effect of chemical parameters on benthic algae, finding that N and P enrichment has a negative effect on benthic algae (Zhang et al., 2014). This study contributes this finding to the fact that the enrichment of N and P promotes the dominance of phytoplankton over benthic algae, because these nutrient rich conditions are more favorable for phytoplankton which then

compete with benthic algae. Regarding pH, nutrient poor conditions have been found in other studies to be generally associated with low pH (Schneider et al., 2013). The same can be said for the opposite relationship: high pH can be associated with nutrient rich conditions, which is what was observed from the LSC results. The effect of pH on benthic algae is difficult to reason through without the known relationship with nutrient conditions, because each species of algae has a different relationship with pH. Some species stop photosynthesis under low pH levels (Schneider et al., 2013), which could be the case here, but could not be determined unless the benthic samples were identified and classified.

Most of the inter-stream regulated parameters that I tried to use to make a relationship with benthic algae at either stream did not give me significant results. The only results that stood out, that were independent of each stream, were the positive relationship with discharge and chlorophyll at Woodcock Creek, and the negative relationship with NO_3 and pH at LSC. Between the streams however, it is worth noting that they had somewhat of a significant difference in chlorophyll levels: Woodcock Creek's chlorophyll concentration was much higher than that of Little Sugar Creek. WC is a significantly wider stream with less canopy coverage, so light had to be the determining factor in the higher levels of chlorophyll-α. This leads me to believe that light is a key regulating factor of algae and chlorophyll abundance in these two systems, and in this geographic area during the season of Spring. In previous studies, algal abundance has been found to be reduced by 40% or more in natural or artificially shaded sections (Hepinstall & Fuller, 1994), which supports the theory that light is important here, as it would account for the difference in chlorophyll levels between my two study sites. Light has previously been found to increase maximum algal growth rates and nutrient uptake rates (Beck et al., 2021), so this conclusion does make sense, however, it is not new information that the presence of light

will lead to an increase in photosynthesis and growth amongst plants. I purposefully measured chemical and physical parameters other than light to see if they had an effect on benthic algae growth in these streams because I assumed that light would of course have some sort of an effect; I did not, however, anticipate that light would be the crucial factor for regulation comparison between these two streams. WC is approximately five times as wide as LSC, and although I did not measure canopy, I noticed that WC has much less coverage from trees than LSC does. The significant difference in width and available light that WC has compared to LSC can account for why Woodcock Creek's samples had much higher chlorophyll values, despite none of the internally regulated parameters giving very conclusive trends. Although I did not find the conclusive results that I was hoping to find, I did find a possible explanation for the difference in chlorophyll levels between both streams: light. Based on my results, I can conclude that, to the extent of my research, in the season of spring, in Northwestern PA, light is the regulating factor that most noticeably causes variance in the abundance of benthic algae and the concentration of chlorophyll-ɑ between these two streams.

My twelve weeks of sampling occurred entirely during the spring season, so I tried to capture whether or not there was some variation in these two streams within one season. This specific research is the beginning of an ongoing, year-long monitoring of these two streams. The ongoing efforts in the lab will look at the full year scale of all of the seasons in relation to variability within these streams and how algae and chlorophyll are impacted. After the year-long sampling concludes and the data is processed, it can be analyzed for trends across seasons, within seasons, etc. The year-long data should give more information to allow for significant findings. I am going to suggest that for the rest of the year-long period, the canopy coverage is measured at both streams to further investigate the difference in and impact of light on these two

stream ecosystems. My sampling also measured all of these parameters instantaneously, once a week, so the results only reflect that; high nutrient levels would need to be sustained constantly to see the effect when sampling is only being done once a week. More constant data would give a better look into these systems individually and compared to one another. My results that light regulates the chlorophyll-α levels in these two streams in the season of spring was not an anticipated finding, and leaves room for further research. The inter-regulated parameters that I measured, slightly and differently impacted each stream independently, but light can be the assumed, unmeasured, parameter that caused the difference in chlorophyll levels between the two streams. Hopefully the year-long sampling can draw more straightforward and significant conclusions as to how benthic algae in these two streams in Northwestern PA is impacted by short-term abiotic factors, as well stream size and light availability.

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