"Patterns in the Abundance of Benthic Algae in Streams"

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Patterns in the abundance of Benthic Algae in Streams

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Western Michigan University
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Introduction:

Benthic, microscopic algae are the main drivers of primary productivity in streams. The relationship between the abundance and productivity of benthic algae in streams and lakes and the availability of limiting nutrients, especially soluble reactive phosphorus (SRP), has been extensively studied (reviewed in Allan and Castillo 2007, Larned 2010). However, nearly all of this research has been conducted on the periphyton assemblage found on hard substrates (e.g., stones, wood), while very little work has addressed the factors affecting the abundance of epipelagic algae (i.e., the algae associated with depositional (soft) sediments such as sands and silts). One of the few studies conducted on epipelagic periphyton (e.g., Vadeboncoeur et al. 2006) documented an interesting pattern in the abundance of benthic algae in temperate zone lakes. They found that the abundance of epilithic algae (i.e., algae growing on stones) was positively correlated with lake total phosphorus concentration (TP). That is, lakes with high TP had relatively high epilithic periphyton abundance, which suggests that the biomass and productivity of epilithic algae in temperate lakes is limited by the availability of phosphorus. Interestingly, a similar correlation was not observed for epipelagic periphyton. Epipelagic periphyton biomass was not correlated with TP and, surprisingly, it was significantly greater than epilithic biomass at all levels of TP observed. Chlorophyll a density is a surrogate measure for biomass, and has a molecular weight of 895 g/mol. Vadeboncoeur et al. (2006) found that chlorophyll a density on sediments was 5 to 100 times greater than chlorophyll density on rocks and wood.

The main objective of this study was to determine if similar patterns characterize benthic algal communities in streams. This research studied three main objectives. The first objective was to determine if patterns in the abundance of periphytic algae on hard and soft substrates in streams were similar to those found in lakes. (Vadeboncoeur et al. 2006) The second objective
was to document the variability in epipel periphyton biomass within streams, and compare and contrast that with within-stream variability in epilithic periphyton biomass. The third objective was to determine whether epipelic periphyton biomass was correlated with abiotic environmental variables such as light availability, water depth, and sediment particle size composition. This study includes a detailed analysis of multiple streams in the Kalamazoo River watershed in terms of the abundance of benthic algae on hard and soft sediments. This study will be used to compare to the minimal amount of research conducted in lakes in comparing the two different biota habitats. Larned (2010) suggested that the abundance of periphyton is based on five key aspects of environmental variation. The five aspects include: disturbances, stressors, resources, hydraulic conditions, and biotic interactions. This study focuses on abiotic factors that may influence epipelic periphyton biomass.

**Methods:**

For this study samples were taken from eight streams. All streams were relatively small and third order cold water streams capable of supporting trout populations. All the streams contain a mixture of erosional and depositional benthic habitats. The streams were sampled once in October-November 2015 to quantify periphyton biomass present on both habitat types. The eight streams are described below:

*Augusta Creek*

Augusta Creek is located within the Kalamazoo River watershed flowing north to south with a drainage area of 77 km². The stream was accessed near C Avenue in Kalamazoo County at 42°23'31.64"N and 85°21'14.66"W. Augusta Creek was selected because it contained a variety of depositional environments, canopy coverage, and depth. Augusta Creek flows through a wooded area.
Portage Creek

Portage Creek is located within the Kalamazoo River watershed. Portage Creek was sampled near the PCB Grist Mill Trail between Milham Road and I-94 in Kalamazoo County at 42° 13' 50.379" N and 85° 34' 35.6658" W. Portage Creek appeared to be a sandy stream with some variation in sediment size. There was a variety of depth and canopy coverage. Portage Creek flows in a residential area and located along a major road.

Lee Creek

Lee Creek is located within the Kalamazoo River watershed flowing south to north with a drainage area of 3.2 km². The stream was accessed off of ML Avenue in Kalamazoo County at 42°15'56.52"N and 85°28'53.52"W. Lee Creek appeared to be a highly shaded stream surrounded by vegetation.

Seven Mile Creek

Seven Mile Creek is located in the Kalamazoo River basin flowing north to south with a drainage area of 40 km². This stream was accessed near Kirby Road in Calhoun County at 42°22'24.29"N and 85°17'26.01"W. In general Seven Mile Creek appeared to be a shady stream.

Trout Run

Trout Run is located within the Kalamazoo River basin flowing west to east with a drainage area of 4.5 km². This stream was accessed in the Kalamazoo Nature Center in Kalamazoo County at 42°21'31.03"N and 85°34'55.71"W. This stream was a highly shaded sandy stream, which made it difficult to get a wide variety of sampling sites.

Spring Brook
Spring Brook is located in the Kalamazoo River watershed flowing from north to south with a drainage basin of 45.4 km$^2$. The stream was accessed near DE Avenue in Kalamazoo County at 42°21'54.63"N and 85°31'39.53"W. Overall, Spring Brook was a sandy stream with a variety of shaded and non-shaded areas.

Silver Creek

Located in the Kalamazoo River watershed Silver Creek flows north to south with a drainage basin covering 54 km$^2$. The stream was accessed near Riverview Avenue in Allegan County at 42°25'25.81"N and 85°35'2.51"W. Even though Silver Creek was a shady stream, there was variety in depth and sediment composition.

East Branch Paw Paw River

The East Branch Paw Paw River is found in the Kalamazoo River basin flowing from south to north with a drainage area covering 30 km$^2$. This stream was accessed near 26th Street and 67th Avenue in Van Buren County at 42°10'39.78"N and 85°48'4.74"W.

Depositional habitats

Twenty samples were taken in the depositional habitats from a 100 meter reach in each stream. At each sampling site three tasks were completed; a sample was collected and the water depth and canopy coverage was measured. The algae samples were taken using a 30cc syringe with the tip cut-off as a small coring device (Vadeboncoeur et al. 2006). Sampling was always done facing upstream to minimize disturbance to the epipelic algae. For each sample the coring device was inserted into the top 1 to 3 cm of sediment. This was done because epipelic algae is only expected to be near the surface due to light availability (Vadeboncoeur et al. 2006). Sediment and water collected in the core were immediately transferred to a 50 mL centrifuge
tube, capped, and placed on ice and in the dark in a cooler. Water depth where the sample was taken was measured using a standard 30 cm ruler. Canopy coverage was measured using a spherical densitometer. Four readings were taken, with the spherical densitometer oriented upstream, downstream, and to each side. The average of the four readings was used to find the proportion of shaded area. Upon return to the laboratory all samples were left on ice, in a dark cooler to settle for two hours. Once the samples had settled the excess water was siphoned off, and the remaining sample was capped and stored at -20°C.

Erosional habitats

Periphyton algae growing on cobbles in erosional habitats were sampled using methods described in Kohler and Wiley (1997). Ten cobbles were randomly selected from erosional habitats in the same 100 m stream stretch from which depositional habitats were sampled. One sample was taken from the top surface of each cobble using a scrub brush sampler. The scrub brush sampler is a 120 mm length wooden dowel with a disposable scouring disc at the bottom, capable of sampling an area of 490 mm² (Davies et al 1993). After a sample was acquired the disposable scoring pad was immediately removed from the sampler and placed in a 15 mL centrifuge tube and capped. The tube was placed on ice in a dark cooler until returned to the laboratory. At the laboratory all samples were immediately stored at -20°C. Canopy cover was estimated in three even sections covering the erosional depositional study reach. The measurements were taken in the center of the channel in each section. Canopy coverage was measured using the same method as stated in depositional environments.

Laboratory procedures

The depositional samples were taken out of the freezer and freeze-dried to remove all water from the samples to prevent water from interfering with chlorophyll analysis (Hansson
1988, Vadeboncoeur et al. 2006). For the epilithic algae samples, 8 mL of 100% methanol was added to the centrifuge tubes containing the disposable scouring disc sample. The samples were stored in the dark at room temperature for four hours then placed in a centrifuge for ten minutes at 4,000 rpm. Chlorophyll α concentration was measured using a fluorometer equipped with filters for performing the Welschmeyer procedure (Welschmeyer, 1994). For the depositional samples, the sediment was transferred in approximately equal amounts into two 15cc centrifuge test tubes. Then, 10 mL of 100% methanol was added to each tube. The samples were capped, mixed and vortexed, then stored in the dark at room temperature for 4 hours. Samples were mixed and vortexed again two and four hours later. Lastly, they were centrifuged and the chlorophyll α concentration was measured as described above.

With the remaining sediment the goal was to estimate the amount of organic matter present in the sediments, and then quantify the size composition of the remaining sediment. Each sample was transferred into a pre-weighed aluminum dish and placed in a drying oven at 65°C to remove the excess liquid. Then, the samples were transferred into a pre-weighed porcelain crucible, dried at 65°C to a constant weight and weighed to the nearest 0.1 mg, to obtain the dry weight of the sediment. The samples were then combusted at 550°C for one hour in a muffler furnace, cooled to room temperature in a desiccator, and reweighed to obtain the mass of inorganic sediment. The amount of organic matter in the sample was estimated as the difference in the sample’s dry weigh and the weight after combustion. The remaining sediment was quantified to find the composition of fine material (silts and clays) and coarse material (sand and gravel). All samples were pre-weighed individually. To separate the fine material from the course material, each sample was poured onto a 62-micron sieve and then deionized water was used to wet sieve the fine material through. The sand and gravel materials were scraped and
cleaned off the sieve and dried for at least 48 hours. The mass of the dried coarse material was measured. The fine sediment fraction was calculated by taking the pre-weights minus the post-weights. This was the standard method explained by the USGS survey report on grain-size analysis.

*Statistical Analyses*

I tested whether periphyton biomass differed between hard and soft substrates using randomized complete block one-way analysis of variance. Stream was used as a blocking factor in the model, and the treatment was substrate type, with two levels (hard, soft). The response variable was periphyton biomass, expressed as chlorophyll $a$ density ($\mu g/cm^2$). Chlorophyll $a$ density was log-transformed to satisfy assumptions of the anova model.

I used regression analysis and analysis of covariance to explore relationships between epipelic periphyton biomass and several environmental variables including water depth, canopy cover, sediment organic matter content, and sediment size composition. For each environmental variable, I first used analysis of covariance to determine if the relationship between epipelic periphyton biomass (expressed as log chlorophyll $a$ density ($\mu g/cm^2$)) and the variable was consistent for all streams (i.e., the slope of the regression of epipelic periphyton biomass on the environmental variable did not differ among streams). If the slopes differed significantly among streams, separate regressions were fitted for each stream.

*Results:*

*Comparison of Erosional and Depositional Habitats*

Periphyton biomass was significantly greater on soft sediments than on hard substrates (Table 1, Figure 1). Mean epipelic periphyton biomass ranged from $8.48\pm0.99$ (mean ±SE) $\mu g$
Chl a/ cm$^2$ in Silver Creek to 27.52±2.67 μg Chl a/ cm$^2$ in Augusta Creek. By contrast, mean epilithic periphyton biomass ranged from 0.08±0.018 μg Chl a/ cm$^2$ in Spring Brook to 11.00±3.44 μg Chl a/ cm$^2$ in Portage Creek. Epipelic periphyton biomass exceeded epilithic biomass by more than 10-20 fold in all streams except Portage Creek and Silver Creek (Figure 1). In general, the standard error of the mean was larger for hard substrates compared to what is found in the soft substrates (Fig. 1). To allow direct comparison of variability between the substrate types, I calculated the coefficient of variation of the estimates for each stream and substrate type (Table 2). The coefficient of variation was always greater on hard substrates than soft substrates. Some places it was 2-fold greater.

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stream</td>
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<td>64.36</td>
<td>9.19</td>
<td>10.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sediment Type</td>
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<td>416.9</td>
<td>491.85</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
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<td>195.79</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Randomized complete block analysis of variance of periphyton biomass on hard and soft substrates. Stream is a blocking factor in the model.

<table>
<thead>
<tr>
<th>Stream</th>
<th>Hard</th>
<th>Soft</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>63.97</td>
<td>43.37</td>
</tr>
<tr>
<td>EBPP</td>
<td>179.89</td>
<td>77.92</td>
</tr>
<tr>
<td>LC</td>
<td>78.07</td>
<td>43.53</td>
</tr>
<tr>
<td>PC</td>
<td>98.77</td>
<td>40.48</td>
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<tr>
<td>SB</td>
<td>69.57</td>
<td>60.74</td>
</tr>
<tr>
<td>SC</td>
<td>100.11</td>
<td>52.27</td>
</tr>
<tr>
<td>SMC</td>
<td>151.89</td>
<td>47.09</td>
</tr>
<tr>
<td>TR</td>
<td>60.16</td>
<td>30.89</td>
</tr>
</tbody>
</table>

Table 2: Coefficient of variation of periphyton biomass on hard (n=10) and soft (n=20) sediments in 8 streams.
Periphyton biomass in depositional habitats

As described in the methods section, canopy cover was measured at each sampling site as a surrogate measure of light availability (i.e., areas with high canopy cover should have relatively low light availability, and vice versa). Figure 2 shows the relationship between chlorophyll a density compared to canopy cover in each stream. The first statistical analysis was done to see how similar the streams overall slope is. To determine if the data can be refitted without the stream by canopy cover interaction the $P$-value of the interaction term must be greater than 0.05. If the $P$-value is greater than 0.05 we conclude that the slopes of the regression
of log chl a on canopy cover do not differ among streams. For this analysis, $P = 0.822$, indicating that the regression slopes did not differ significantly among streams. The overall regression slope was $-0.0034$ ($P=0.0449$), indicating that epipelic periphyton biomass declined significantly with increasing canopy cover. This relationship is most evident for streams with a wide variation in canopy cover among sampling locations (Fig. 2). Silver Creek, Trout Run, and Lee Creek are heavily shaded streams and it was harder to find variation in canopy coverage for the sampling sites.

Another comparison is involved the relationship between water depth and chlorophyll $a$ density (Figure 3). A statistical test was done to see if the streams together showed a significant
relationship and the data revealed a statistical test of $P=0.119$. Since $P$ is greater than 0.05 we conclude that the regression slopes of chlorophyll $a$ density on depth did not differ among streams. The overall regression slope was 0.0153 ($P=0.0501$). Even though streams such as the East Branch Paw Paw River and Silver creek appeared to have a weak negative slope, there was considerable scatter in the data for those streams in comparison with streams such as Augusta Creek, Portage Creek, and Seven Mile Creek for which there was less scatter in the data. The positive slope indicates that as depth increases, epipelic periphyton biomass increases as well.

![Figure 3: Epipelic periphyton biomass (as log(chlorophyll $a$ density)) as a function of water depth at each sampling location (n=20 in each stream).](image)

The relationship between epipelic periphyton biomass and the proportion of very fine sediment ($< 63 \mu m$; silts and clays) in a sample was not consistent across streams (Figure 4). The
slopes of the regression relationships differed significantly among streams \((P = 0.0197)\), which indicates that each stream needs to be considered individually. Similar to previous graphs, the more variety across the x-axis the better the correlation is shown. The East Branch Paw Paw River is a sandy stream with little diversity, so it was hard to get a broad range in the proportion of fine sediment in the samples. Silver Creek had a nice range of sediment composition. Based on the limited range in the proportion of very fine sediment in most streams, conclusions were not able to be drawn about the overall relationship between epipelic periphyton biomass and the silt/clay content of samples.

Figure 4: Epipelic periphyton biomass (as log [chlorophyll a density]) as a function of the percent of total sediment weight from particles < 63 \(\mu\)m (sils and clays). Seven streams have twenty samples. Augusta Creek has 12 samples because 8 sediment composition samples were lost after periphyton biomass had already been measured.
The relationship between organic matter content of soft sediments to sediment particle size composition was not consistent across streams (Figure 5). The slopes of the regression relationships differed significantly among streams ($P = 0.0012$) and therefore each stream needed to be considered individually. Several streams exhibited very strong positive correlations between sediment organic matter content and the percent of silt and clay in sediment samples (e.g., Augusta Creek, Lee Creek, Portage Creek, Seven Mile Creek, Silver Creek; Fig. 5). Silver Creek was the most significant, and it also had the widest distribution in sediment composition. Portage Creek and Trout Run showed a high enough $P$-value to be counted as significant; however, there was not enough variation in sediment composition for the results to be noteworthy. The percent of fine material at East Branch Paw Paw River was 10% or less in all samples. Since there were no samples that contained more than 10% fine material, it is impossible to draw strong conclusions from this relationship. Overall, there is a positive correlation between sediment organic matter content and fine fraction. The slope is nearly one or greater than one in most streams, meaning for every percent increase in fine sediment fraction there is approximately an equal increase in percent sediment organic matter content.
Figure 5: The organic matter content of depositional samples compared to percent of silt and clay sediments in the sample (n=20 for all streams except Augusta Creek (n=12)).

Discussion:
There were three main objectives in this project. The first objective was to determine if patterns in the abundance of periphytic algae on hard and soft substrates in streams were similar to those found in lakes (Vadeboncoeur et al. 2006). The second objective was to document the variability in epipelic periphyton biomass within streams, and compare and contrast that with within-stream variability in epilithic periphyton biomass. The third objective was to determine
whether epipelic periphyton biomass was correlated with abiotic environmental variables such as light availability, water depth, and sediment particle size composition.

Comparison between erosional and depositional habitats

I found that periphyton biomass in depositional environments (sand, silts, and clays in regions of relatively slow flow) was consistently much greater than that observed in erosional environments (rocks in regions of relatively fast flow). To my knowledge, this pattern has not been documented in streams before my study. Interestingly, Vadeboncoeur et al. (2006) also observed this pattern in temperate freshwater lakes, reporting that chlorophyll $a$ concentrations on soft substrates were over five times greater than chlorophyll $a$ contents found on hard substrates. The results of this study show this to be true as well in streams. In fact, in most streams epipelic periphyton biomass was over 10 times greater than epilithic periphyton biomass.

One hypothesis to explain why epipelic periphyton biomass is greater than epilithic periphyton biomass is that epipelic periphyton growth is not limited by the availability of nutrients, especially phosphorus, while epilithic periphyton growth is strongly nutrient limited. Vadeboncoeur et al. (2006) reported that chlorophyll concentrations on hard substrates in lakes was strongly correlated with total phosphorus concentration in the water column while chlorophyll $a$ concentration on soft sediments showed no change with increasing total phosphorus. Moreover, numerous experiments in both streams and lakes have demonstrated that the growth of epilithic algae is commonly limited by nutrient (especially phosphorus) availability (Hart, 2012, Larned, 2010, Wessel, 2013). Epipelic periphyton may be less susceptible to nutrient limitation because it could obtain nutrients from two sources: the overlying water column and by diffusion through the underlying soft sediments. Epilithic algae can only obtain
nutrients from the overlying water column. Experimental manipulations of nutrient availability in soft sediments are needed to test this hypothesis.

A second hypothesis for the substantially greater biomass of algae on soft than hard substrates is that epipelic periphyton biomass is not limited by predation on it by periphyton-grazing invertebrates, while epilithic is often limited by grazing pressure. There is substantial evidence that periphyton grazers often limit the abundance of epilithic periphyton in streams (Kohler and Wiley, 1997, 1992). I found that epilithic periphyton biomass was much greater in the streams that had less grazing activity such as Portage Creek. Streams such as Spring Brook had much lower chlorophyll \( a \) concentrations with a higher amount of grazers present. (Kohler and Wiley, 1997), Experimental manipulations of grazer abundance in soft sediments are needed to test this hypothesis.

Finally, another hypothesis is that both higher nutrient availability and lower grazing pressure in soft sediment habitats account for greater periphyton biomass on soft sediments compared with hard substrate habitats. There is considerable evidence that both top-down (grazing) and bottom-up (nutrient availability) forces can act simultaneously in limiting the abundance of epilithic algae. Similarly, in depositional habitats the combined effects of relatively high nutrient availability and low grazing pressure could account for the relatively high biomass of epipelic algae. Sophisticated factorial experiments manipulating both nutrient availability and grazing pressure would be needed to test this hypothesis.

*Why is there high variability on hard substrates?*
I found that within-stream variability in periphyton abundance was consistently greater on hard substrates than on soft substrates. Prior to conducting the study, my hypothesis was that there would be a higher variability in depositional habitats because there were more opportunities for disturbance and other factors. Disturbances and other factors that could affect the variability on hard substrates include: grazing activity, water flow hitting the top of cobbles, and higher light availability. The data showed low mean values, which indicates that there were not rocks with high epilithic periphyton biomass, but some cobbles showed extremely low values for chlorophyll $a$ density.

There appeared to be more grazing activity on hard substrates as explained in the previous section. Since, there is more grazing activity it is hard to control for whether the site sampled had not been recently grazed. This would cause local patchiness on the cobbles in the 100 meter stretch. Previous data have shown certain streams have much higher amounts of grazers such as *Glossosoma nigrior*. *Glossosoma nigrior* is the dominant grazer on hard substrates in cold water streams throughout Michigan. They can strongly depress epilithic periphyton biomass (Kohler 1992) and, as a consequence, affect the entire benthic community structure (Kohler and Wiley, 1997). Lower amounts of periphyton at a patch may reflect recent grazing activity. For example, if a grazer such as *Glossosoma nigrior* were to graze on the rock shortly before sampling that would give the data a lower amount than might be present on a nearby rock that had not been grazed recently. This could account for the wide variety that was at each stream showed in Table 2. Certain streams tend to have more grazers: the East Branch Paw Paw River, Lee Creek, Spring Brook, Seven Mile Creek, and Trout Run. Possibly because of the larger population of *Glossosoma nigrior* these streams showed a much greater range between hard and soft substrates. These streams would have smaller amounts of epilithic periphyton
biomass because of the greater grazing intensity on hard substrates. By contrast, I observed streams like Portage Creek to have very few grazers which would explain why it has the highest concentration of epilithic periphyton biomass compared to other streams. Silver Creek and Augusta Creek have minimal amounts of grazers because of a parasite-induced disease that has begun to wipe out the population (Kohler and Wiley, 1992). Seven Mile Creek and Spring Brook had already had a major decline dealing with Cougourdella sp.; a parasite that caused Glossosoma nigror to reach almost zero population in the streams. (Kohler and Wiley, 1992) Since then, the populations have risen causing there to be more grazing activity. This could account for a major disturbance that would cause high variability within streams on hard substrates.

In a 100 meter stream reach, some rocks may experience much greater light availability than others, and light availability can limit periphyton biomass and productivity (Hart, 2012, Larned, 2010). Thus, spatial variation in light availability could account for some of the within-stream variability in epilithic periphyton abundance. However, the same considerations apply to epipelic periphyton, so it seems unlikely that differential light availability could account for the observed differences in variability in periphyton abundance between depositional and erosional habitats in my study.

Do environmental variables help explain within-stream variation in epipelic periphyton abundance?

The last objective was to find patterns and correlations between chlorophyll α concentrations and abiotic environmental variables such as light availability (using canopy cover as a surrogate measure), water depth, and sediment particle size composition. Beginning with
canopy coverage, I attempted to sample over a variety of shading conditions in each stream in order to see if a pattern could be found. The streams that exhibited the widest range of shading conditions showed a negative correlation between epipelic periphyton abundance and canopy cover. Since algae needs light for photosynthesis, the negative correlation makes sense. As a stream gets limited light availability the concentration of chlorophyll $\alpha$ becomes less abundant.

In general, epipelic periphyton biomass increased with increasing water depth. Originally, this correlation seemed opposite of what I expected. A hypothesis to explain this is very shallow depositional areas are typically near the bank and therefore potentially subject to increased frequency of disturbance (e.g. from fluctuations in stream discharge resulting in the areas periodically drying and then rewetting). High disturbance frequency should reduce periphyton biomass in those areas, resulting in increased biomass with increasing depth. However, because light attenuates with depth, periphyton biomass should eventually decline with increasing depth due to light limitation. Thus, over a broad range of depths, one might expect a hump-shaped relationship between periphyton biomass and depth, where epipelic periphyton biomass is maximized at an intermediate depth.

In most streams, epipelic periphyton biomass was not strongly correlated with sediment particle size composition, particularly the proportion of very fine sediments (silts and clays) in the habitat. There was only one stream that showed a highly significant positive correlation. Possibly the main reason such a strong correlation was observed in Silver Creek was because the range in sediment particle size composition was much greater there than in the other streams (Figure 4), which made it easier to detect a correlation. When looking at these streams individually, there were a couple streams (e.g., Augusta Creek and Lee Creek) that needed to be looked at closer to tell if influential points were the reason behind making an observed
correlation significant. Starting with Augusta Creek the significance showed a negative slope meaning as sediment becomes a larger finer fraction the amount of epipelic biomass decreases. However, the outlier point was thought to be a strong influential point and when you take that point out the graph shows a no longer significant relationship. Lee Creek appeared to have some influential points however, when the point farthest to the right was taken out, it still showed a significant positive relationship. Then when the next point farthest to the right was also thought to be an influential point was taken out it was still a significant positive slope. This suggests that Lee Creek does have a significant positive correlation between epipelic periphyton biomass and fine sediment fraction. The overall conclusion that can be drawn from this graph though is that in general the data shows no significant relationship between epipelic periphyton biomass and fine sediment fraction.

Finally, I found a strong positive relationship between organic sediment composition and fine sediment fraction. Since, the correlation is so significant these values are almost interchangeable. The advantage for this is if organic matter composition was put in place of fine sediment in Figure 4, it would show patterns very similar to those described by Vadeboncoeur et al. (2006) for lakes. They showed that chlorophyll a concentration was weakly but significantly related to sediment organic matter content. This similarity between lakes and streams is a very interesting finding.

In conclusion, streams demonstrate similar patterns to those found in lakes. Epipelic periphyton biomass always exceeds epilithic periphyton biomass. I found significant, but relatively weak, relationships between epipelic periphyton biomass and canopy coverage, water depth and, to a lesser extent, sediment composition. None of these factors appeared to exert strong effects on epipelic periphyton biomass, and they do not account for the substantial
differences in periphyton biomass between adjacent depositional and erosional habitats. Future research will be needed to study these patterns and how they compare with nutrient availability and other factors such as grazing intensity. An example of a future study could include measuring the diffusion gradient of nutrients from in the pore water beneath the stream. This would answer the question is since epipelic periphyton appears to not be nutrient limited where is the excess nutrients coming from?

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