

Seasonal Progression of Algal Development and Quality in Streams that Vary in Timing of Springtime Peak Flow

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Abstract:

Climate driven shifts in stream hydrology from early, rapid snowmelt may have implications for algae growing within high altitude montane streams. Peak flow scours the benthic layer and clears senesced algal growth and sediment, effectively priming the substrate for new algal growth following peak flow. An earlier peak flow allows for a longer season for algae to accrue biomass, but the implications for algal resource quality are unknown. Algae are crucial components of aquatic ecosystems because they are the basal food resource for animals, and algal nutrient uptake can dominate stream nutrient cycling. This study considers how variation in the timing of peak streamflow of different streams in the southern Rocky Mountains could impact the resource quality and development of algae. Algal samples from these streams were analyzed for biomass, chlorophyll-a, community composition, and percent phosphorous. *Baetis bicaudatus*, a grazing mayfly, was also sampled as they can selectively feed on nutrient dense algae and therefore their developmental stage and body chemistry may characterize among stream variation in algal resource quality more effectively than bulk algal samples. I hypothesized that with shorter and earlier peak flows, algae will (1) accrue more biomass, (2) have higher amounts of chlorophyll, and (3) have poorer elemental resource quality than algae in a stream with a later peak flow. These hypotheses were tested along seven streams in the southwestern part of Colorado, USA. Analysis of variance (ANOVA) was used to see if there were differences in each variable among streams and dates. Biomass, phosphorus content, chlorophyll-A, and cell counts of algal phyla differed among streams, but only cell counts differed amongst dates. Although concrete conclusions about the effects of peak flow cannot be evaluated until streams have all been sampled to base flow this autumn, the differences detected among streams thus far suggest that an unknown factor is influencing algal development, and patterns in biomass and chlorophyll accrual are consistent with predictions based on these rivers' melt order.

Introduction:

Algae play many roles within aquatic ecosystems that impact the systems' overall structure, function, and productivity. In high altitude montane streams, algae are the predominant food source for heterotrophic organisms, and therefore algae are a key basal resource for consumers in aquatic food webs (Caro-Borrero & Carmona-Jimenez, 2018). Furthermore, algae play an important role in nutrient cycling. Nutrient cycling is a critical foundational ecosystems process, and nutrient availability (primarily nitrogen (N), phosphorus (P) and sulfur (S) in the case of aquatic ecosystems) is a limiting factor for organismal growth (De Groot, Wilson, & Boumans, 2002). Algae help drive these nutrient cycles via nutrient uptake in streams (Mulholland, Steinman, Marzolf, Hart, & DeAngelis, 1994). Algal biomass is directly linked to nutrient uptake rates, and following uptake, higher concentrations of N and P in algal tissues improve algae's resource quality for macroinvertebrate consumers (Sterner & Elser, 2002). Furthermore, algae can act as a natural purification system for stream water by removing excess nutrients. The ability of algae to improve water quality offers an economically favorable option for water purification through natural processes as opposed to sewage treatment plants (Sabater, Guasch, & Muñoz, 2002).

Over the past few decades, the Western United States has had lower levels of snowpack and earlier snowmelt times; both of which are prompted by climate change (Safeeq, Grant, Lewis, & Tague, 2012). These changes directly impact freshwater aquatic ecosystems by shifting the spring peak flow times of rivers and streams to occur earlier in the year (Ryberg, Akyüz, Wiche, & Lin, 2016). Peak flow causes a streambed scour that primes the benthos for algal accrual by removing sediment and any remaining growth from the previous year (Peterson, 1992). In snowmelt dominated streams, earlier peak flows allow algal development to initiate earlier in the year, allowing for a longer growing season and therefore a larger accumulation of algal biomass (Davis et al. 2013). Climate driven increases in algal biomass have the potential to enhance nutrient uptake (Mulholland et al. 1994). Davis et al. (2013) also suggests that despite this potential increase in algal quantity, the resource quality of algae for invertebrate

consumers will become less predictable year to year. Inconsistency in resource quality could directly impact aquatic trophic pathways for stream invertebrates and subsequently trout.

Algal resource quality may also be impacted by climate-driven changes in the timing of nutrient availability. Snowpack accumulation during the winter holds nutrients frozen in the snow; these nutrients then enter streams during the melting period. When there is an early and rapid melting period, this can cause an “ionic pulse” where a high concentration of nutrients held in the snowpack is released in a short period of time (Wolford & Bales, 1996). Historically, later and more protracted peak flow times would cause these snowpack nutrients to slowly titrate into montane streams throughout the growing season. Release of a large majority of nutrients in a shorter period of time may have implications for the timing of nutrient availability in streams for algal growth and resource quality.

Longer growing seasons for algae may also impact algal taxa present in streams at different times following peak flow. During post flood stream recovery, diatoms are the first periphyton to colonize, followed by green algae and cyanobacteria (Fisher, Gray, Grimm, & Busch, 1982). Cyanobacteria is, in most cases, not useful for macroinvertebrates to use as a food source, and furthermore, excessive amounts can lead to the production of harmful chemicals that can impact the aquatic food web (Murdock, Roelke, & Gelwick, 2004). Following cyanobacteria, later algal successions are dominated by red algae, which, similarly to cyanobacteria, are too large for macroinvertebrates to use as a food source (Murdock, Roelke, & Gelwick, 2004). With shifts in peak flow timing, there are many opportunities for taxa present throughout succession to shift because there will not only be more time for taxa to diverge, but also differences in light, temperature, and nutrient availability could influence competitive outcomes. In turn, these diverging algal communities may have different stoichiometric and elemental resource qualities (Stelzer & Lamberti, 2001).

Here, my primary objective was to determine if varying stream peak flows impact the growth, development, and resource quality of algae. Examining the effects of climate driven shifts in stream hydrology on algae as basal resources for aquatic foodwebs and as drivers for ecosystem-level nutrient uptake will inform subsequent predictions linking climate driven changes in hydrology to aquatic

foodwebs and ecosystem functions. I addressed these questions in seven streams near the Rocky Mountain Biological Laboratory that vary in the timing of their peak flows but are similar in other biological and physical conditions. Specifically, I hypothesized that with earlier, more rapid peak flows: (1) algae will reach a greater biomass, (2) chlorophyll would be higher and (3), the elemental resource quality of algae in these streams will be poorer in comparison to algae in streams with longer, more protracted peak flows. I followed biomass accrual because we expect a larger accrual of biomass when there is an earlier peak flow that allows for a longer season for algal development (Davis et al. 2013). Chlorophyll a is an index that can tell us how much algal tissue is living, and therefore measuring both AFDM and chlorophyll-a allowed me to understand how much of the algal mat is living/growing algae (Peterson, 1992). I measured algal %P because it is unclear how algal resource quality will respond to changes in peak flow, and furthermore, any divergence of taxa following peak flow may have different elemental resource qualities.

Methods:

The seven streams I sampled in this study are: the East River, Copper Creek, Washington Gulch, Coal Creek, Cement Creek, Poverty Gulch, and the Slate River. Three locations for sampling were chosen per stream. Starting at the most downstream location, I randomly selected 3 softball sized rocks and collected algae by scrubbing all algal material from the rocks into a pan following the methods described by Steinman, Leavitt, & Uzarski (2017). Image J was used to measure the area of the rocks (Rueden et al. 2017). All streams were sampled at least 3 times as of August 11th, 2019, but sampling will continue through base flow conditions in late September. At each sampling site, three 56 mL falcon tubes were filled with collected algal scrubate: one for Ash Free Dry Mass (AFDM), one for % phosphorous, and one for community composition algal cell counts. All samples were centrifuged and aspirated down to 10mL. Subsamples of 1 mL were taken from the community composition vial and frozen for chlorophyll a analysis. AFDM samples were dried and ashed at 500C to calculate Ash-Free Dry Mass (AFDM) using the methods of Steinman et al. (2017). Chlorophyll-A was measured following the methods of Arar &

Collins (1997) using a Turner Trilogy fluorometer. Algal P content was determined via standard colorimetry following acid-persulfate digestion using methods outlined by Steinman and Duhamel (2017). Algal phyla (diatoms, green filamentous, green non-filamentous, didymo, stacked diatoms, and cyanobacteria) present were enumerated following the methods of Lowe and LaLiberte (2017).

In addition to scrubbing rocks, I also collected *Baetis bicaudatus*, a grazer mayfly taxa found in each of my study streams. These invertebrates sample the algae selectively for nutrient dense algae, and therefore may better characterize algal nutrient contents. Each baetis collected from each stream was also measured for developmental (instar) stage because stage may indicate the growing conditions; mayflies consuming algae with higher nutrient concentrations may pupate earlier.

Preliminary data were analyzed using Analysis of Variance (ANOVA) to test for differences among streams and dates in %P, chlorophyll-a, AFDM, and cell counts of live diatoms, dead diatoms, filamentous green algae, and non-filamentous green algae. I am stopping at the use of an ANOVA for now because more advanced time-series analysis tools would be more appropriate when I have a complete dataset after the streams have been sampled to base flow. These results could potentially be followed up with a statistical test that tells us which streams and dates differed from each other in %P, chlorophyll-a, AFDM, and cell counts, and by how much.

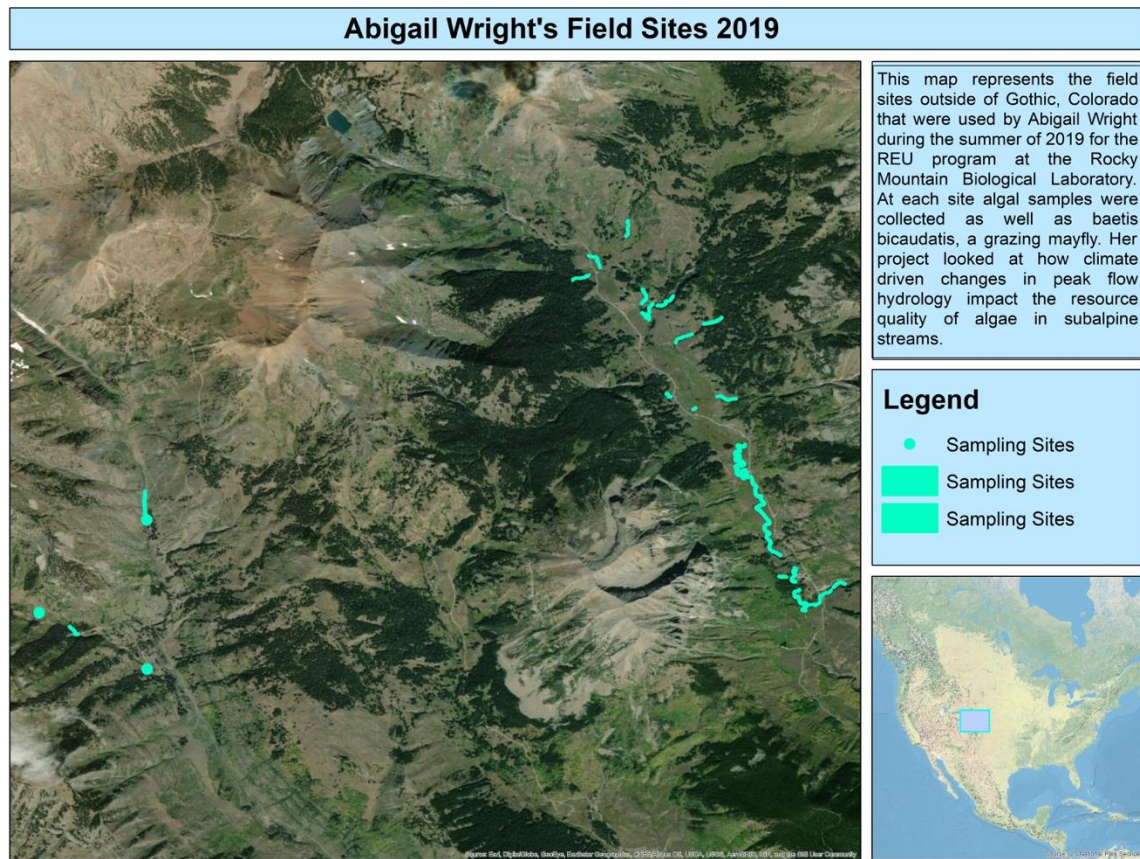


Fig 1. Sampling sites surrounding the East River valley.

Results:

AFDM differed among streams ($F_{(6,32)} = 21.891$, $p < 0.05$) and bordered on significance across dates ($F_{(1,32)} = 3.515$, $p = 0.06996$). The interaction between stream and date was also significant ($F_{(4,32)} = 7.87$, $p < 0.05$). Chlorophyll-a also differed among streams ($F_{1/37} = 20.319$, $p < 0.05$), and the interaction between stream and date was significant as well ($F_{6/37} = 13.330$, $p < 0.05$). Chlorophyll-a was not different among dates ($F_{1/37} = 0.016$, $p = 0.899$). Phosphorus amounts also differed among both streams and the interaction between stream and date (streams: $F_{6/22} = 25.161$, $p < 0.05$, stream/date interactions: $F_{3/22} = 3.188$, $p < 0.05$). Phosphorus did not differ among dates ($F_{1/22} = 1.442$, $p = 0.2427$).

For cell counts, live diatom counts were different among both streams and dates (streams: $F_{6/34}=24.862$, dates: $p<0.05$, $F_{8/34}=5.903$, $p<0.05$). Live diatoms were not different between stream date interactions ($F_{2/34}=1.012$, $p=0.374$). For filamentous green algae, there were differences between streams, dates, and also stream date interactions (streams: $F_{6/34}=4.767$, $p<0.05$, dates: $F_{8/34}=4.010$, $p<0.05$, stream/date interactions: $F_{2/34}=4.263$, $p<0.05$). Dead diatoms were different across streams and dates (streams: $F_{6/34}=16.004$, $p<0.05$, dates: $F_{8/34}=2.416$, $p<0.05$), but not among the interaction between streams and dates ($F_{2/34}=0.188$, $p=0.8296$). There was not enough data to run an ANOVA on green non-filamentous algal cell counts, as well as for Didymo counts, so differences in variance among those algal taxa for dates and streams was not calculated.

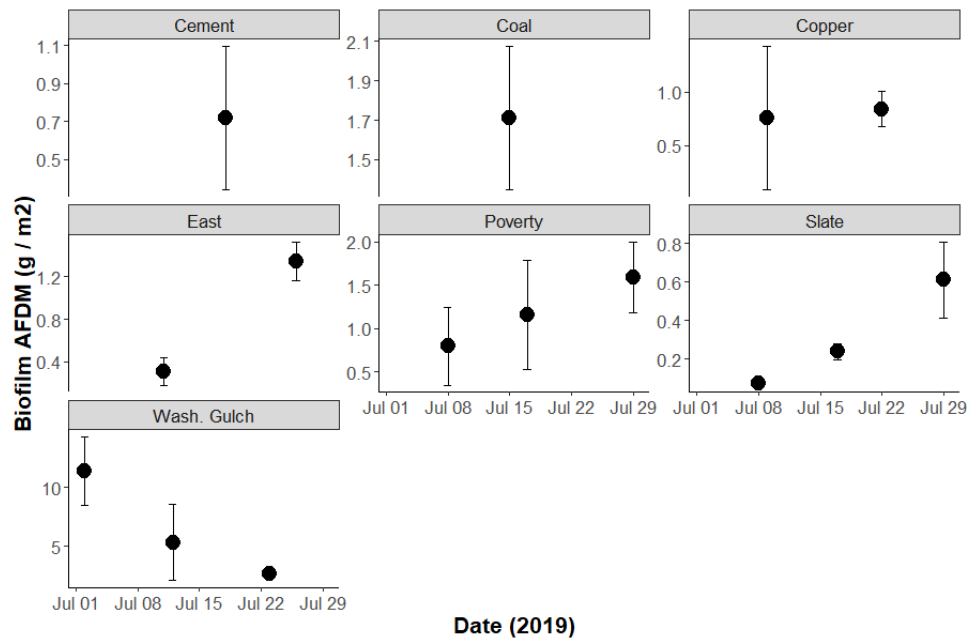


Fig 2. Temporal pattern of AFDM from rocks in seven streams following the springtime peak in streamflow. Points are means with standard error. There is a difference in scale of AFDM amounts among sites (e.g., Washington Gulch and Slate). Patterns between East, Poverty and Slate (rivers with later peak flow times) also show a gradual increase in AFDM while AFDM in Washington Gulch decreases (a river with an earlier peak flow).

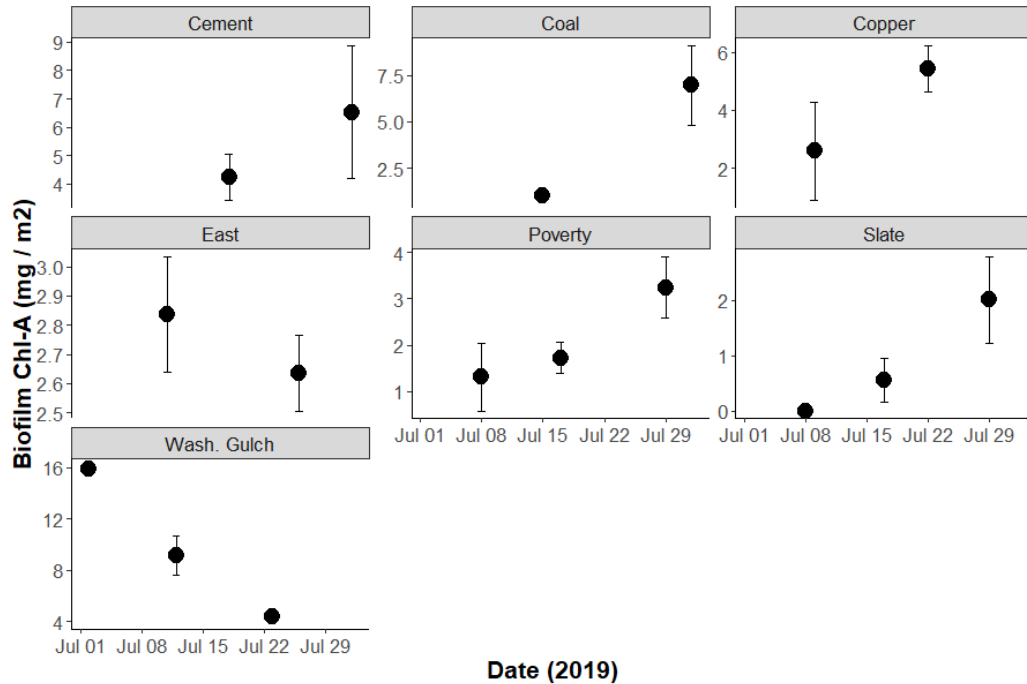


Fig 3. Temporal pattern of chlorophyll-a on rocks in seven streams following the springtime peak in streamflow. Points are means with standard error. The patterns in Poverty and Slate suggest that the amount of living algae increases as expected while amounts of living algae in Washington Gulch (a river with an earlier peak flow) decreases.

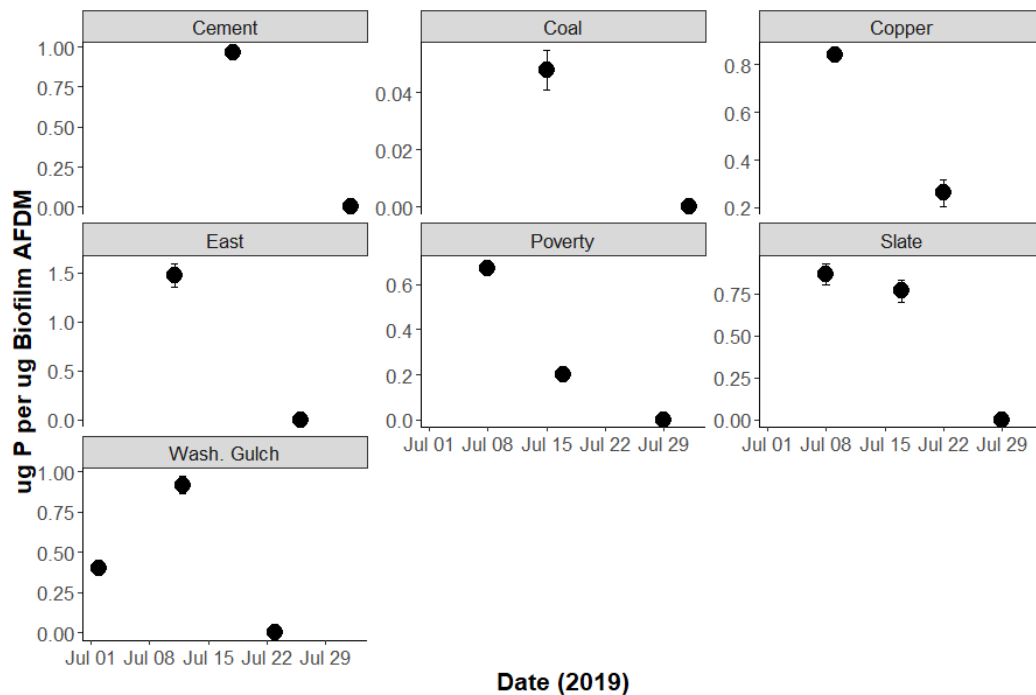


Fig 4. Temporal pattern of %P in sampled biofilms from seven streams following the springtime peak in streamflow. Points are means with standard error. Most streams are showing a steady decrease in %P over time from peak flow.

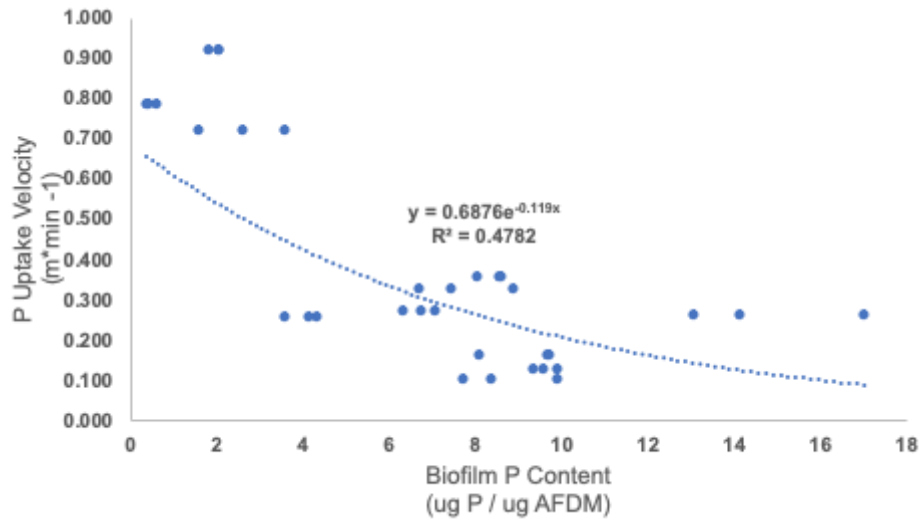


Fig 5. Negative relationship between algal phosphorus content and ecosystem (stream reach) scale phosphorus uptake velocities.

Linear regression was used to test for mechanistic links between algal nutrient contents and ecosystem (stream reach) scale nutrient uptake (Fig 5). There was a strong negative correlation between log-transformed algal phosphorus content and reach-scale phosphorus uptake velocities ($F_{(1,31)}=28.38$, $p<0.05$, $R^2=0.4782$).

Discussion:

Every single variable tested (AFDM, chlorophyll-a, %P, live diatom cell count, dead diatom cell count, and green filamentous cell count) differed among streams. In the case of AFDM and chlorophyll-a there were also significant differences across dates within streams. So, in some of the streams, there were differences in AFDM and chlorophyll-a between the sampling dates.

All cell counts were significantly different across both streams as well as dates. For green filamentous algae, there was also significance across the interaction between streams and dates. Variance in live diatom cell counts among streams had the highest significance (lowest P value), followed by the variance of dead diatoms among streams. There were also differences in live diatom counts among dates, but the strength of significance for variance among dates was not as strong for both green filamentous and dead diatoms.

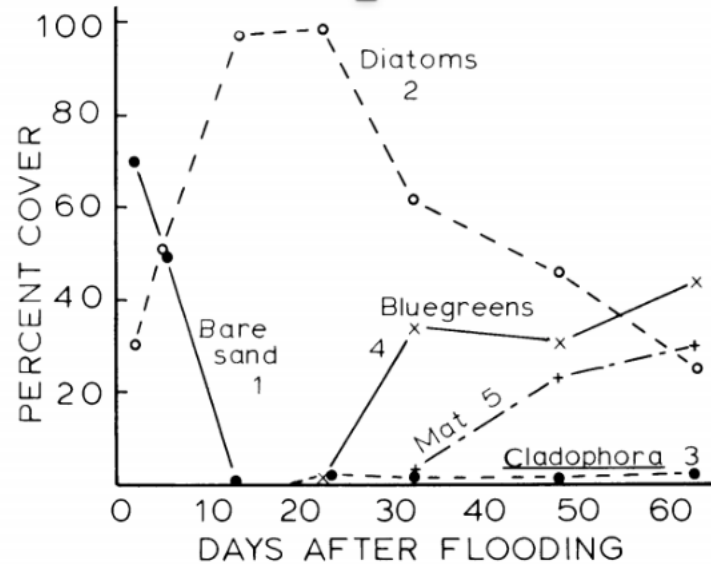


Fig 6. This graph (Figure 3 from Fisher et al. 1982. represents a potential way to graph cell counts when data collection is complete. The x-axis would be changed to days after peak flow. This plot may give clarity on how the community composition of algae within streams changes over the period of time from peak to base flow.

These results are a start for answering my original question about if variance in peak flow timing would affect algal biomass accrual, chlorophyll-a, %P, and cell counts of different algal taxa. While conclusions cannot be made about how shifts in peak flow timing may impact the variables of concern, the significant differences between variables among streams suggests that there is an unknown factor impacting the %P, AFDM, chlorophyll-a, and cell counts of biofilms amongst the differing streams. However, the results thus far are supporting my hypotheses in that streams with earlier peak flows (ie Washington Gulch), whereas decreasing in AFDM and chlorophyll-a, still have higher amounts of both biomass and chlorophyll than streams with later peak flows (Poverty and Slate, Fig 2 and Fig 3). In regards to Fig 4, the negative relationship between date and %P could mean that with an earlier peak flow, %P will reach lower concentrations sooner than it might have in historic years. Although I am beginning to see relationships between peak flow and biomass, chlorophyll-a, and %P, it is a stretch to use this data to answer my original hypotheses because there is not enough data to establish a significant

relationship. Not all streams have been sampled the preferred four times yet, and it is unknown if the results after finished sampling will continue to uphold the current pattern, or if there is potential for the relationships to change.

This outcome strongly suggests that further statistical testing (e.g., a timeseries analysis) is needed when all data has been collected, particularly on variance among the different streams rather than variance among dates. Figure 6 shows a potential format that I could use to graph out my algal cell counts when data collection is complete to see if the community composition changes between peak flow and base flow, and if it is different compared to data on algal community composition from historic years.

Analysis of my *Baetis bicaudatus* samples for dry mass, body chemistry, and instar could also strengthen my interpretations of my biofilm scrub data. If the *Baetis* body chemistry is highest in %P in rivers with the highest %P biofilms, that will suggest that streams with higher %P biofilms will have better resource quality for macroinvertebrates. This claim could further be supported by the instar data; if the streams with higher %P biofilms contain *Baetis* that have reached the later development stages earlier, this would support that streams with higher %P biofilms are of better resource quality because they support faster growth and development. Conversely, if *Baetis* body chemistry is lowest in %P in rivers with the highest %P biofilms, it may suggest that *Baetis* either are not selecting for more nutrient dense algae, or that %P is not an accurate representation of resource quality for macroinvertebrates within streams.

The relationship between biofilm P content and P uptake velocity in streams (Fig. 5) suggests that algal communities with low amounts of P have a higher demand for P, while algae with higher amounts of P do not have as high of a demand. This may be because the biofilms with high amounts of P have already absorbed enough P for successful growth and reproduction, and so therefore are not absorbing as much as those biofilms with a possible deficit in P.

One interpretation of this relationship is that in streams with slower P uptake, the nutrient content of the biofilms within the stream would be higher, offering a better food source for macroinvertebrates. Conversely, streams with faster P uptake may have biofilms that are of a lower resource quality for the

macroinvertebrates within the stream. When data collection on the developmental stage, dry mass, and body composition of the collected *baetis bicaudatis* is complete, this could be tested to see if there is a relationship between P uptake and resource quality of algae for macroinvertebrates.

Conclusion:

This study, although still underway, shows a strong beginning to answering the overall question of if climate driven changes in peak flow timing of subalpine streams will impact the resource quality and community composition of algae within said streams. My results thus far are consistent with my predictions about how shifts in peak flow may impact algal resource quality. Regardless of if it is indeed shifts in peak flow that are impacting the differences between the variables of concern among streams, of interest is to find out what factor is causing the differences in streams. Identifying the variable that is impacting the differences among streams could potentially give insight into how to better protect these crucial aquatic environments through environmental policy. If the results suggest that there is a significant relationship between the variables of concern and either peak flow timing or another unknown factor, new studies could be expanded to other montane regions to compare if the streams are responding in a similar way to the said factor in order to better understand the effects of climate change in subalpine aquatic environments. It is important to consider furthering the reaches of this study to other areas not only because of the prospective impacts on the trout fishing industry across the western United States, but also to preserve these algal communities that are vital to aquatic ecosystem functioning through nutrient cycling and water purification.

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