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Environmental Controls on Didymosphenia geminata Bloom Formation

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ENVIRONMENTAL CONTROLS ON *DIDYMOSPHENIA GEMINATA* BLOOM FORMATION

by

Lindsay Capito

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ABSTRACT

Environmental Controls on *Didymosphenia geminata*

Bloom Formation

by

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Climate change induced loss of glacial extent and shifts to earlier snowmelt timing will have profound implications for a suite of hydrologic and biogeochemical riverine processes. We evaluated how temporal shifts in stream habitats affect phenological characteristics in *D. geminata* and initiate bloom formation. We used three complementary approaches, experimental studies, high-frequency observations, and a space-for-time substitution. We used experimental flumes to mimic the effects of glacier recession on stream habitats, especially the loss of glacial flour and the increase in dissolved organic carbon and/or earlier snowmelt. In the flume studies, the high photosynthetically active radiation (PAR) and limited ultraviolet radiation (UVR) treatment had the highest *D. geminata* biomass, followed by the full spectrum-high light treatment, and the most shaded treatment had the least biomass ($p<0.001$). We sampled the snowfed Logan River, in Utah weekly from May to December of 2019 and found that a *D. geminata* blooms were triggered under similar circumstances, specifically at the juncture of low turbidity and low phosphorous concentrations at a time when the

photoperiod was near its maximum. Finally, we employed a space-for-time substitution study by sampling 53 streams across a gradient in glacial cover in British Columbia and Alberta, Canada to evaluate the relations between physical and chemical parameters and *D. geminata* bloom formation. *D. geminata* blooms were found in streams where conditions favored both nutrient limitation and high light, which were more prevalent in streams with little to no glacial cover ($p<0.10$). Our combined results show that environmental conditions linked to glacial recession and earlier snowmelt are driving the formation of *D. geminata* blooms in British Columbia, Alberta, and the Logan River, respectively, due to the mismatch in timing of high light and low nutrient conditions. These observations have wide reaching implications for climate adaptation and mitigation in aquatic systems. As climate change alters the timing of biologically important environmental cues, biota at the base of the food web are responding in unforeseen ways and have the potential to significantly alter stream ecosystems. Understanding how biota is responding to climate change and the underlying mechanisms driving phenological mismatch is critical for agencies and land-managers to take effective actions to combat the ecological implications of climate change.

(63 pages)

PUBLIC ABSTRACT

Environmental Controls on *Didymosphenia geminata*

Bloom Formation

Lindsay Capito

Climate change is causing rapid glacial recession and earlier snowmelt, which alter the physical and chemical properties of rivers. As a result, organisms at the base of the food web are responding in unforeseen ways. We use the nuisance algae *D. geminata* (Didymo) as a case study for how climate induced shifts in the timing of glacial and snowmelt runoff are affecting river ecosystems. We evaluated how shifts in the timing of nutrient concentrations and light availability affect nuisance blooms of Didymo in three complementary ways. These are, field studies across streams in various stages of glacial recession, weekly measurements at one section of river, and experimental studies. We used a range of streams in different stages of glacial recession as a proxy for decadal scale climate change to evaluate the relationship between chemical and physical stream characteristics and nuisance blooms. Blooms were found in streams with low nutrient and high light, and with less glacial cover. We sampled the snowfed Logan River, in Utah weekly from May to December of 2019 and found that blooms were triggered under similar circumstances, specifically at the juncture of clear water and low phosphorous concentrations at a time of year when day length was near its maximum. Finally, we used experimental flumes to investigate only the role of light on blooms. We found the highest algal growth with the high light treatments. Our results show that environmental conditions linked to glacial recession and earlier snowmelt are driving the formation of

Didymo blooms through shifts in the timing of high nutrient, turbid water, occurring earlier in the year when light availability is lower. Didymo serves as a case study for how climate change alters the timing of important environmental conditions resulting in unforeseen effects to stream ecosystems. This study exemplifies the need to study how altered glacial and snowmelt timing will change stream ecosystems.

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INTRODUCTION

Climate change is rapidly altering stream and river ecosystems through changes in temperature, flow magnitude, and discharge timing (McGregor and others 1995; Poff 2002; Stahl and Moore 2006; Rahel and Olden 2008; Brahney and others 2017b; Milner and others 2017). These hydrologic changes can alter nutrient fluxes and lead to shifts in aquatic species composition and density (Hawkings and others 2016; Milner and others 2017). Predicting how aquatic communities will respond to changes in the physical and chemical characteristics of watercourses is difficult because of competing environmental controls on production and species ranges (Rahel and Olden 2008). For example, increases in temperature can lead to greater production of native species, a reduction in native habitat, and expansion of habitat for non-native species (Poff 2002; Rahel and Olden 2008). Further, native species may have detrimental effects on their surroundings by expanding their habitat or distribution in deleterious ways (Rahel and others 2008).

In recent decades, *Didymosphenia geminata* has become notorious for producing substantial amounts of unsightly stalk that can overtake streambeds, altering benthic community structure and function (Gillis and Chalifour 2010; Anderson and others 2014). *D. geminata* was initially believed to be invasive given that reports of overgrowths (hereafter "blooms") are constrained primarily to the last 30 years (Bhatt and others 2008; Kirkwood and others 2008; Blanco and Ector 2009; Bergey and others 2010; Kilroy and Unwin 2011; Taylor and Bothwell 2014). Though blooms appear to be a new occurrence, evidence from historical and fossil records indicates that *D. geminata* has been present in North America for hundreds and in some regions thousands of years (Taylor and Bothwell 2014; Spaulding and others 2020). *D. geminata* cells do not always

produce elongated stalks resulting in blooms, rather *D. geminata* is often present in low densities and therefore can remain undetected in the absence of microscopic analysis.

Because blooms occur in both native and non-native habitat ranges, it is hypothesized that the shift from *D. geminata* being present but unseen to blooming depends on environmental conditions (Bothwell and Kilroy 2011; Taylor and Bothwell 2014). Many studies have found significant relationships between various environmental conditions and *D. geminata* blooms including low phosphorous (P) (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011, 2012; Bothwell and others 2014; James and others 2015), high organic P (Ellwood and Whitton 2007; Bray and others 2017), nitrogen (N) limitation (Hix and Murdock 2019), high iron concentrations (Sundareshwar and others 2011), stable flow regimes (Miller and others 2009; Cullis 2011), low stream temperatures (Kumar and others 2009), high stream temperatures (Bothwell and Kilroy 2011) ion concentrations and bedrock geology (Rost and others 2011), and high light (Kilroy and Bothwell 2011; James and others 2014). Although these significant relationships to environmental conditions have been observed, a unifying explanation for excessive stalk production and a coherent driver for the recent occurrence of blooms are lacking. To uncover the mechanism underlying the excessive production of carbohydrate stalk it is crucial to understand the controls on primary production in aquatic systems.

The biotic structure and function of aquatic ecosystems are fundamentally controlled by the relative abundance of nutrients and light (Sterner and others 1997). Sterner et al. proposed that under phosphorus limited and high light conditions, the base of the food web (i.e. primary producers) would be carbon rich and phosphorus poor and have high nutrient use efficiency (NUE). NUE is a measurement of how efficiently

organisms use nutrients to produce biomass (i.e., carbon) (Sterner and others 1997). However, high ultraviolet radiation (UVR) can inhibit production likely due to the damage of protein molecules important in the PSII phase of photosynthesis (Schofield and others 1995; Herrmann and others 1997; Krause and others 1999; Hessen and others 2008). Many studies have linked *D. geminata* blooms to conditions of low phosphorus and high light (Bothwell and Kilroy 2011; Kilroy and Bothwell 2011, 2014), where low nutrient concentrations limit cellular division, but ample light allows for photosynthesis and carbon production as carbohydrate stalk, termed 'photosynthetic overflow' (Kilroy and Bothwell 2011). Although these observations support the hypothesis that *D. geminata* stalk production is likely the result of photosynthetic overflow, the explanation for a likely climate related link to *D. geminata* overgrowths is unclear.

Climate change can alter fundamental environmental conditions such as streamflow and temperature. In nival and glacial systems these shifts can be profound and include the timing of snowmelt runoff and nutrient pulses (McGregor et al. 1995, Hood and Berner 2009, Milner et al. 2017, Brahney et al. 2020 in review). Further, in-stream light characteristics are influenced by properties of the water such as dissolved and suspended solids tied to runoff (Lewis and Grant 1979), and external conditions related to photoperiod which are not subject to climate change.

Climate induced changes to co-varying hydrologic, chemical, and biological conditions (Preston and others 2016) can result in substantial shifts in species phenology which can uncouple trophic interactions (Winder and Schindler 2004; Donnelly and others 2011). Species specific responses to altered seasonality are highly variable and therefore hard to predict, however, many case studies have emerged as climate change

unfolds and may serve as a template for understanding future phenological shifts (Stenseth and Mysterud 2002; Donnelly and others 2011). For example, increased temperature in some lakes has caused thermal stratification to occur earlier in the season resulting in an earlier diatom bloom (Winder and Schindler 2004). These changes resulted in uncoupled trophic interactions due to the lack of a corresponding shift in zooplankton emergence (Winder and Schindler 2004). Further investigation into phenological mismatches is necessary to predict and understand how climate change may impact ecosystems in non-linear ways (Donnelly and others 2011).

A recent hypothesis links *D. geminata* blooms to temporal shifts driven by changes in glacial contributions to streamflow and earlier snowmelt (Brahney et al. 2020 in review), however, the hypothesis has not yet been rigorously tested. Glaciers contribute inorganic phosphorus, typically from the apatite-rich bedrock physically weathered by glacier movement and freeze-thaw cycles or from dust deposited on the glacier (Hodson and others 2004; Hood and Scott 2008). Therefore, reduced glacial meltwater input may decrease phosphorus concentrations and contribute to bloom formation at a critical time in the year. Additionally, the weathered rock particles from glacial movement create turbidity as fine sediment and organic material. Turbidity reduces photosynthetically active radiation (PAR), which may encourage bloom formation (Kawecka and Sanecki 2003; Kirkwood and others 2009) when paired with low nutrient conditions (Kilroy and Bothwell 2011). Moreover, the loss of glacier melt leads to earlier flow and turbidity peaks resulting in lower flows, higher light, and higher temperatures later in the summer (Brahney and others 2017b), conditions linked to *D. geminata* stalk production (Kilroy and Bothwell 2011; Cullis and others 2012; Hix and

Murdock 2019). Brahney et al. (2020 in review) have identified declining glacial melt as a potential driver for bloom formation, but observations were limited spatially and lacked quantitative sampling methods for *D. geminata* specifically. Their study also lacked experimental studies under controlled conditions.

Global warming induced changes in snowmelt timing may have similar effects on nival streams as glacial recession has on glacierized systems. In nival systems, peak streamflow occurs during spring runoff, when sediments and organic material enter the stream and reduce light penetration. If runoff occurs earlier in the season, the pulse of turbid water comes and goes before the prime growing season in late June when the photoperiod is the longest in northern latitudes, resulting in high light, nutrient limited conditions. These conditions are becoming increasingly widespread in the mountainous regions of the northwestern U.S. and Canada primarily due to increased temperatures associated with climate change (Regonda and others 2005; Brahney and others 2017b). Brahney et al. (2017b) found that climate change has altered both the volume and timing of streamflow in the Canadian Columbian Basin, where peak flows are reduced in magnitude and occurring earlier in the year (Figure 1). These observations indicate that the conditions necessary for photosynthetic overflow are becoming more prevalent in many of the regions where *D. geminata* blooms are reported.

We hypothesize that *D. geminata* blooms in native environments are driven by a phenological mismatch wherein climatic warming has altered shifted the delivery of nutrient-rich turbid water to an earlier period in the year that does not overlap with the peak photoperiod and encourages *D. geminata* bloom formation. To test this hypothesis, we took a three-pronged approach examining the role of light and nutrients on *D.*

geminata growth via experimental analysis and then across space and time. First, we experimentally tested the relationship between light attenuation, including the full visible spectrum and UVR, and *D. geminata* growth to isolate the role of solar energy on *D. geminata* bloom formation. Second, we conducted a high-frequency sampling study to determine the environmental conditions that initiate *D. geminata* blooms in the Logan River. Finally, we conducted a space-for-time study to relate chemical and physical stream conditions and *D. geminata* presence to gradients in catchment glacierization.

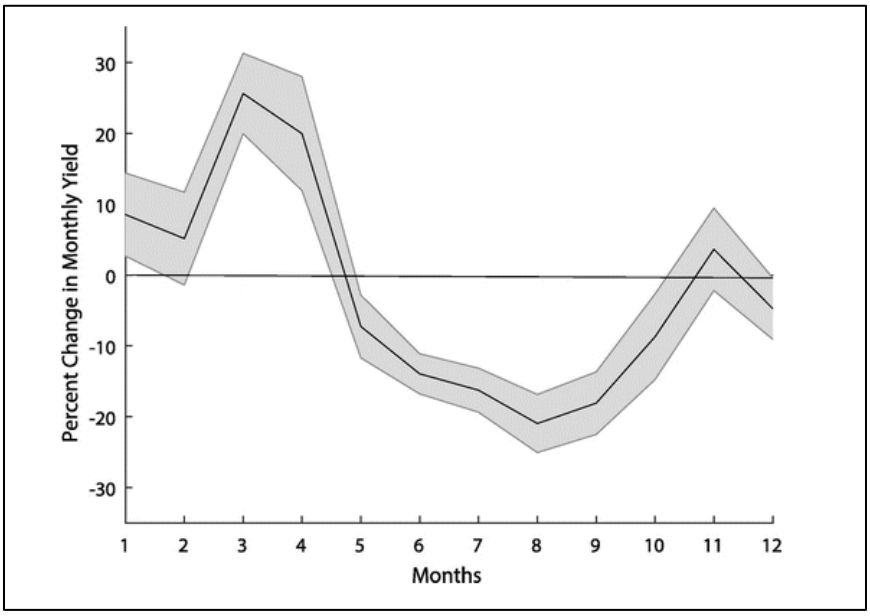

Figure 1. Percent change in streamflow for snowmelt dominated streams in the Canadian Columbia Basin from Brahney et al 2017.

METHODS

We conducted three studies because each addresses a different aspect of our hypothesis. To mimic the conditions created by earlier snowmelt timing and reduced glacial input in a controlled environment we conducted a flume experiment wherein we isolated the role of light on *D. geminata* stalk production. We conducted high-frequency sampling at one site to provide insight into the conditions preceding, during, and following the shift from *D. geminata* presence to *D. geminata* overgrowth (i.e., bloom). Finally, the space-for-time study allowed us to observe the role of glacial recession over decadal timescales on both stream characteristics and *D. geminata* presence. By pairing these three studies we were able to overcome the limitations posed by each individually and comprehensively address our hypothesis.

Sample collection and processing

We used similar collection and processing methods for each study, therefore, the common methods are described in this section. Where methods differ for a study, the differences will be explained in that study's sampling section. Data analysis will be described for each study specifically.

Water chemistry

Turbidity, temperature, and, specific conductance (SPC) were measured on-site using a Hach 2100Q handheld turbidity meter and YSI probe, respectively. Light attenuation was measured using a LI-COR meter by taking readings at the surface and throughout the water column in 5cm increments to determine extinction coefficients (k). We collected dissolved and total nutrient samples to measure soluble reactive phosphorus

(SRP), total phosphorus (TP), and nitrate (NO_3^-). Water samples were collected in acid-washed and triple DI rinsed Nalgene bottles by placing the bottle just below the surface of the water upstream of the collector at a 45-degree angle. We rinsed each bottle three times with stream water before collecting the sample. Samples for SRP and NO_3^- analysis were filtered on-site through a 0.45 μm nylon filter and stored on ice for transport to the USU Environmental Biogeochemistry and Paleolimnology Laboratory (EBPL) where they were refrigerated until analysis. SRP and TP samples were measured using EPA method 365.4 on a Lachat Quickchem 8500 Flow Injection Analyzer in the USU EBPL. NO_3^- was analyzed by the Utah State University Aquatic Biogeochemistry Laboratory (EPA method 353.2).

Periphyton

We collected periphyton scrubbings to analyze for chlorophyll-a, ash-free dry mass (AFDM), autotrophic index values (AI), algal C:P ratios, and *D. geminata* cell identification. To collect periphyton, we used a 50 ml sample cup cut in half as a template to sample a known area of a cobble sized rock. The visible periphyton from within the cup was removed using a metal scraper and placed in a centrifuge tube. The area was then scrubbed with a toothbrush and rinsed with stream water. The slurry was aspirated and transferred to the centrifuge tube. We repeated this process for each rock a total of four times per site.

Processing

We analyzed periphyton samples for chlorophyll-a concentrations by fluorometry on a SpectraMax M2E plate reader (Ritchie 2008) at the USU EBPL. Biomass was

measured using the loss on ignition (LOI) method (Heiri and others 2001). To prepare samples for LOI and chlorophyll-a analysis, the slurry collected from periphyton scrubbings was homogenized in a food processor and filtered through pre-combusted 0.7 µm filters to retain the biomass. To desiccate the samples, we freeze-dried the filters for 24 hours and recorded the mass. For LOI the filters were then heated in a muffle furnace at 550 °C for 4 hours to combust organic matter. The filters cooled in a desiccator to prevent water reabsorption from the air and were then re-weighed. The difference from the initial dry weight is the weight lost on ignition. The ratio of AFDM to chlorophyll-a concentrations was used to calculate an autotrophic index (AI) (Biggs and Kilroy 2000). High AI values indicate large amounts of non-photosynthetic material (Biggs and Kilroy 2000) rather than living algae. *D. geminata* blooms will have high AI values because stalks are composed of EPS (extracellular polymeric substances) and lack chlorophyll, therefore, the more stalk *D. geminata* produces the higher the AI values.

The percentage of organic carbon and nitrogen were determined using a Costech 4010 elemental analyzer in the Geology Department at USU. Samples were freeze-dried and homogenized then ~0.4mg of each were placed in Ag capsules for analysis. SRP stalk concentrations were measured by first freeze-drying and homogenizing the stalk material. A subsample of the stalk was weighed and combusted at 550°C to oxidize organic matter. The stalk was then transferred to centrifuge tubes and reweighed. We added trace element analysis grade HCL in 1:25 mass ratios for digestion. Samples were heated to expedite digestion in a hot water bath at 50°C for 2 hours. We then pipetted the supernatant and filtered through 0.45µm nylon filters. SRP was analyzed by the Aquatic Biogeochemistry Laboratory at Utah State University (EPA method 365.1).

To create microscope slides for diatom analysis, we first evaporated and freeze-dried the slurry samples. Then a subsample of the dried slurry, about 5 mg, was weighed and digested using a 30% hydrogen peroxide solution. The sample was rinsed with DI water and aspirated three times to remove residual hydrogen peroxide, waiting 24 hours between each aspiration. After rinsing, the sample was agitated and poured into Battarbee trays for even distribution across 18mm glass coverslips. The trays were left to evaporate in a fume hood until dry. Once dry, the coverslips were mounted on glass slides using Naphrax and heat. The diatoms slides were then viewed under microscopy and enumerated to determine the presence and proportion of *D. geminata*. We scanned each microscope slide for *D. geminata* and counted a minimum of 400 diatoms per site.

Flume experiments

We conducted flume experiments to investigate the role of changing light conditions associated with phenological mismatch on *D. geminata* growth. To examine the role of light in our experiments we attenuated light intensity with several types of neutral density shades and one that selectively filtered out UVR. The treatments included weed barrier cloth (WB, 93% reduction), window screening (SS, 72% reduction), Alcar™ (M1, 8% reduction), Courtgurard™ (M2, 22% reduction + UVR blocking), and a control (C, 0% reduction) with no cover (Table 1). The WB and SS treatments represent light reduction from varying degrees of turbid snowmelt and glacial runoff. The control has no cover and because the flumes are very shallow, this represents high elevation catchments with extreme UVR exposure, whereas the M1 treatment more accurately mimics the clear water phase of phenological mismatch in high elevation catchments with high UVR exposure due to sparse vegetation. Finally, M2 mimics the clear water phase in

forested catchments where dissolved organic carbon (DOC) selectively attenuates UVR. We used the UVR reducing film to accurately represent the catchments that have lost glacial cover and are increasingly forested as well as snowmelt driven systems that are frequently forested, as these are the types of habitat where *D. geminata* blooms. Each treatment was replicated twice per flume in a randomized design in a total of three flumes (Figure 2). By using treatments that reduce light evenly at several intensities and manipulating the attenuation of UVR, we observed in detail the role of light and changing habitat conditions due to glacier recession and earlier snowmelt on *D. geminata* growth.

Table 1. Shade treatments and their respective reduction in overall light.

Treatment	Reduction in light %
Control-C	0
Apgar-M1	8
Courtguard-M2	22
Shade screen-SS	72
Weed barrier-WB	93

The flumes were constructed from a 20cm (8-inch) diameter PVC pipe that was cut in half and assembled so that water would flow through the flumes in a horseshoe pattern, then empty into a reservoir before being pumped back into the PVC trough (Figure 2). Each reservoir held 132 L of water from the nearby Logan River and was covered with a shade screen to prevent evaporation and reduce thermal fluctuations. Each reservoir had a Hoboware V2 Pro Temperature Logger which recorded water temperature every 15 minutes for the duration of the experiment. We added Alum to the reservoirs (10g $AlSO_4$ per 132L) to ensure consistently low phosphorus concentrations throughout the experiment. SRP concentrations at the time of sampling were 1.87µg/L and 2.19µg/L

for flumes 1 and 3, respectively. NO_3^- concentrations were 9.2µg/L and 11.8µg/L and molar NO3-:SRP ratios were 10.8 and 11.9, respectively. Flumes were placed on the roof of the Utah Water Research Laboratory (UWRL) at Utah State University for optimal sun exposure. We used 2.5cm (~1 inch) ceramic tiles as a substrate in the flumes. The tiles were conditioned to allow a biofilm to form by anchoring them in the Logan River for 3 weeks before the experiment. The flumes were also conditioned with river water at the same time. After the conditioning phase, we colonized ceramic tiles with *D. geminata* cells. To isolate live *D. geminata* cells, we blended and filtered *D. geminata* stalk collected from nearby rivers. The blending process separates live *D. geminata* cells from excess stalk material. We caped the ends of the flumes and poured the filtered slurry material over the ceramic tiles, then the slurry sat for 1-2 days allowing live *D. geminata* cells to attach to the tiles. After 1-2 days the slurry was flushed from the flumes and the flumes were filled with low-nutrient Logan River water. Once the colonization period was complete, we placed the shade treatments over the flumes.

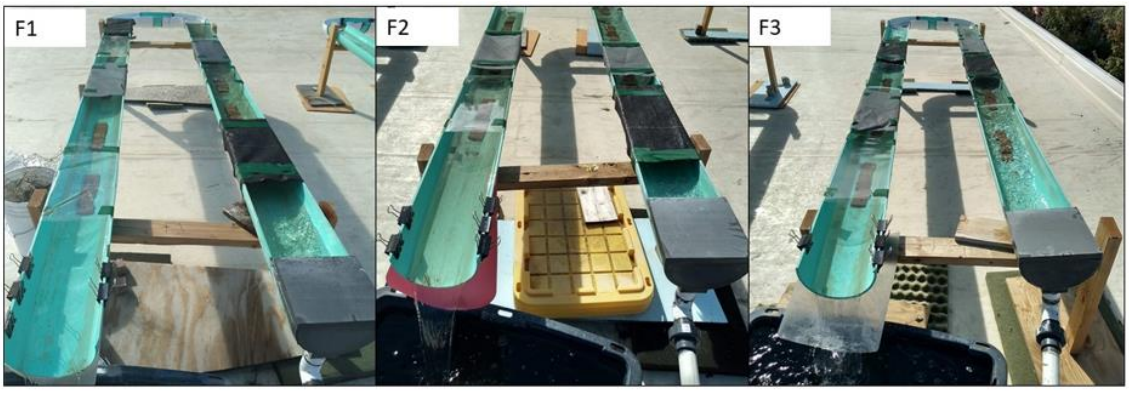

Figure 2. Flumes with shade treatments in a randomized design.

We visited the flumes daily to replace water lost from evaporation and monitor algal growth. We periodically viewed scrapings of algae under microscopy to determine the species and confirm that *D. geminata* cells were alive. The experiments began in July of 2019 and ran until the end of October 2019. We removed the tiles on September 6th and collected the periphyton. *Cymbella* species were the dominant taxa at this sampling and *D. geminata* was not present. We re-started the experiment with newly collected *D. geminata* cells on newly colonized tiles on September 24th. On October 23rd we identified *D. geminata* as being present and producing stalk. Due to the onset of winter and ice buildup in the flumes, we removed the tiles, collected the periphyton for analysis, and concluding the experiment on October 26th.

Sampling

To remove periphyton from the tiles we used the method outlined in the sample collection and processing section except we scrubbed the whole tile, not a subsection. Stalk production was measured by the loss on ignition method, chlorophyll-a, and C:P ratios were measured as described in the sample collection and processing section (Biggs and Kilroy 2000). Nutrient ratios were monitored and recorded using the methods described in the field sampling section.

Data analysis

We used one-way-ANOVA to analyze differences in AFDM, chlorophyll-a, AI, and C:P ratios between treatments. To determine which treatments were significantly different we applied a post-hoc Tukey test.

High-frequency sampling

Weekly observations at one location on the Logan River allowed us to record the physical and chemical conditions preceding, during, and after a *D. geminata* bloom event. We took weekly measurements from one 10-meter stretch of river with a history of *D. geminata* blooms from May to December of 2019. We measured SRP, TP, NO_3^-, chlorophyll-a, and biomass using the sampling methods listed in the sample collection and processing section.

Study site

We sampled the Logan River, a tributary to the Bear River, located in Cache Valley Utah near Utah State University. The Logan River is fed by snowmelt from the Bear River Range and experiences an average peak runoff of 27.89 cubic meters per second (cms) between May 18th and June 10th (U.S. Geological Survey 2019). 2019 was an average year with a peak of 27.29 cms on June 8th. The study site is just downstream a reservoir and a Logan River Observatory monitoring location that continually records discharge, temperature, turbidity, and dissolved oxygen (DO). We selected a ~10-meter reach in full sun and which included an outside bend (Figure 3).

Sampling

We visited the site weekly from May through December of 2019 at approximately mid-day. During each visit, we took pictures of the reach and periphyton coverage. SRP, TP, NO_3^-, and periphyton samples were collected and processed using the methods described in the sample collection and processing section. Quality controlled discharge,

Figure 3. High-frequency monitoring study site located in Logan UT.

dissolved oxygen (DO), SPC, and turbidity measurements were downloaded from the Hydroshare database (Logan River Observatory 2020).

Data analysis

We regressed AFDM, algal C:P ratios, and AI values against all environmental variables then used stepwise AICc with forward selection to identify key variables for each response metric. Additionally, we used changepoint analysis (package "Changepoint" in R) to identify key shifts in environmental variables throughout the year. We then compared the changepoints to the onset of the *D. geminata* bloom.

Space-for-time study

We conducted a space-for-time substitution study across southeastern British Columbia and western Alberta (Figure 4) where we both categorized streams based on glacial cover and tested for a linear relationship along a gradient in glacial cover. Space-

for-time substitutions have been used extensively in ecology when long term studies are not feasible (Blois and others 2013). Space-for-time substitution is an appropriate method to simulate glacial recession and examine the effect of reduced glacial input on *D. geminata* because it would be otherwise impossible to replicate exactly the hydrogeologic and biogeochemical properties of glacial systems as they recede over decadal timescales. Glacial systems lend themselves well to space-for-time studies and others have successfully used this type of analysis to infer climate-induced changes in glacial streams (Hood and Berner 2009; Wilhelm and others 2013; Zimmer and others 2018).

To define the gradient of glacierization we categorized streams as “glacierized”, meaning catchments with large active glaciers, “transitional”, that have residual ice, and

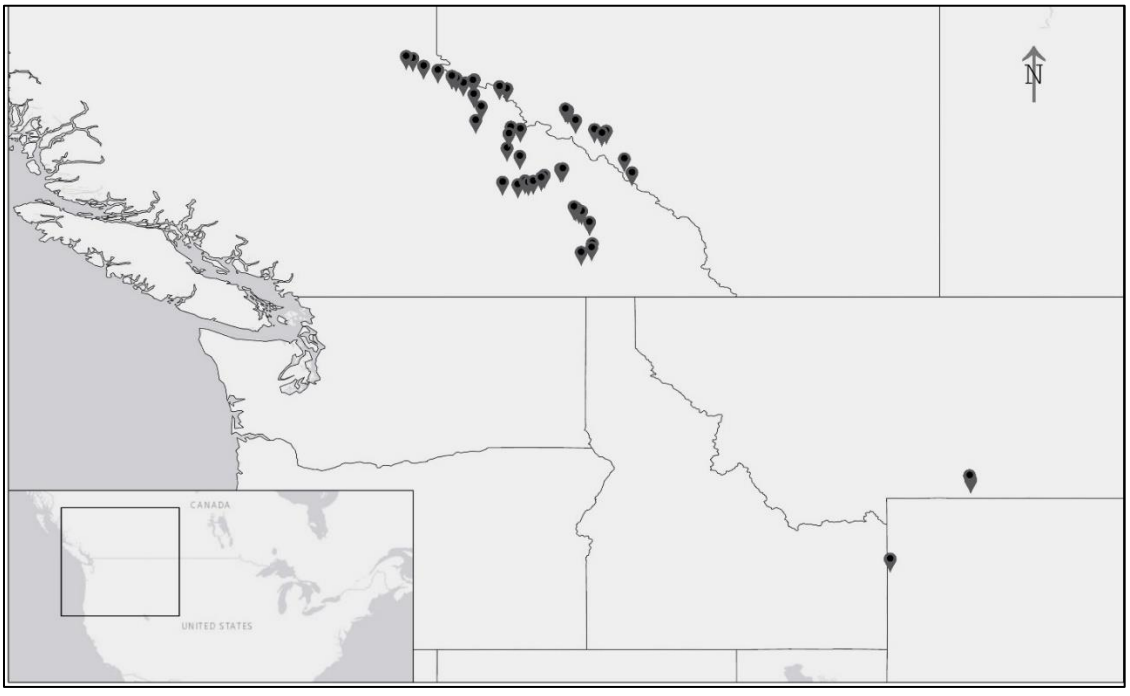

Figure 4. Space-for-time sampling locations. Southernmost sites in WY and MT, USA. Northernmost sites in Southeastern BC and Western Alberta, Canada.

"snowfed", including either historically snowfed streams or those that have lost glaciers. The threshold for "transitional" was 2-5% glacial cover, based on observed hydrologic impairment of British Columbia streams from (Brahney et al. 2020 in review). We used the 2-5 % cutoff in the initial designation between "glacierized" and "transitional", however, we also analyzed our data to determine ecological thresholds.

We used the Global Land Ice Measurements from Space (GLIMS) (https://www.glims.org/) Randolph Glacial Inventory 6.0 (RGI 6.0) dataset to determine glacial area and delineated watersheds upstream of the point of sampling using ArcPro GIS software. Watershed delineation was performed using methods described by Chinnayakanahalli and others (2006). First, we obtained 90m digital elevation models (DEMs) covering the sampling area from https://www.altalis.com/. The DEMs "filled" using the TauDEM toolbox. Filling depressions in the DEM corrects for both natural depressions and artifacts due to modeling and allows for seamless flow from one cell to the next. Flow directions are then calculated using the TauDEM toolbox via the D8 flow direction method. This step produces a flow direction raster from which flow accumulation can be derived. The final step is to use the flow accumulation grid to backtrack upstream and delineate the watershed. These processes can be automated in TauDEM and produce multiple watershed polygons. Each watershed was assessed visually for accuracy after processing. The watershed polygons were then intersected with the GIS layer to determine the percent of the watershed covered by glaciers.

Sampling

We sampled 53 streams across the glacierization gradient for water column nutrients, light, temperature, specific conductance (SPC), and turbidity. Turbidity,

temperature, and SPC were measured on-site using a Hach 2100Q handheld turbidity meter and YSI probe, respectively. Light attenuation was measured using a LI-COR meter by taking readings at the surface and throughout the water column to determine extinction coefficients (k) for each site. We obtained the LI-COR meter just before the second field season; therefore, all k values are for 2019 (31 sites).

At each site, we collected dissolved and total nutrient samples to measure soluble reactive phosphorus (SRP), total phosphorus (TP), and nitrate (NO_3^-). Water samples were collected and analyzed using the methods described in the sample collection and processing section.

Each site was characterized as "Bloom" (B), "No bloom or cells" (N), or "Cells only" (C). The "Bloom" classification was determined on site whereas the "Cells only" and "No Bloom or cells" categories were determined later through microscopy. We collected periphyton scrubbings to analyze for chlorophyll-a, AFDM, autotrophic index values, algal C:P ratios, and *D. geminata* cell identification using the methods described in the sample collection and processing section. Whitton et al. (2009) caution that if *D. geminata* is localized to a site, standard periphyton collection procedures may fail to capture the presence of cells. To remedy this, we performed a visual assessment of approximately 50 meters of stream to look for macroscopic colonies and determine the percent of benthos covered with algae using a constructed bathyscope calibrated with a dot grid. Where macroscopic colonies were found, 4 cobble sized rocks with attached colonies were selected for collection. We did not sample randomly due to the heterogeneous distribution of periphyton in a stream reach and the likelihood that random sampling will fail to accurately capture the presence of *D. geminata* (Whitton and others

2009). Instead, we used purposive sampling to select cobble with macroscopic periphyton communities. Where the reach was devoid of visible periphyton, we sampled randomly. To sample randomly we walked upstream in a zig-zag pattern taking one step then reaching into the stream to retrieve the first rock touched. This process was repeated four times per site.

We analyzed periphyton samples for chlorophyll-a concentrations and biomass using the fluorometry and loss on ignition (LOI) methods respectively (Heiri and others 2001) described in the sample collection and processing section. The percentage of organic carbon and nitrogen were determined using the methods described in the sample collection and processing section. Finally, we created microscope slides for diatom analysis using the methods described in the sample collection and processing section.

Data analysis

To test if a combination of low nutrient and high light conditions co-occur with *D. geminata* blooms we used both binomial and multinomial generalized linear models (GLM's). We took two approaches to test differences between the sites with blooms and sites with cells but not blooms and additionally compared sites with blooms to all sites without blooms regardless of the presence of cells. We created binomial logistic regression models with bloom vs no bloom as the response variable for sites with *D. geminata* cells, meaning we excluded sites without blooms or cells, and again for all sites regardless of the presence of cells. We then created multinomial logistic regression models with each category as the multinomial response variable, using "no cells no bloom" as the reference condition. Predictor variables were assessed for collinearity by creating a correlation matrix (Figures A-1 and A-2 in appendix) and removing models

with related variables. We used Akaike's Information Criterion for small sample sizes (AICc) and Bayesian Information Criterion (BIC) for model selection. We also compared sites with *D. geminata* blooms to sites without blooms (all no bloom sites regardless of whether cells were present) using Welch's two-sample t-test to see if average conditions differed between bloom and no bloom sites. Additionally, we compared sites with blooms, cells only, and no blooms or cells with one-way-ANOVA using all environmental variables independently as predictors.

All statistical calculations were performed in R (3.5.2) (R Core Team 2018). After AICc and BIC selection, top models were assessed for multicollinearity using a variance inflation factor test (VIF). Significance is indicated as 1) $^{+}$marginally significant $p<.10$, *significant $p<0.05$, and ***highly significant $p<0.01$. McFadden R^2 values were calculated for models selected by AICc.

RESULTS

Flume experiments

During the experiment we lost water from Flume 2, causing the tiles to become dry, therefore we have disregarded Flume 2 data.

July-September

Water temperatures ranged from a minimum of 7.6 ˚C to a maximum of 44.5˚C with an average of 22.4 ˚C (Figure A-3 in appendix). All flumes were within 1˚C for average, maximum, and minimum water temperatures. We examined periphyton from the tiles under microscopy and determined that >90% of biomass was Cymbella stalk and that D. geminata was not present. We observed significant differences in biomass between the M1 treatment and the UV blocking treatment (M2) ($p<0.04$) and between M2 and the weed barrier (WB) ($p<0.005$) (Figure 5). There were no significant differences in chlorophyll-a concentrations or AI values between the treatments.

September-October

Water temperatures ranged from a minimum of 0˚C to a maximum of 28.6˚C with an average of 9.2˚C during the final experiment from September 24th to October 26th, 2019 (Figure A-4 in appendix). All flumes were within 1˚C for average, maximum, and minimum water temperatures. We examined the periphyton under microscopy and found that *D. geminata* was present and producing stalk which was >90% of the biomass.

We observed significant differences in biomass between the UV blocking treatment (M2) and the control (C) ($p<0.01$), between M2 and window screening (SS) ($p<0.01$), between M2 and weed barrier (WB) ($p<0.001$), and the difference between M2

and M1 was approaching marginal significance (p=0.10) (Figure 6). The UV blocking treatment (M2) had the most biomass and the weed barrier (WB) had the least (Figure 6). There were no significant differences in chlorophyll-a concentrations or AI values between the treatments.

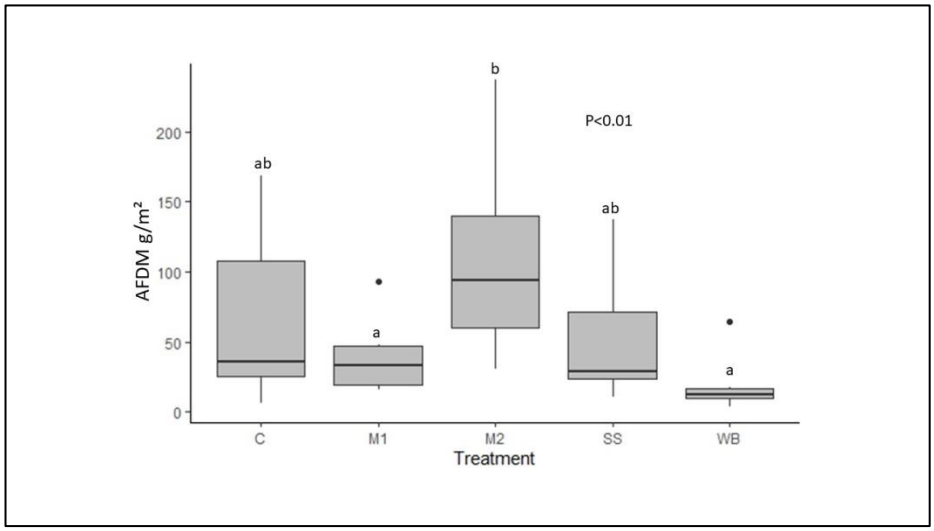

Figure 1. ANOVA results for AFDM between treatments. Post hoc Tukey test shows significant differences between M1 and M2 and M2 and WB.

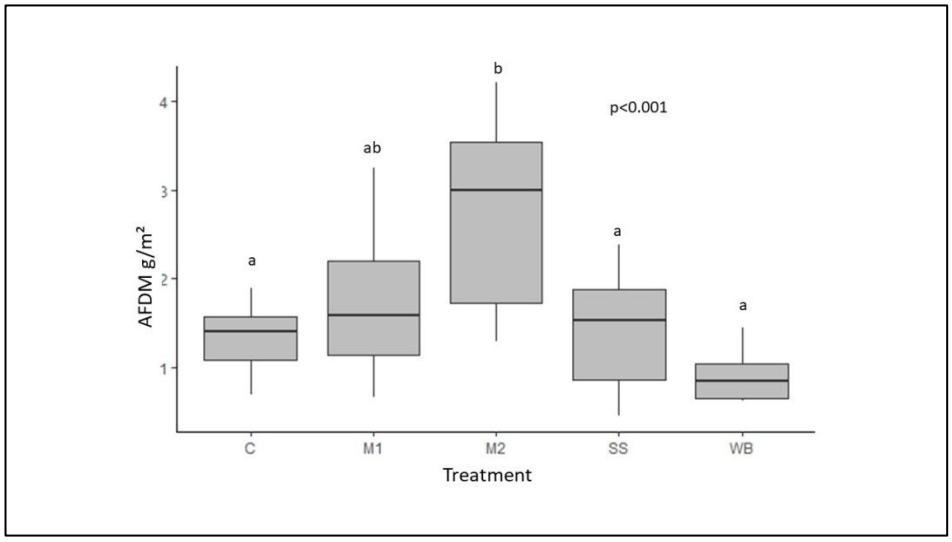

Figure 2. ANOVA results for AFDM between treatments. Post hoc Tukey results show significant differences between M2 and all treatments except M1.

High-frequency sampling

In 2019, the Logan River reached a maximum instantaneous discharge of ~27 cms (964 cfs) on June 8th as measured at the USGS gage 10109000 upstream of the study site (U.S. Geological Survey 2019) (Figure A-5 in appendix). Macroscopic colonies of *D. geminata* appeared in the study reach beginning in late July. At that time turbidity, flow, and SRP were decreasing as NO^{3-}: SRP ratio, AFDM, and AI values were increasing (Figure 7). The peak photoperiod in Logan, Utah occurs on approximately June 20th but the photoperiod exceeded 14 hours until August 12th and overlaps with the decline of turbidity, flow, and SRP (Figure A-6 appendix). We assume a lag between the initiation of photosynthetic overflow and *D. geminata* stalks becoming visible, but the duration of the delay is uncertain. We used changepoint analysis to estimate the onset of photosynthetic overflow and the analysis identified shifts in turbidity and SRP four and two weeks before the detection of the bloom, respectively (Figure A-8 in appendix). AFDM and algal C:P ratios were significantly negatively related to SRP, flow (Q), and turbidity during the sampling period (Table 2, Figure A-7 in appendix). AI was significantly negatively related to only SRP and flow (Figure A-7 in appendix). AICc selected turbidity as the strongest predictor for AFDM while SRP was the strongest predictor for both C:P ratios and AI values (Table 2).

Space-for-time

Of the 53 sites, 27 had *D. geminata* blooms, 26 did not have blooms. Of the 26 without blooms, 13 had cells and 13 did not have *D. geminata* cells visible under microscopy. One site was excluded from analysis due to unusually high P concentrations,

likely from agricultural activity upstream, bringing the number of sites without *D. geminata* cells to 12.

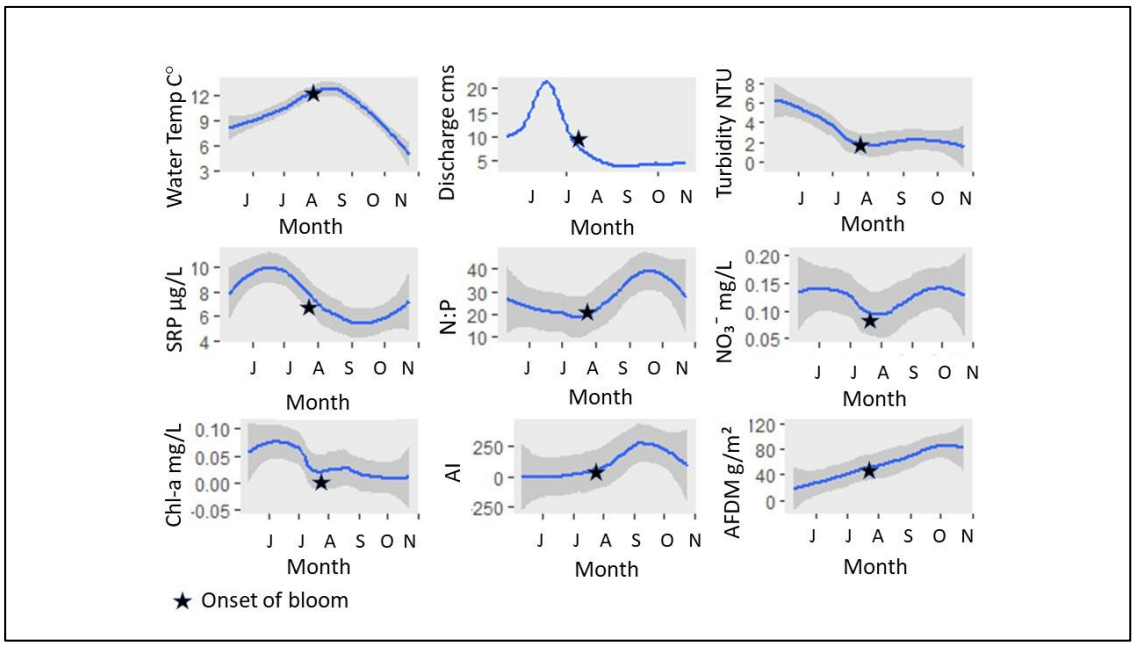

Figure 7. Time series of measured environmental variables at the high-frequency study site. Star is the onset of D. geminata bloom. Bands are standard errors.

Table 2. Pearson correlation coefficients for the space-for-time study. Significance indicators $p<0.1$ ($^{+}$) $p<0.05$(*).

	Turb	Temp	SRP	NO_3^-	N:P	TP	k	Elevation
AFDM	-0.22	0.26^{+}	0.1	-0.1	-0.13	0.03	-0.43*	- 0.32*
AI	0.20	-0.04	0.19	-0.22	-0.06	-0.17	-.07	0

All sites

Table 3 lists the best fit logistic regression models as ranked by AICc. We considered these three models as they were within 2 AICc points of one another. All models had nutrient concentrations and low k values and were significant ($p<0.05$).

Interestingly, the individual variables were not significant on their own, except for k which was marginally significant ($p<0.10$) (Table 3). Further, the models that contained only nutrient concentrations had very low McFadden R^2 values, whereas the additive models with k had much higher McFadden R^2 values (Table 3). The McFadden R^2 values increased further when elevation was added to the models (Table 3).

Table 3. Binomial logistic regression models for all sites in space-for-time study.

	All sites binomial				
Model	Variable	Coefficient	SE	AIC	R^2McFadden
NO_3^-:SRP	Intercept	-0.019	0.40	74.29	0
	NO_3^-:SRP	0.001	0.003		
SRP	Intercept	0.927	0.64	72.12	0.03
	SRP	-0.304	0.226		
NO_3^-	Intercept	-0.500	0.561	72.83	0.02
	NO_3^-	8.932	7.11		
k^+	Intercept	0.999	0.62	46.007	0.36
	k	-16.68$^+$	9.34		
Elevation	Intercept	2.458	0.97	62.04	0.03
	Elevation	-0.002	0.001		
NO_3^-: SRP+k *****	Intercept	0.377	0.72	44.77	0.41
	NO_3^-:SRP	0.017$^+$	0.01		
	k	-23.23*	10.75		
SRP+k *****	Intercept	2.641*****	1.32	44.87	0.41
	SRP	-0.512*	0.35		
	k	-20.61*	10.07		
NO_3^-+k *****	Intercept	0.049	0.89	45.37	0.4
	NO_3^-	19.43	13.29		
	k	-18.51$^+$	9.73		
NO_3^-: SRP+k +Elevation*	Intercept	3.009*	1.52	41.92	0.51
	NO_3^-:SRP	0.019$^+$	0.01		
	k	-29.51*	12.32		
	Elevation	-0.002*	0.001		

Table 3. (cont.)

SRP+k +Elevation*****	Intercept	5.559**	2.06	41.55	0.51
	SRP	-0.529	0.37		
	k	-25.73*	11.18		
	Elevation	-0.003*	0.001		
NO_3^-+k+Elevation *****	Intercept	2.593	1.57	42.75	0.49
	NO_3^-	20.46	14.07		
	k	-24.27*	11.33		
	Elevation	-0.002*	0.001		

Multinomial regression models between sites with blooms, cells only, and sites without cells or blooms, were not significant. However, we compared the same multinomial response categories using one-way-ANOVA and found significant differences in turbidity between the bloom sites and sites with cells that were not blooming, whereas sites without cells were not significantly different from bloom sites (Figure 8).

Sites with D. geminata cells

The light extinction coefficient values (k) tended to be lower in bloom sites than sites with cells but without blooms ($p<0.10$) indicating that in bloom sites light penetrated deeper into the water (Figure 9). Additionally, when comparing only sites that had D. geminata present, sites with D. geminata blooms were on average less turbid ($p<0.10$), had less glacial cover (GC)($p<0.10$), higher NO_3^- concentrations ($p<0.05$), and higher NO3-:SRP ratios ($p<0.10$) than sites without blooms (Figure 10). For sites with D. geminata cells, AICc selected three models within 2 AICc points (Table 4). Each model contained nutrient concentrations and low k values and was marginally significant ($p<0.10$). We were also interested in how the environmental conditions affect biomass,

therefore we used AFDM and AI as continuous response variables. Linear models with AFDM as the response and k ($p<0.05$, $r = -.43$), elevation ($p<0.05$, $r = -.32$ and temperature ($p<0.10$, $r = 0.26$) as predictors were significant (Figure A-12 in appendix). No linear relationships with AI were significant.

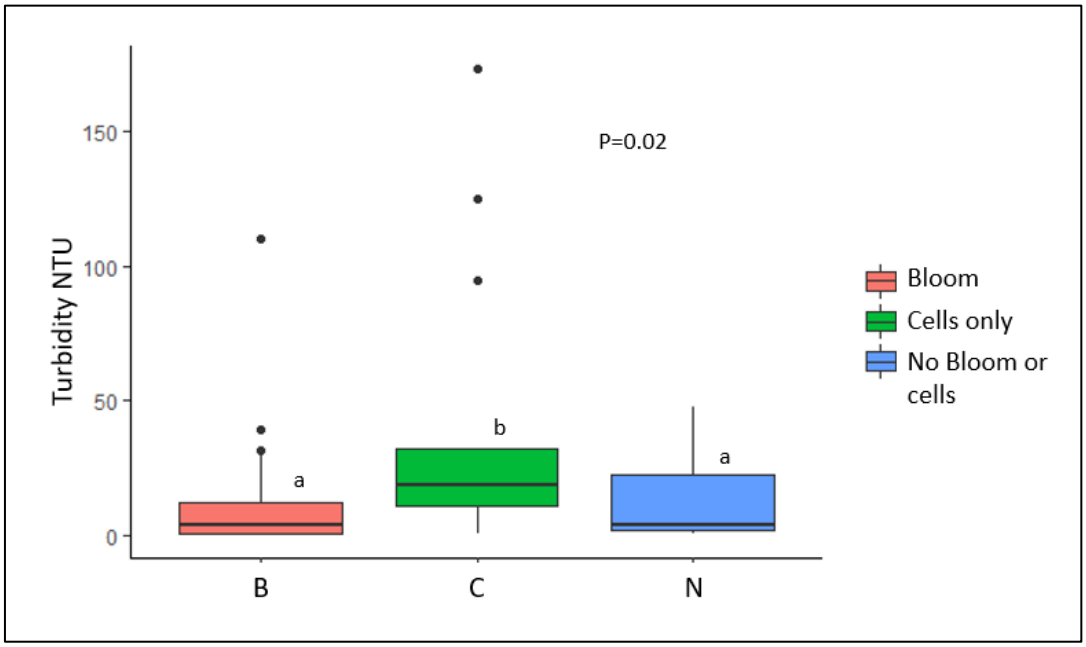

Figure 8. ANOVA results showing differences in turbidity between sites with blooms, sites with cells only, and sites without blooms or cells. Post hoc Tukey test results show significant differences between bloom sites and sites with cells and between sites with cells and sites with no cells or blooms.

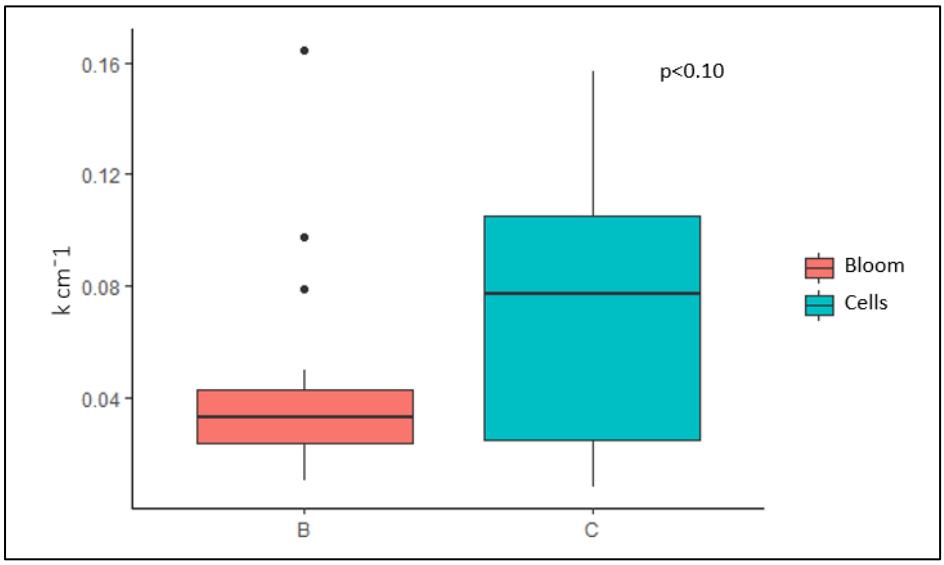

Figure 9. Extinction coefficient (k) for sites with blooms vs sites with cells that are not blooming.

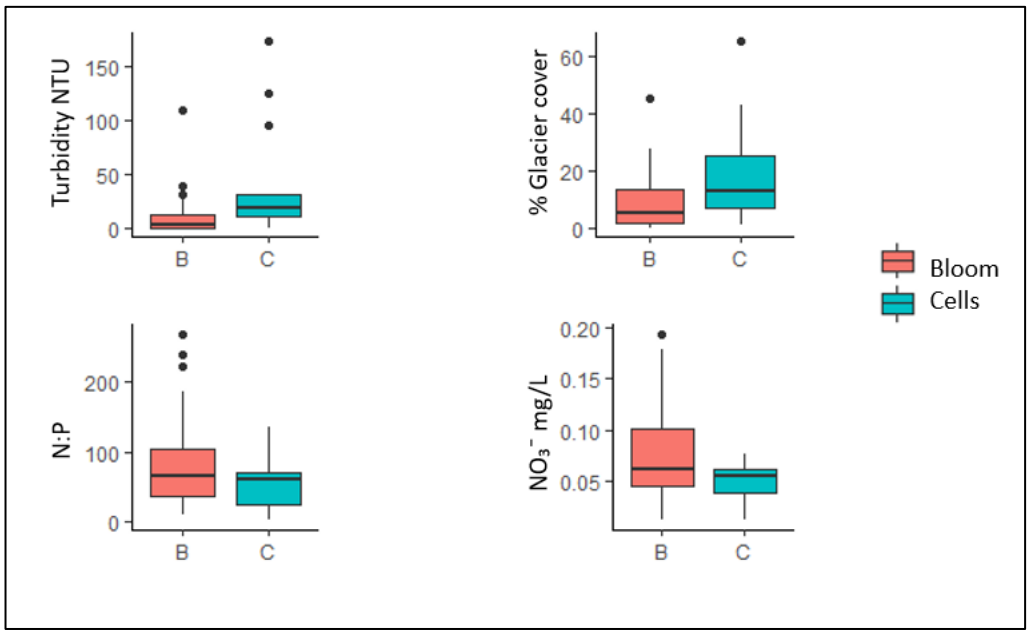

Figure 10. Differences in turbidity, N:P, glacial cover, and NO_3^- for sites with blooms vs sites with cells not blooming.

Table 4. Binomial logistic regression models for sites with D. geminata cells.

	Sites with cells binomial				
Model	Variable	Coefficient	SE	AIC	R^2McFadden
NO_3^-:SRP $^+$	Intercept	-0.031	0.58	51.72	0.03
	NO_3^-:SRP	0.011	0.007		
SRP	Intercept	1.481 *	0.70	52.54	0.03
	SRP	-0.279	0.23		
NO_3^- *	Intercept	-0.636	0.77	50.06	0.07
	NO_3^-	21.84$^+$	12.01		
k$^+$	Intercept	1.353*	0.68	38.36	0.29
	k	-16.12 $^+$	9.66		
Elevation$^+$	Intercept	2.378*	1.05	49.43	0.04
	Elevation	-0.002$^+$	0.001		
NO_3^-:SRP+k $^+$	Intercept	0.796	0.78	38.14	0.34
	NO_3^-:SRP	0.017	0.01		
	k	-24.60*	12.18		
SRP+k $^+$	Intercept	2.690 $^+$	1.43	38.26	0.33
	SRP	-0.402	0.37		
	k	-20.49 $^+$	11.008		
NO_3^-+k $^+$	Intercept	0.298	1.01	37.86	0.34
	NO_3^-	22.90	16.88		
	k	-19.82	10.43		
NO_3^-:SRP+k +Elevation*	Intercept	2.94$^+$	1.59	37.26	0.45
	NO_3^-:SRP	0.016	0.012		
	k	-28.57*	13.13		
	Elevation	-0.002	0.001		
SRP+k +Elevation*	Intercept	5.14*	2.006	36.57	0.44
	SRP	-0.38	0.31		
	k	-24.82*	11.57*		
	Elevation	-0.002$^+$	0.001$^+$		
NO_3^-+k +Elevation*	Intercept	2.43	1.73	37.22	0.44
	NO_3^-	21.04	17.31		
	k	-23.99*	11.63		
	Elevation	-0.002	0.001		

LIMITATIONS

We used three complementary approaches to address the limitations posed by each of the three studies. Some inherent limitations persist and are discussed here. In the flume experiments, our objective was to isolate the role of light on *D. geminata* stalk formation by approximating light reduction in the phases of phenological mismatch, i.e. high light versus low light conditions. The shade treatments were chosen to represent real world conditions most accurately, therefore, we used Courtgurard™ film to selectively attenuate UVR while allowing PAR to penetrate, which simulates preferential UVR reduction via DOC in forested catchments. We recognize that UVR varies throughout the year, therefore, the July through September experiment received more absolute UVR than the experiment that ran September through October. Further, the flumes were very shallow which likely resulted in greater light penetration than many streams, although in the field *D. geminata* was observed on rocks protruding from the water, covered only by a shallow sheet of flow which is similar to the flume conditions, so this effect varies by stream type. Finally, we recognize that replication of these flume studies would help overcome the inherent limitations posed by experimental approximation of real-world conditions.

The high-frequency study was limited in that we were only able to collect data for one year. For a complete picture of changing stream conditions, repeated multi-year studies would be ideal. High frequency, multi-year measurements at one site with a complete record of hydrologic changes and nutrient concentrations would comprehensively address the limitations in our study.

The space-for-time study was subject to the limitations posed by point sampling including the uncertainty associated with field studies. For example, we may have measured conditions at bloom sites that were not associated with the onset that bloom because we could not know when the bloom began, only that it was present at the time of sampling. Further, the sites without blooms may have been in the early stages of photosynthetic overflow and not yet visible. There is also a chance that sampling will miss a bloom. We walked up and downstream of the sample site to look for blooms but the distance was limited by topography and access, therefore, we may have failed to identify a bloom up or downstream of our sample site. Further, we suggest additional space-for-time studies covering a gradient of streams with altered snowmelt timing.

DISCUSSION

To explain the recent occurrence of *D. geminata* blooms we evaluated how phenological shifts in nutrient concentrations and light availability interact to initiate *D. geminata* bloom formation. In the space-for-time, high-frequency, and experimental studies we found that *D. geminata* stalk production occurred under high light and nutrient limited conditions and that in the space-for-time study these conditions were more prevalent in sites without glaciers due to the decline or disappearance of glacial meltwater during the late summer months. Building upon previous research linking *D. geminata* blooms to low nutrient and high light conditions, our study found that shifts in the timing of turbidity and nutrients are the most parsimonious explanation for the widespread increase in *D. geminata* overgrowth (Blanco and Ector 2009; Kilroy and Bothwell 2011, 2012, 2014; Cullis and others 2012; Bothwell and others 2014; Taylor and Bothwell 2014; James and others 2015; Bothwell and Taylor 2017; West and others 2020). Our study shows that in mountain environments, earlier snowmelt and glacial loss are changing the timing and magnitude of turbidity and nutrient pulses that discourage bloom formation in *D. geminata.* Because these pulses no longer coincide with the mid-summer peak photoperiod or are diminished in magnitude, photosynthetic overflow is stimulated and *D. geminata* blooms can form.

High light conditions are central to understanding the drivers *D. geminata* blooms (Whitton and others 2009; Kilroy and Bothwell 2011; James and others 2014; West and others 2020) and are a key piece of the photosynthetic overflow equation (Kilroy and Bothwell 2011). We observed the role of light both experimentally in flumes and observationally in the field studies. The flume experiments demonstrated that light

reduction limits *D. geminata* stalk production but that high UVR can have an inhibitory effect as the most growth occurred where PAR was high and UVR was absent. The effect of UVR was greater in the September through October study and only marginally significant in the October sampling. This is consistent with higher absolute UVR exposure during summer months and likely influenced by the shorter duration of the October experiment. In rivers, DOC preferentially attenuates UVR, therefore, vegetated catchments, like those that have lost glacial cover, may experience conditions similar to the M2 treatment, i.e., higher *D. geminata* biomass. This may explain why *D. geminata* was observed primarily in the lower reaches of the mountain rivers and not at the headwaters, where UVR is still high and there is little DOC to attenuate light, an observation supported by the significant effect of elevation on *D. geminata* blooms. We also considered the role of topographic shading as a source for light reduction but did not find significant effects. We believe this is because our sites exhibited little variation in the degree of topographic shading, but we recognize that in other regions with varied topography this effect may be significant and should be considered.

In the space-for-time study, our sampling locations were primarily near bridges for convenience and we repeatedly observed *D. geminata* growth on either side of the bridge but never under the shaded portion of the stream. Likewise, in streams with dense canopy cover, we only found *D. geminata* blooms in the openings where light reached the streambed. These observations corroborate other studies that have observed the absence of *D. geminata* under bridges (James and others 2014). These findings give support to our experimental results that show light reduction, independent of abrasion from turbidity,

significantly reduced *D. geminata* biomass, supporting the photosynthetic overflow hypothesis.

Much *D. geminata* research has focused on phosphorus concentrations as the primary driver of *D. geminata* blooms (Kilroy and Bothwell 2012; Bothwell and others 2014; James and others 2015; Bothwell and Taylor 2017). However, there are examples of blooms in rivers where phosphorous concentrations are above oligotrophic thresholds, and blooms have been linked to low nitrate conditions in the southeastern U.S. (Kunza and others 2018; Hix and Murdock 2019). Our high-frequency measurements show a decrease in SRP preceding the bloom in the Logan River, however, when the bloom began SRP was between 6-8µg/L which is above the proposed 2µg/L threshold for bloom formation (Kilroy and Bothwell 2012). Our observations do not support an absolute SRP threshold for bloom formation, rather, they suggest that the necessary conditions are nutrient limitation in conjunction with high light.

Understanding the environmental conditions involved in *D. geminata* bloom formation is important, however, it is critical to understand how those key environmental conditions are synchronized in space and time. Many studies, including ours, have used point sampling to identify environmental conditions that cause *D. geminata* blooms. As noted in the limitation section, when point sampling, all conditions responsible for initiating bloom formation may no longer be present when sampling. High-frequency measurements addressed this limitation in our study and showed that the onset of the bloom corresponded with the co-occurrence of low nutrient and high light conditions. Further, after the bloom began, turbidity increased but the bloom persisted. Our results suggest that once photosynthetic overflow begins, conditions may change while the

bloom persists and therefore point sampling may fail to accurately capture the conditions responsible for bloom formation. This “moment in time” hypothesis has implications for study design and interpretation. If the conditions that initiate photosynthetic overflow are constrained temporally, sampling after that “moment” may produce misleading results. Note that the “moment” we refer to is not defined by a specific season or day, but rather, it refers to the time when light penetration and low nutrient conditions co-occur in a given stream. Therefore, this “moment” may be different for different streams. The connection to phenology is that when this “moment” occurs early in the season, *D. geminata* has an extended growing period and can form larger blooms.

D. geminata does not only bloom in glacial and snowfed montane streams. Many occurrences of *D. geminata* blooms are below dams (Kirkwood and others 2009; Miller and others 2009; Hix and Murdock 2019). Reservoirs have a similar influence on downstream water quality as early snowmelt and glacial recession with respect to turbidity, nutrient concentrations, flow, and temperature. Impoundments slow the flow of the river such that suspended particles settle out of the water column, reducing turbidity downstream. Settling of suspended particles also reduces phosphorus concentrations downstream as phosphate tends to bind to particles. Furthermore, reservoirs alter downstream water temperatures by either decreasing or increasing the temperature depending on the release of either hypolimnetic or epilimnetic water, respectively. The occurrence of blooms below dams can likely be attributed to the mechanism of photosynthetic overflow initiated by low nutrient and high light conditions. The difference is that these changes are brought about by the presence of a dam rather than climate related changes as observed in our study.

As our planet rapidly warms, the fundamental characteristics of aquatic ecosystems are shifting physically, chemically, and temporally (Hood and Scott 2008; Rahel and others 2008; Donnelly and others 2011; Brahney and others 2017a, 2017b; Chmura and others 2019) and organisms are responding in unforeseen ways (Stenseth and Mysterud 2002; Visser and Both 2005). Phenological mismatches are difficult to anticipate and predict as they often involve competing controls and non-linear relationships (Stenseth and Mysterud 2002; Visser and Both 2005). We use the enigma of *D. geminata* as a case study for how organisms respond to altered phenology due to climate change in unforeseen ways. The initial designation of *D. geminata* as an invasive species exemplifies the need to look deeper into the emergent properties of climate change before drawing conclusions. By uncovering the complex relations between biota and climate change we can direct resources toward management strategies aimed at the mechanistic causes of a given problem. For example, we found that *D. geminata* blooms are responding to high light and low nutrient conditions. In terms of adaptation strategies, nutrient additions are problematic for many reasons most notably eutrophication and therefore not a feasible management strategy. Whereas light reduction could be provided by shading streams with riparian vegetation, a much less problematic adaptation strategy. This study exemplifies the need to consider not just how environmental conditions will change but the associated shifts in temporal dynamics in our efforts to anticipate and mitigate the ecological implications of climate change.

CONCLUSION

We evaluated how temporal shifts in nutrient concentrations and light availability affect phenological characteristics in *D. geminata* and initiate bloom formation through space-for-time substitution, high-frequency observations, and experimental studies. We found that *D. geminata* stalk production occurred under high light and nutrient limited conditions and that in the space-for-time study these conditions were more prevalent in sites without glaciers which we attribute to the decline or disappearance of glacial meltwater during the late summer months. The flume experiments demonstrated that *D. geminata* blooms occur under high light conditions and disintagnled the potential effect of abrasion via suspended particles. The high-frequency study verified that a combination of declining nutrient concentrations and high light via a reduction in turbidity during the peak photoperiod, directly preceded bloom formation. Finally, the space-for-time study tested these concepts over a gradient in glacial cover and connected the mechanism of photosynthetic overflow to phenological mismatch due to climate change. Building upon previous research linking *D. geminata* blooms to low nutrient and high light conditions, our study found that shifts in the timing of turbidity and nutrients are the most parsimonious explanation for the widespread increase in *D. geminata* overgrowth in snowfed and glacial systems. Our study provides a case study for climate induced phenological mismatch and exemplifies how climate change is affecting aquatic ecosystems unforeseen ways.

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APPENDIX

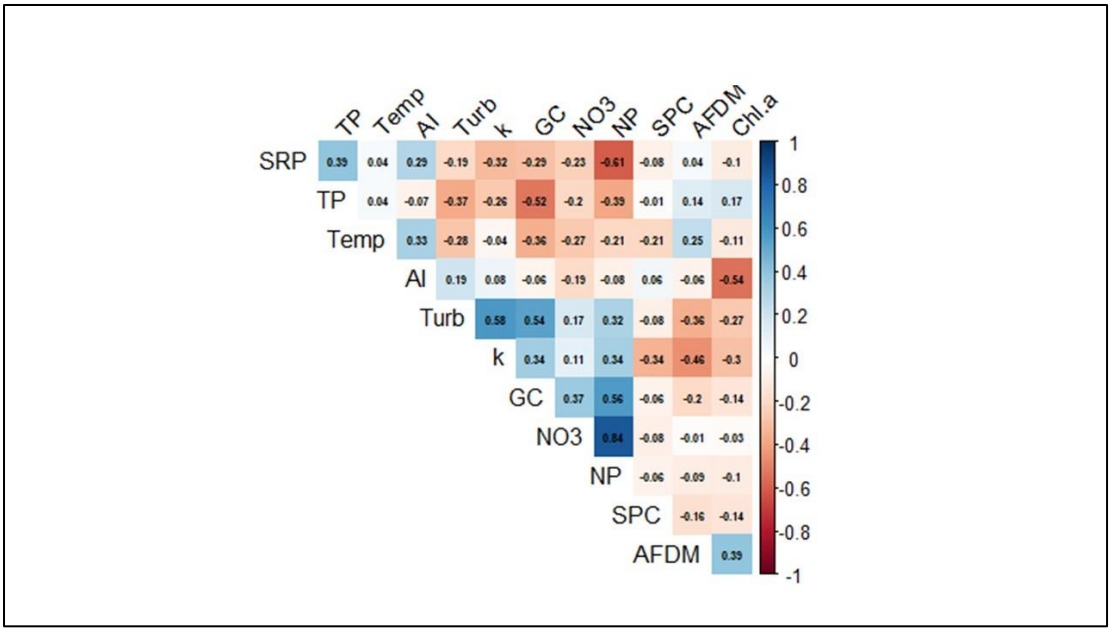

Figure A-1. Correlation matrix for 2019 space-for-time sampling which includes extinction coefficient (k). Full abbreviations given in figure A-10.

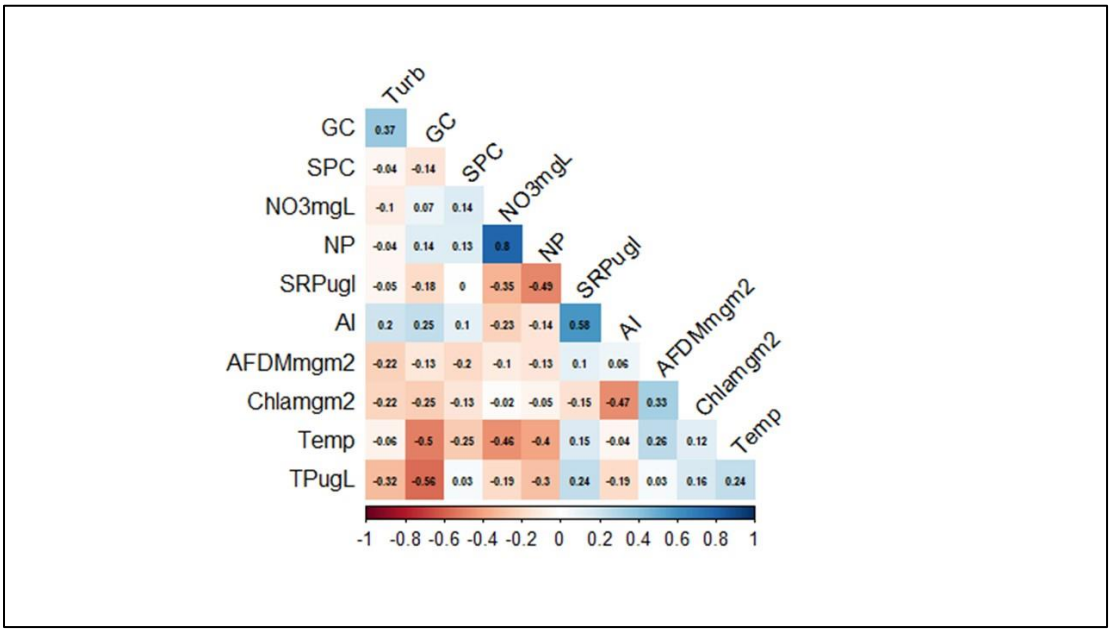

Figure A-2. Correlation matrix for 2018-2019 space-for-time sampling. Excluding extinction coefficient (k). Full abbreviations given in figure A-10.

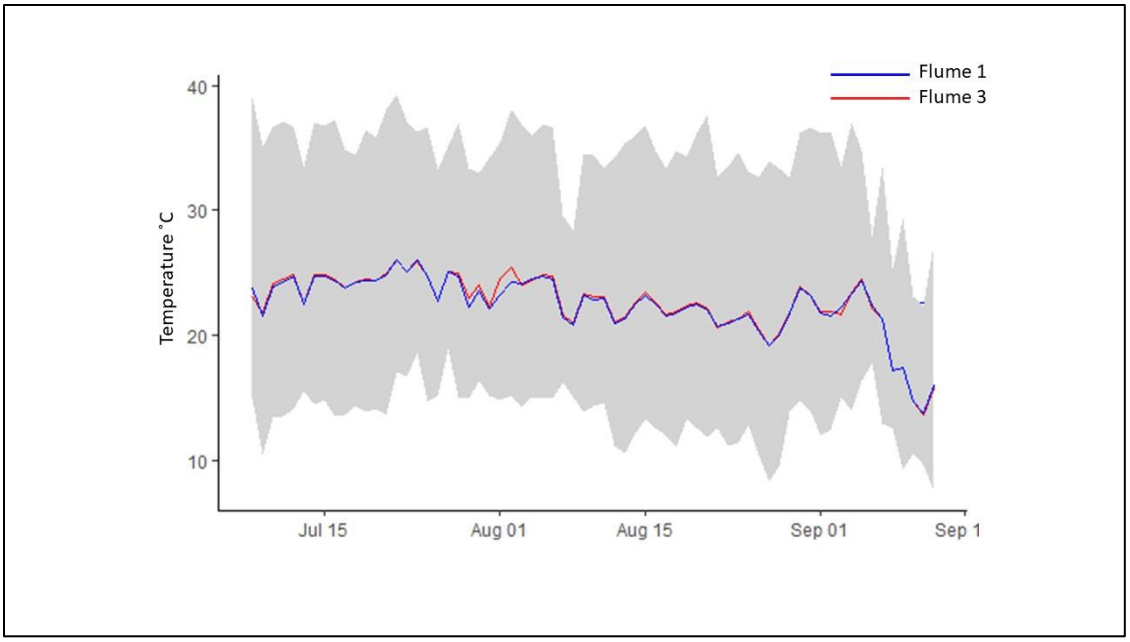

Figure A-3. Temperatures from July-September flume studies. Blue line is average of flume 1 temperatures, red line is average of flume 3 temperatures. Grey shadow is range of maximum and minimum temperatures.

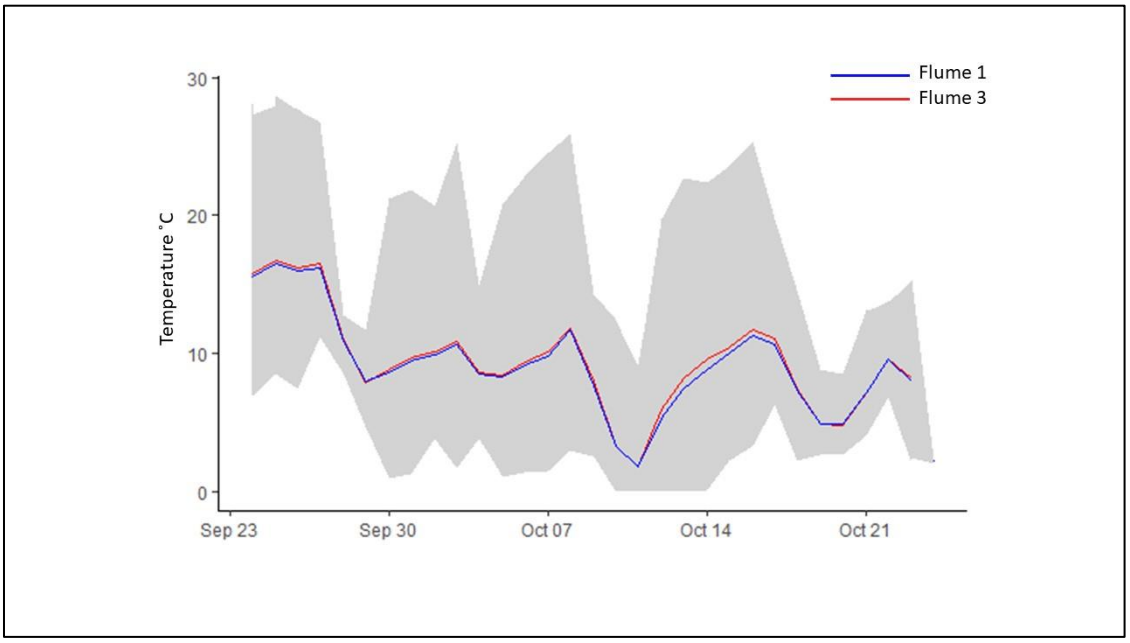

Figure A-4. Temperatures from September-October flume study. Blue line is average of flume 1 temperatures, red line is average of flume 3 temperatures. Grey shadow is range of maximum and minimum temperatuers.

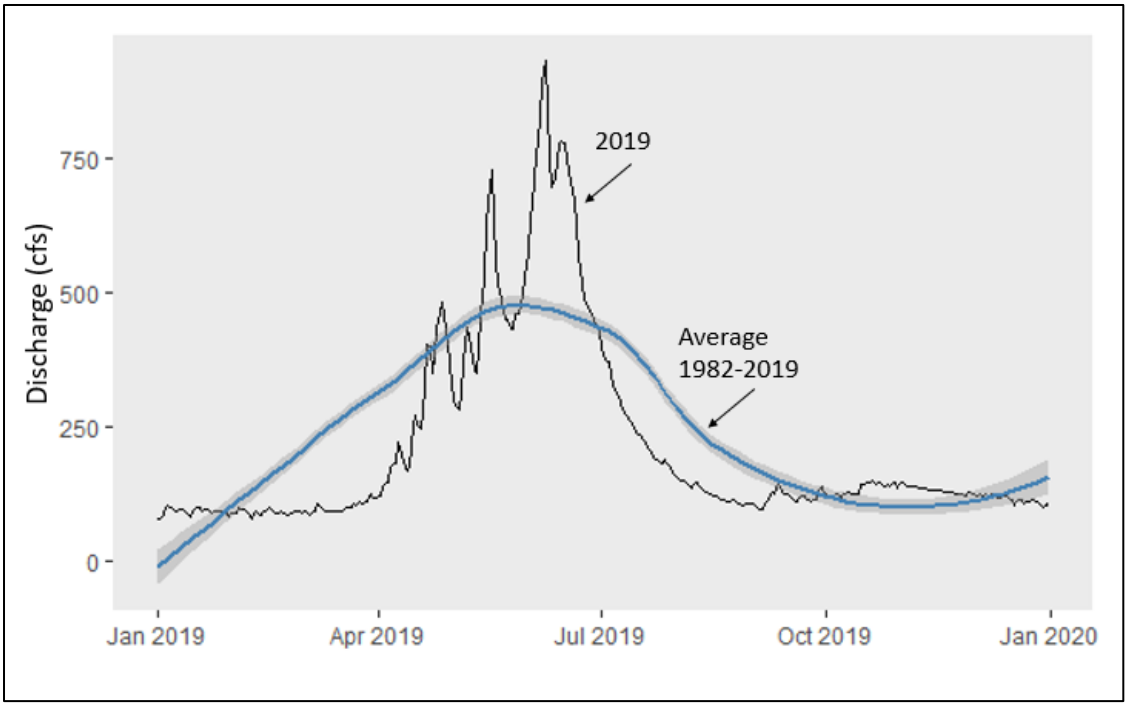

Figure A-5. 2019 Logan River hydrograph (black line) with averaged monthly flow from 1982-2019 (blue line).

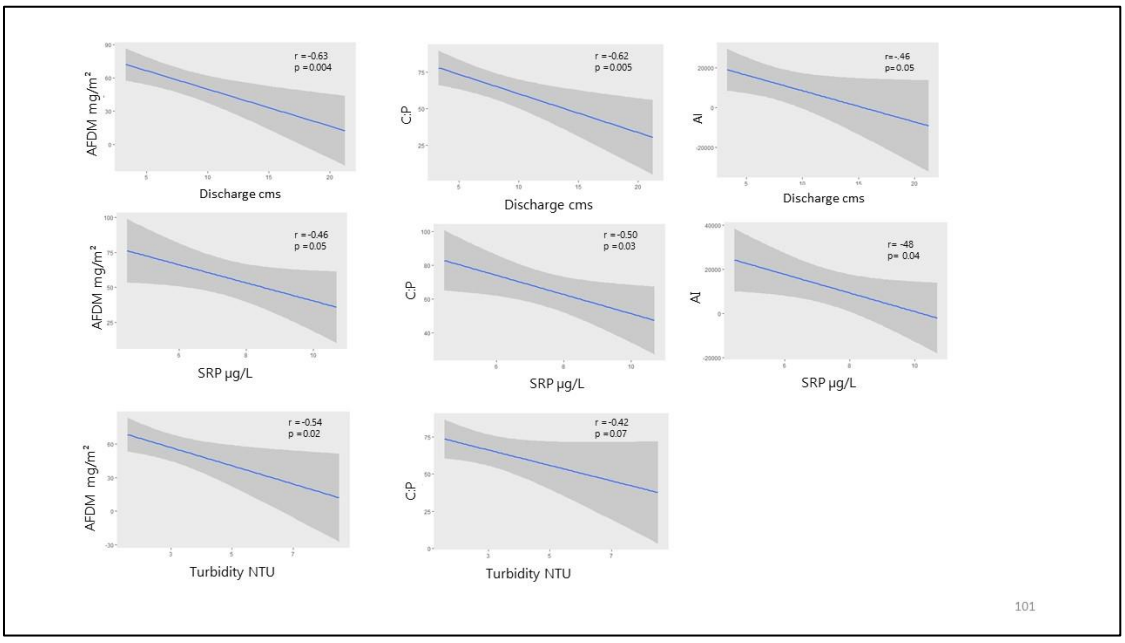

Figure A-6. Linear models between biomass (AFDM first column), (C:P second column),(Autotrophic Index (AI) third column) and Discharge, SRP, and Turbidity for high-frequency study.

All data							
	Q	TURB	Temp	SRP	NO_3^-	N:P	TP
AFDM	-0.63**	-0.54*	-0.04	-0.46^+	-0.13	0.27	0.07
C:P	-0.62**	-0.42^+	0.13	-0.50*	-0.22	0.23	0.07
AI	-0.47^+	-0.26	0.2	-0.48*	-0.01	0.35	0.07
Post Bloom							
	Q	TURB	Temp	SRP	NO_3^-	N:P	TP
AFDM	-0.18	0.46	-0.36	0.04	0.36	0.21	-0.20
C:P	-0.25	0.64*	0.06	-0.14	0.14	0.16	0.02
AI	-0.53^+	0.60*	0.16	-0.30	0.17	0.27	0.00

Figure A-7. Pearson correlation coefficients for AFDM, C:P, and AI and measured environmental variables in high-frequency Logan River study. Significant relationships are highlighted and noted by (**p<0.01, *p<0.05, $^+$p<0.10).

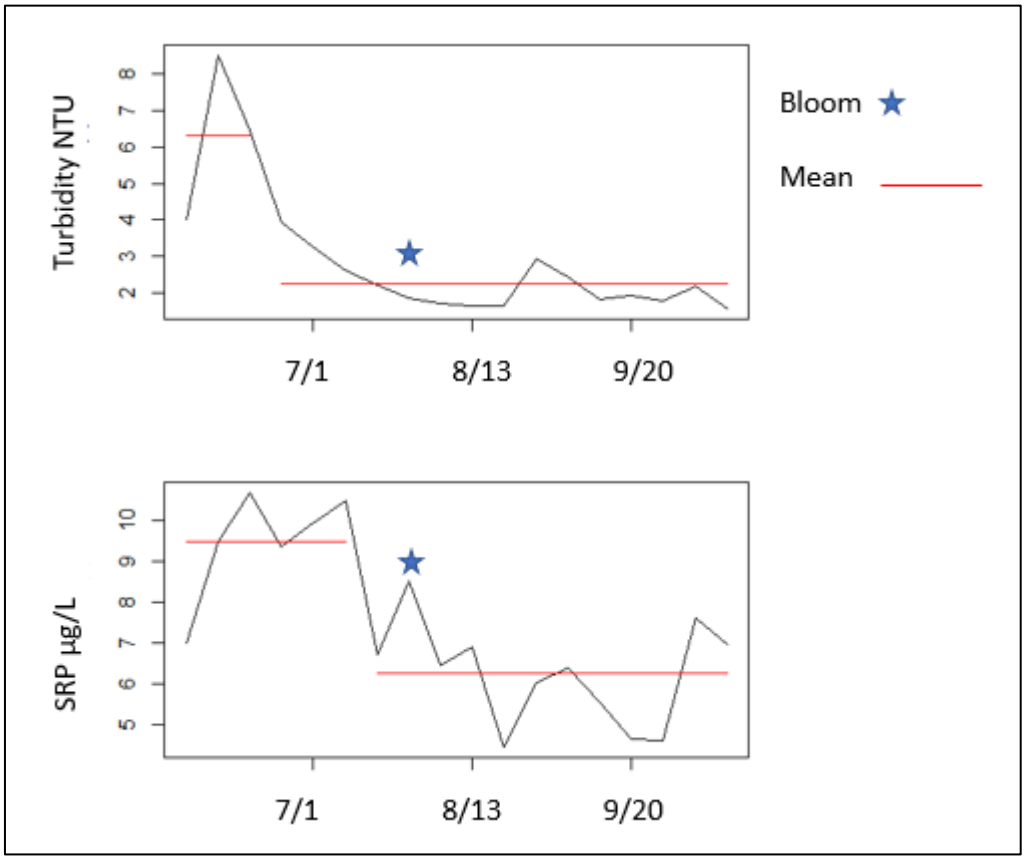

Figure A-8. Changepoint detection results for Logan River high-frequency study for turbidity and SRP. Mean for distinct periods is red line. Bloom appearance is blue star.

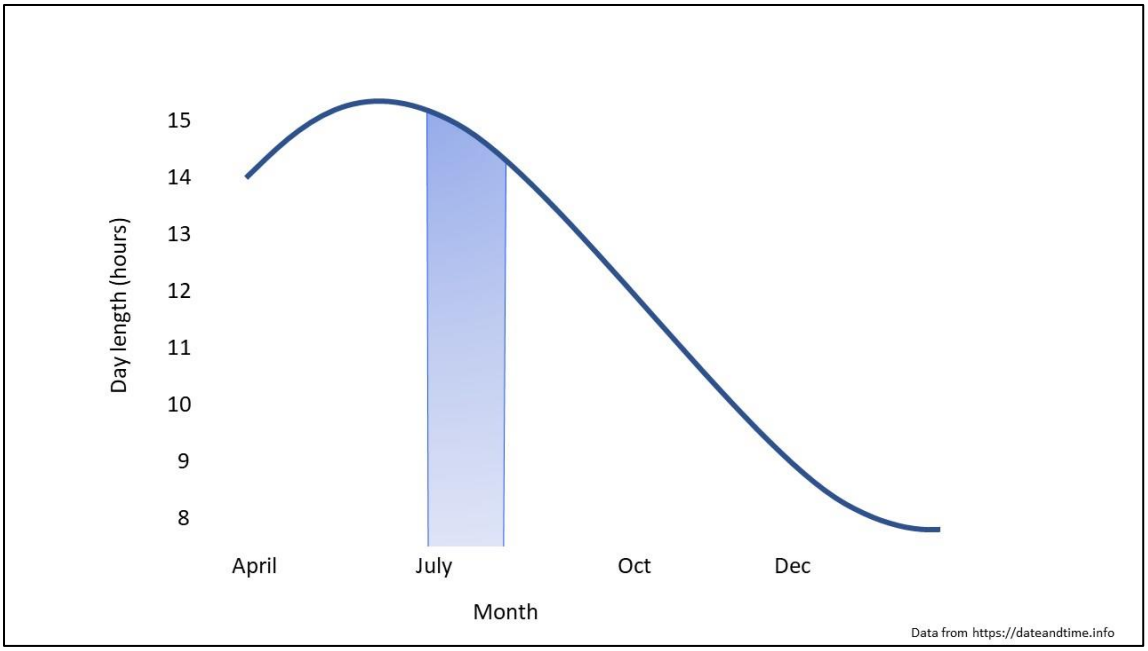

Figure A-9. Photoperiod in Logan UT. Blue shading is time frame for D. geminata bloom.

Variable	**Abbreviation**	**Range**	**Units**
Turbidity	Turb	0.23-173	NTU
Temperature	Temp	4.7-12.6	C°
Specific Conductance	SPC	32-264	µS/cm
Soluble Reactive Phosphorus	SRP	0.84-11.7	µg/L
Total Phosphorus	TP	12-129	µg/L
Nitrate	NO_3	0.01-0.19	mg/L
Nitrogren to Phosphorus Ratio	N:P	2-467	NA
Glacial Cover	GC	0-66	%
Extinction Coefficient	k	0.007-0.16	cm
Ash-free-dry-mass	AFDM	0-300	g/m^2
Chlorophyll-a	Chl-a	2-795	g/m^2
Autotrophic Index	AI	10-200	/

Figure A-10. Range and units of measured variables in space-for-time study.

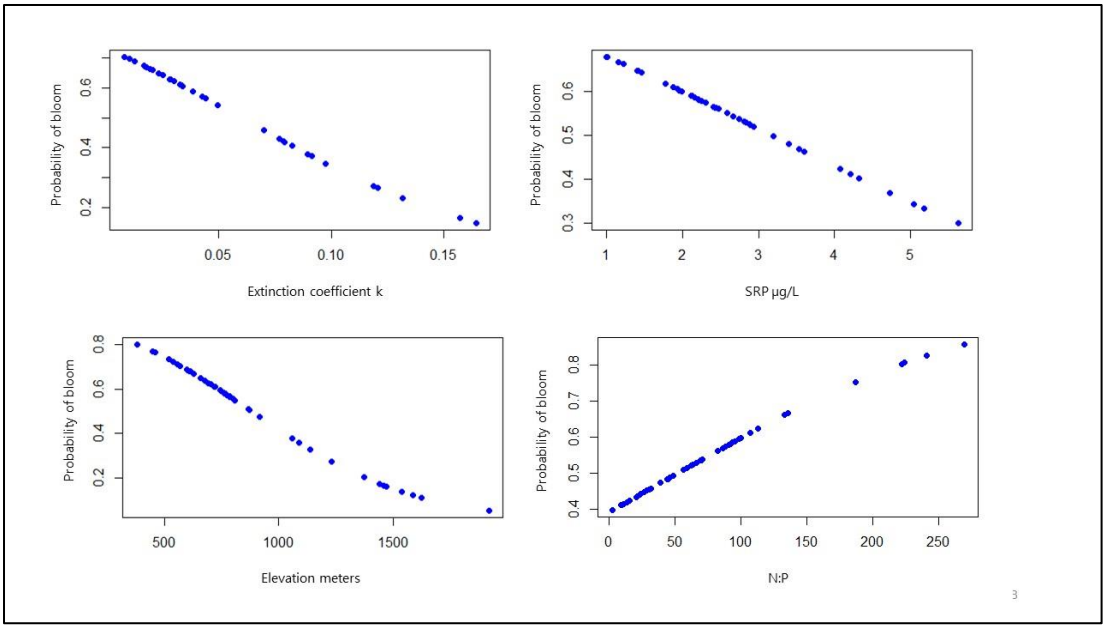

Figure A-11. Independent probabilities for the extinction coefficient (k), elevation, SRP, and N:P based on logistic regression.

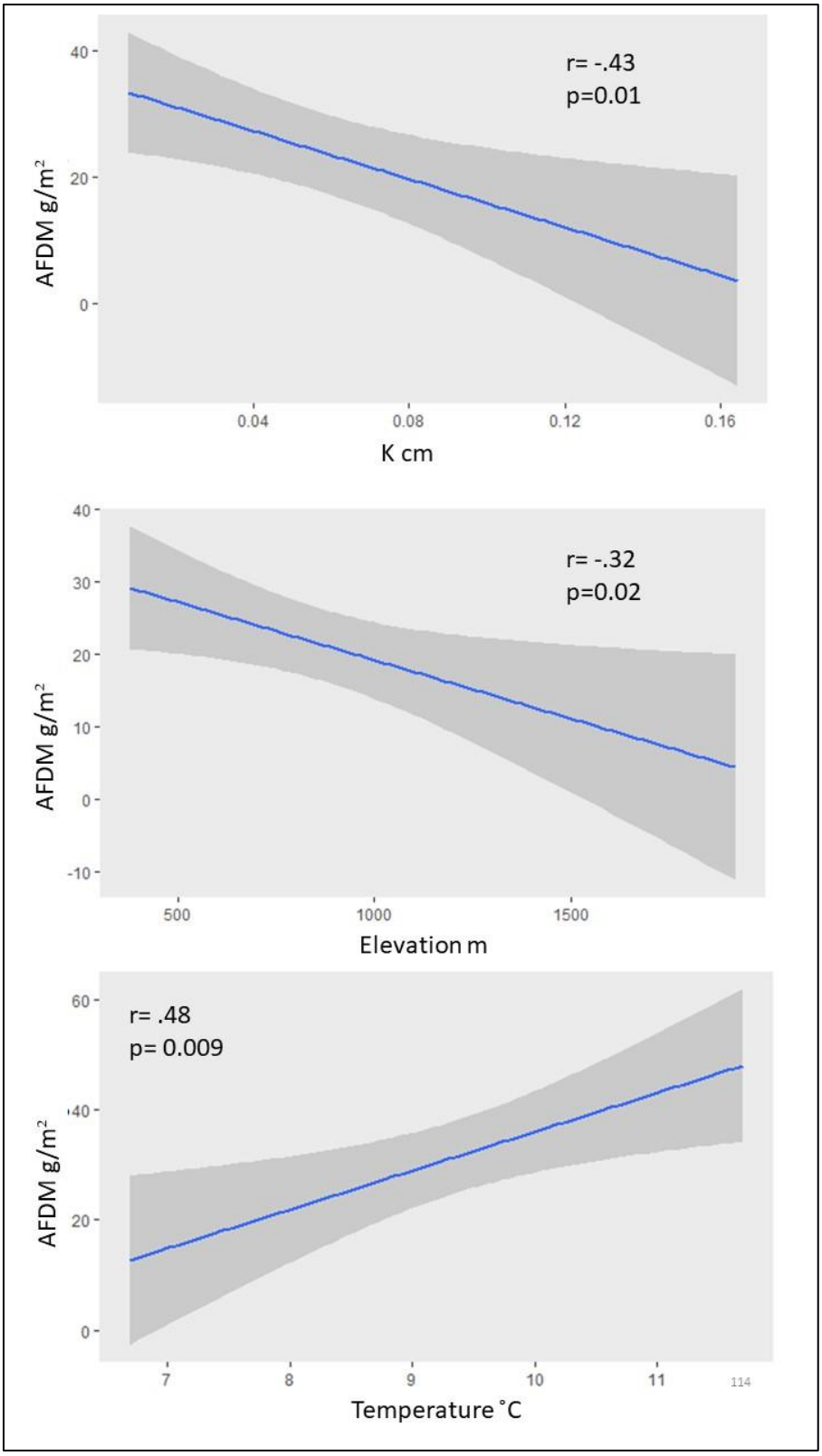

Figure A-12. Linear relationships between AFDM (g/m²) on y axis and k, elevation, and temperature.