

THESIS

AQUATIC INSECT RESPONSE TO PREDATION AND TEMPERATURE: VARIATION IN  
CONTEXT DEPENDENT TROPHIC INTERACTIONS

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## ABSTRACT

### AQUATIC INSECT RESPONSE TO PREDATION AND TEMPERATURE: VARIATION IN CONTEXT DEPENDENT TROPHIC INTERACTIONS

Trophic cascades, the indirect effects of carnivores on primary producers mediated by herbivores, remains a central theme of ecological theory. How climate change will alter the mechanisms controlling such interactions remains largely unexplored, certainly in stream ecosystems. In montane streams, stonefly predators have been documented to indirectly affect algal biomass by influencing the distribution, abundance, behavior, and life histories of invertebrate grazers. Density mediated indirect interactions (DMII) occur when primary producer biomass is primarily influenced by changes in herbivore abundance due to consumption by predators. Trait-mediated indirect interactions (TMII) alter primary producer abundance through non-consumptive interactions such as anti-predatory behaviors. In this research, I conducted mesocosm experiments on stonefly predators and mayfly prey to determine the relative importance of grazers on regulating algal production under three temperature treatments intended to simulate climate warming. Furthermore, I examined the influence of both DMII and TMII on algal production through consumptive and non-consumptive predatory treatments. I found algal biomass to decrease as temperature increased, however found no differences among grazer-alone treatments versus DMII or TMII on algal production.

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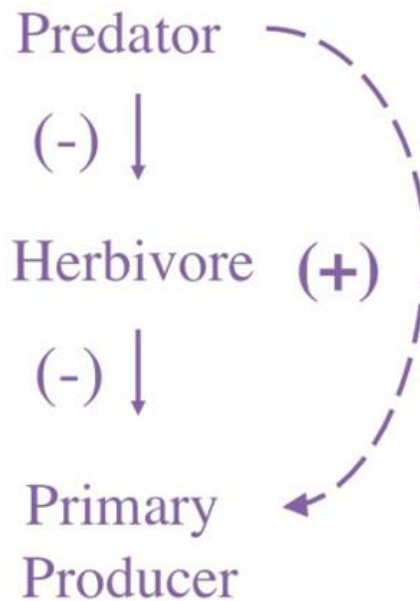
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CHAPTER 1: AQUATIC INSECT RESPONSES TO PREDATION AND TEMPERATURE:  
VARIATION IN CONTEXT DEPENDENT TROPHIC INTERACTIONS

**Introduction**

Trophic cascades – the indirect effects of predators on primary producers mediated by herbivores, is a central idea of ecology (Shurin *et al.* 2002). First described by Carpenter *et al.* (1985), cascading trophic interactions are important mechanisms that contribute to overall



**Figure 1. Classic trophic cascade model showing predators having a positive indirect effect on primary productivity.**

ecosystem structure. Cascading interactions can influence food web dynamics by altering population abundances or inducing herbivore behaviors that either promote or inhibit primary productivity (Figure 1). Theories regarding food chain interactions have come from two main perspectives, population ecology and evolutionary ecology (Schmitz *et al.* 2004). Both agree that predator-prey interactions lead to trophic cascades, but differ in respects to the causal mechanisms. Classical population ecology suggests that decreases in herbivore numbers cause an

increase in primary producer abundance ( Hairston et al. 1960). This reduction in herbivore numbers leads to predators having a positive indirect effect on primary producers. Evolutionary theory suggests that prey should adopt anti-predatory behaviors that reduce or eliminate the risk of predation (Schmitz et al. 2004). These defensive strategies result in foregone foraging opportunities and lead to an indirect positive affect on primary productivity (Preisser et al. 2005). Thus, the presence of predatory species alone should result in prey having to choose between vital activities (e. g. feeding and mating) and avoiding predation in a fashion that maximizes fitness (Schmitz et al. 2004).

Two major mechanisms play critical roles in determining cascading effects, density-mediated indirect interactions and trait-mediated indirect interactions. Density mediated indirect interactions (DMII) occur when predators have an overall positive influence on primary producer abundances through a reduction in herbivore numbers by direct mortality (Werner and Peacor 2003). For example, non-native trout in New Zealand have been shown to cause an increase in primary producer biomass through direct consumption of aquatic grazers (Biggs et al. 2000). When behavioral changes, such as anti-predatory behaviors, lead to fluctuations in prey availability, trait-mediated indirect interactions (TMII) become important (Werner and Peacor 2003). The adoption of anti-predatory behaviors in herbivores can cause decreases in grazing, leading to predators having a positive effect on primary producer abundance. For example, work done by Carlson and Langkilde (2014) found that tadpoles exposed to predation spent more time in refuge and less time foraging. Predators induced a TMII that lead to grazing tadpoles needing to choose between gaining resources and reducing predation risk.

Both TMII and DMII can be important mechanisms in top-down cascading systems. Likely, the best-known example of cascading effects was documented after the reintroduction of



gray wolves to Yellowstone National Park. As wolves began reducing elk populations through direct consumption, heavily grazed aspen stands were released from elk grazing resulting in an increase in the total aspen abundance in the Park (Smith, Peterson, and Houston 2003, Mao et al. 2005, Ripple et al. 2001, Fortin et al. 2005). Gray wolves induced a DMII through direct mortality of elk. Concurrently, elk began changing their foraging behaviors by avoiding riparian areas which provides productive habitat for young aspen trees but where wolves are likely to hunt (Ripple and Beschta 2004). This change in behavior demonstrates how TMIIIs can have significant effects on primary producer biomass by creating a “landscape of fear” rather than direct mortality. A meta-analysis of trophic dynamics by Preisser *et al.* (2005) concluded that TMIIIs are just as effective, and in some cases, more effective at reducing herbivory rates than DMIIIs. Both are important to understanding trophic networks and in this example, it was the combination of both TMIIIs and DMIIIs that promoted aspen growth in the park.

In stream communities, species interactions can also lead to cascades. Algae are consumed by grazers, some of which are vulnerable to predation (Flecker and Townsend 1994, McIntosh and Townsend 1996, Peckarsky 1982). There is a large body of literature on how fish predators mediate primary productivity in streams (McIntosh and Townsend 1994, 1996, Flecker and Townsend, 1994, Biggs *et al.* 2001), but few studies empirically test how invertebrate predators directly or indirectly regulate algal production. Grazing macroinvertebrates respond to predation by fish and invertebrate predators in different ways. Nymphs of *Baetis bicaudatus* have been shown to perceive predatory stoneflies and fish through chemoreception, supporting the argument that grazers will change their behavior even when they have not directly perceived or physically detected predators (Peckarsky and Dodson 1980). *Baetis bicaudatus* commonly avoid stonefly predators by engaging in drift behaviors (McIntosh and Peckarsky 1999). This

behavior allows grazers to be swept downstream and relocate to less risky or higher quality habitat patches (McIntosh and Peckarsky 1999). Predator avoidance behavior can reduce time for foraging and therefore lead to a reduction in herbivory and an indirect increase in primary producer biomass (Pressier et al. 2005). *Baetis bicaudatus* mainly avoid fish predators by reducing drift behaviors (Hernandez and Peckarsky 2014). These two distinct strategies can have differential impacts on primary producer biomass. Understanding the mechanisms controlling invertebrate trophic interactions and to what degree TMIIIs or DMIIIs influence trophic structure is vital to gaining a deeper perspective of lotic food webs.

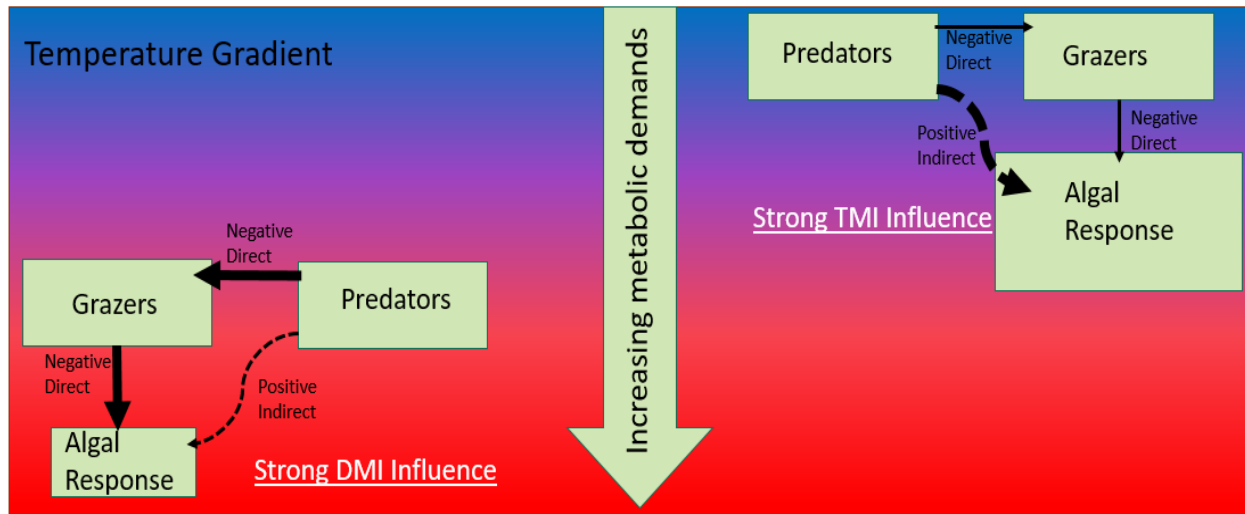
Projections indicate that climate change will cause stream temperatures to increase (Pyne and Poff 2017). Temperature is a key environmental factor affecting all ectotherms by directly altering metabolic demands and influencing their distribution and abundances (Vannote and Sweeney 1980). Invertebrate grazers have been documented to respond to elevated water temperatures by increasing their consumption rates (Gutow et al. 2016, Petraitis 1992). Water temperature influences metabolic rates of grazing insects by increasing their hunger and energy demands (Ward and Stanford 1982) and therefore may alter their risk avoidance behavior. Water temperature can directly affect how much time grazing macroinvertebrates spend foraging and how much algae they ultimately consume. Water temperature thus creates a context that can influence the outcomes of species interactions. Aquatic insect grazers have been shown to change their foraging behavior in the presence of predators (McIntosh and Townsend 1994, Peckarsky 1980, 1982), but how temperature affects the degree of these behavioral changes is largely unexplored.

Rising temperatures may increase the effects of DMIIIs caused by greater consumption due to higher energy requirements of both predators and prey. Increased metabolic demands

require more energy to maintain survival and reproductive efforts, but acquiring this energy may put prey species at increased risk of predation through conspicuous foraging (Matassa and Trussel 2015, Bale et al. 2002). Higher resting metabolic rates require more energy and thus greater acquisition of resources throughout an individual's lifespan. This could have an overall negative affect on algal production. Temperature has direct effects on all ectotherms including predatory stoneflies, mainly through their growth rate (Cather and Gaufin 1975). Marine invertebrate predators have been shown to increase predation rates at elevated temperatures (Sanford 2002, Pincebourde *et al.*, 2008). Per the temperature size-rule, ectotherms at ambient temperatures will grow slower yet attain a larger body size at the time of maturation as compared to projected thermal increases (Zuo et al. 2012). Warmer temperatures lead to less absolute growth, so for organisms to maintain the same growth rates at elevated temperatures, they must consume more resources (Sweeney and Vannote 1984). Climate warming could shift some species closer to their thermal optimum allowing for more efficient foraging (Matassa and Trussell 2015). Yet, for species that are already pressed for metabolic efficiency, the increase in energy demands may exacerbate the tradeoffs between foraging and energetically-costly behaviors that reduce predation. Consumptive and non-consumptive effects of predators may differ in their relative influence depending on temperature. This idea has been tested in intertidal communities (O'Connor 2009, Kordas, Harley and O'Connor 2011, Sanford 1999), but to my knowledge has not been examined in streams.

I hypothesize predators at elevated temperatures will have increased activity through increased metabolism, further increasing mortality risk of mayflies. It may be that the increase in predation risk through exposure and increased predatory activity does not outweigh the

metabolic needs of grazers at greater than ambient temperatures (Figure 2). Grazing mayflies may continue to forage at higher temperatures in the face of increased predation risk. This



**Figure 2. Conceptual diagram of species interactions leading to changes in total algal abundance. Temperature creates a context dependent stage upon which species interactions play out. Predictions suggest density mediated indirect interactions (DMII's) are highest at warmer temperatures whereas trait mediated indirect interactions (TMI's) are more prominent at ambient temperatures. Temperature acts as a master variable controlling metabolic demands and can mask the indirect effects of predation.**

implies that the consumptive effects of grazers on algae should be strongest where the metabolic demands of grazers are large enough to keep them foraging while experiencing predation.

Grazing demands at elevated temperatures are likely too great for predator presence alone to cause grazers to engage in anti-predatory behavior but rather remain foraging. Thus, with predators present at high temperatures, mayflies are likely to remain grazing. At ambient temperatures, mayflies may adopt antipredator behaviors that reduce the risk of predation as energy demands are not being elevated beyond average conditions. This suggests trait-mediated indirect interactions are strongest when metabolic demands are at ambient levels. The risk of predation outweighs the energy demand to forage and anti-predatory behaviors should be adopted to reduce predation risk, decreasing herbivory. Figure 2 represents a conceptual model of cascading effects across thermal regimes. Indirect effects are represented by dashed lines and

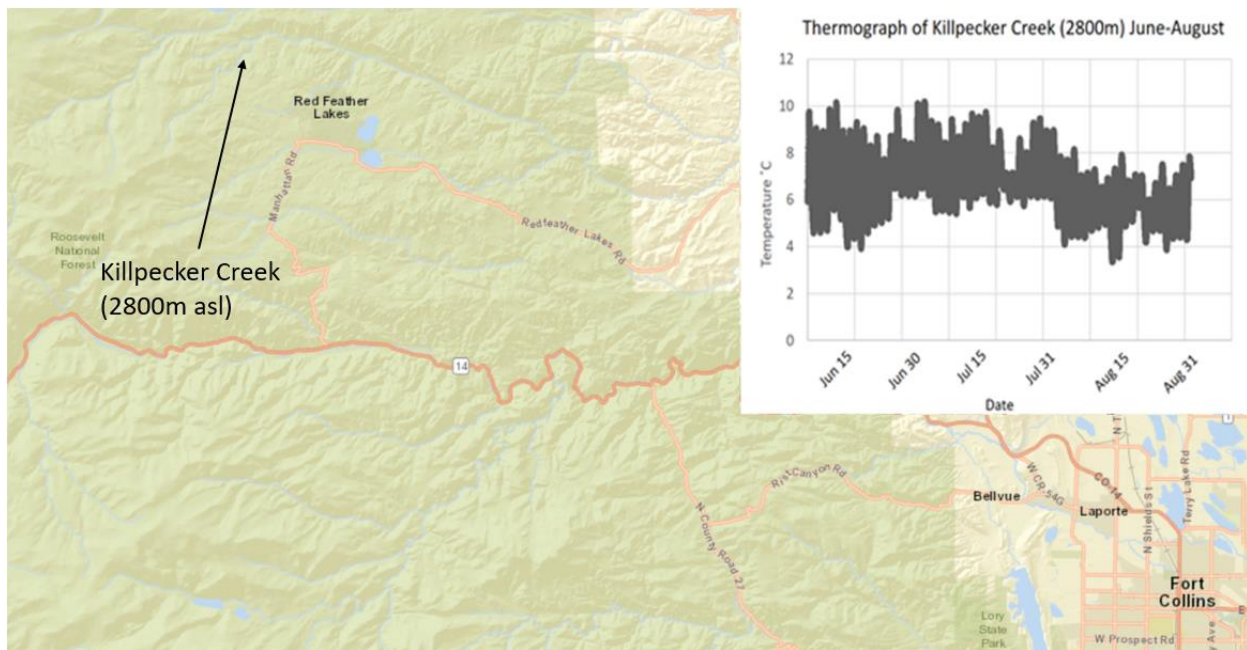
direct effects are seen by solid arrows. Arrow thickness corresponds to the strength of interactions; at warmer temperatures, direct consumptive effects are greater than cooler temperatures where metabolic demands are lower. The relative size of algal response boxes show that total algal biomass is reduced at elevated temperatures due to increased grazing pressure. At cooler temperatures, algal biomass is greater due to strong TMII.

Trophic networks are conceptualized through species interactions; these interactions are context dependent (Chamberlain *et al.* 2014). Predation introduces a “landscape of fear” where in prey species exhibit tradeoffs between growth and predation risk through conspicuous foraging. Behaviors that mitigate the risks of predation often come at the expense of gaining resources. I predict at cooler temperatures where metabolic demands are low; grazers are perhaps more likely to forgo foraging and adopt anti-predatory behaviors, increasing the relative influence of trait-mediated indirect interactions. Conversely at high temperatures, where metabolic demands are greatest, grazers may be forced to forgo anti-predator behaviors and continue foraging. This would lead to an increase in the relative effects of density-mediated indirect interactions. Increasing stream temperatures will cause a decrease in primary producer abundance through increased metabolic requirements of grazing ectotherms. This will lead to systems shifting away from highly influential trait-mediated indirect interactions to more density-mediated controls.

## Methods

### Study area

Killpecker Creek begins at 3,200m asl and joins the North Fork of the Cache la Poudre River at 2800m asl in northern Larimer Co., Colorado. The sample location at Killpecker Creek was 2,800m asl where average summer temperatures were 6°C (range 3-10°C), between June and August (Figure 3), with a mean temperature of 6°C. Killpecker Creek is dominated by two



**Figure 3. Thermograph and map of Killpecker Creek, Larimer Co., Colorado sampling location (2,800m asl). Average stream temperature is 6°C from June through August 2016.**

major stonefly predator species; nymphs of *Megarcys signata*, are abundant in the early summer (June-August) and is replaced by *Kogotus modestus*, in the late summer and early fall (August-October). *Kogotus modestus* and *M. signata* are found in relatively high abundances, approximately 17 individuals/m<sup>2</sup> and 15 individuals/m<sup>2</sup>, respectively. These two species usually do not co-occur at high population numbers (personal observation) as seen by a sharp transition in dominance around mid-August. In addition, the stream is inhabited by high larval densities of

*Rhyacophila*, species a predatory caddisfly. Killpecker Creek has an herbivore composition composed of two dominant mayfly groups *B. bicaudatus* and *Epeorus spp.* The cumulative grazing of these two species likely comprise most of the top down control on algal standing biomass in this system. Killpecker Creek does not possess any trout predators making stoneflies the top predators in this stream. Killpecker Creek is a step pool stream dominated by a heterogeneous cobble substrate and continuous flows.

### *Study species*

*Megarcys signata* is a tactile predator, actively touching the substrate with its antennae to locate prey (Peckarsky 1982). Later instars of these stoneflies primarily consume mayflies and chironomids, whereas earlier instars tend to be generalists consuming organic material (Richardson and Gaufin 1971, Palmer et al. 1993, and Tomanova et al. 2006). *Baetis bicaudatus* is a highly mobile grazer, moving actively through the water column (Poff *et al.* 2003) and capable of escaping tactile predators. *Baetis* have also been shown to detect predators through chemoreception thus anti-predatory responses can be elicited without the physical presence of predatory species (Peckarsky 1999). This mayfly species is a common prey animal for many stonefly predators (Sheldon 1969).

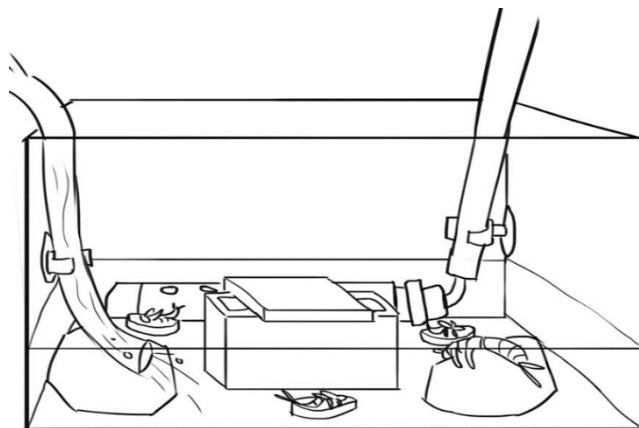
### *Specimen collection and acclimation*

Insects were collected by gently disrupting the substrate, so as not to damage individuals, and collected in a 500 $\mu$ m D-frame kicknet. The kicknet contents were placed in sorting trays where individuals could be identified and transferred to life-support chambers. Target taxa (*M. signata* and *B. bicaudatus*) were sorted stream-side using an available key (Ward et al. 2002). Individuals were identified to the species level streamside. Both predators and grazers had

comparable size distributions among mesocosms treatments. However, larger stoneflies were prioritized when possible as they exhibit greater predatory behavior (Taylor et al. 1999). Mayflies with darkened wing pads were not considered and returned to the stream, as it has been shown that nymphs that are close to emergence do not actively forage (Cummins 1973). *Baetis bicaudatus* were collected using plastic pipettes, as to not damage gills, and transferred into life-support chambers. Organisms were then transported to the laboratory while being provided oxygen-saturated water kept at 6°C during transport. Stream water was also collected on site and used for both acclimation and experimentation. Insects were then acclimated for 48 hours, in experimental mesocosms, at one of three specified test temperatures (ambient, +2, or +4°C) before experimentation began. Predators were starved for at least 24 hours (Peckarsky et al. 1993) prior to experimentation to ensure hunger.

#### *Mesocosm design*

Mesocosms consisted of 29L X 21W X 12D (cm) plastic, translucent, and smooth cornered rectangular mouse cages with solid supports in the center creating a racetrack-like feature (Figure 4). Each mesocosm contained three 25mm diameter ceramic algal discs, two



**Figure 4. Mesocosm design showing algal discs, cobble refuge and circulating flow. Resource patches induce conspicuous foraging and refuge occurs in resource poor locations.**

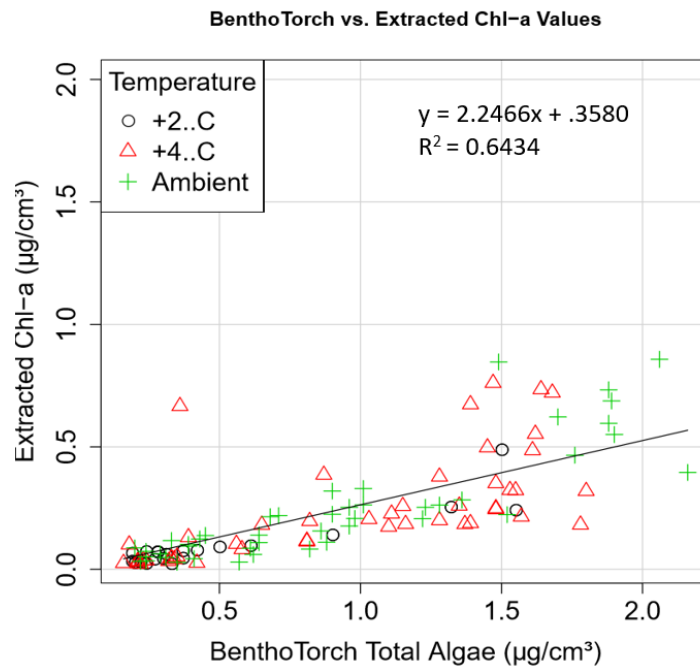


7.5cm cobbles (refuge), and small gravel pieces covering the bottom. Each mesocosm contained 3.2L of unfiltered stream water. Water was segregated between mesocosms containing chemical predator cues and no-predator treatments to prevent the false detection of predation risk by grazing mayflies. Each trial contained 15 *B. bicaudatus* and predator treatments received one *M. signata*.

#### *Diatom growth and chlorophyll- $\alpha$ analysis*

Killpecker Creek's algal resource base is dominated by diatoms. To control for food quality and quantity, *Navicula* spp., a common freshwater diatom that can be successfully cultured in the lab, was chosen as the experimental food resource. Only one diatom species was used to control for preferential grazing of algal species by mayflies. Diatoms were cultured at 22.5°C for two weeks in glass 5000ml conical flasks. This culture was then used to seed autoclaved crucible covers, in large autoclave-safe tubs containing 120 individual discs, and allowed to grow for at least seven days. Control treatments at each experimental temperature ran with only colonized algal discs, these mesocosms ran concurrently with experimental treatments (Poff *et al.* 2003). This was done to document the average growth rate of algae discs across the temperature gradient. Trials were conducted at 6°C (ambient), 8°C, and 10°C. Initial chlorophyll- $\alpha$  measurement, a proxy for biomass of algae present, was taken after the 48hr acclimation period at each temperature. All chlorophyll  $\alpha$  readings were confirmed with standard fluorometric methods. Because fluorometer readings are destructive, initial chlorophyll  $\alpha$  values were calculated from three randomly selected discs at each experimental temperature; this average was used as a pre-reading for that week/temperature combination. After experimentation, each algal disc was analyzed for chlorophyll  $\alpha$ , yielding before and after measurements, the difference of which could be attributed to *Baetis* grazing. Consumption in each mesocosm was determined using an average of

pre-vs-post measurements taken from all three algal discs, ensuring that disc placement was not confounding. All algal readings were taken using the bbe-BethoTorch, including individual pre-disc measurements. The BethoTorch is an *in situ* fluorometer used to quantify algal biomass through chlorophyll- $\alpha$  concentrations. BethoTorch measurements are non-destructive and allow for *in situ* before and after readings, thus each algal disc could be tracked for changes in abundance. However, this method was deemed biased at higher chlorophyll- $\alpha$  readings and not used in the analysis (Figure 5).



**Figure 5. Relationship between BethoTorch total chlorophyll- $\alpha$  readings and extracted chlorophyll- $\alpha$  using standard methods.**

*Treatments and replicates*

All insects and algal discs were acclimated for 48hr prior to experimentation. Trials then ran for 24 hours on a 16:8 light:dark cycle, mimicking natural light conditions. Chlorophyll- $\alpha$  readings were taken at time zero and at the end of experiments.

*Control; Algal Growth in the Absence of Grazers (Treatment 1)*

At each experimental temperature 6°C (ambient), 8°C, and 10°C trials were run containing only algal discs. This yielded average algal accrual at each of three temperatures to determine if algal growth and accrual (and hence *Baetis* food level) were accelerated by warming.

*Algae and Grazers; Consumption in the Absence of Predation (Treatment 2)*

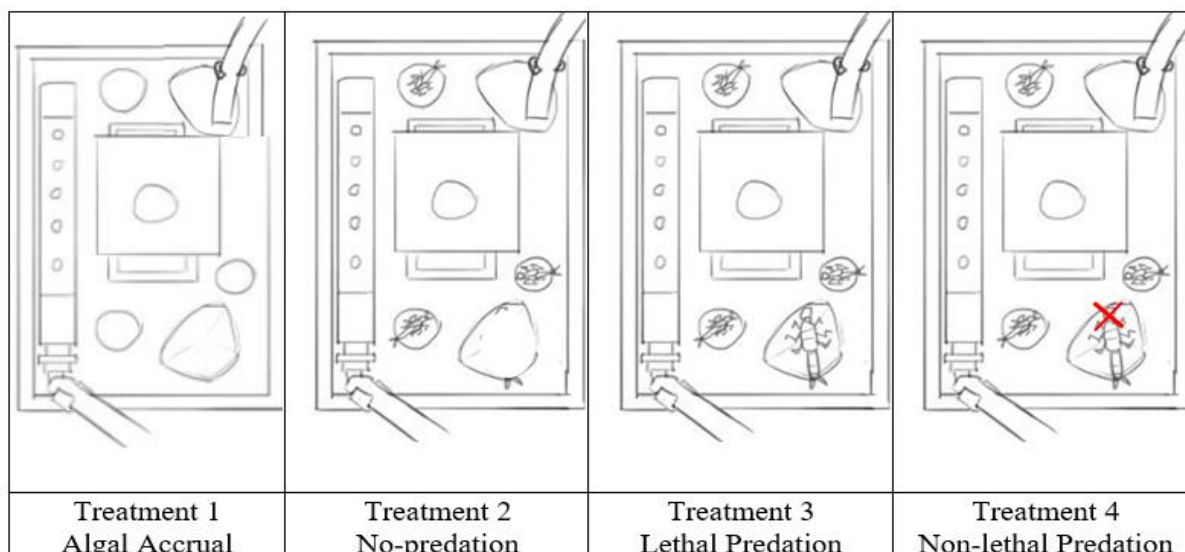
At each experimental temperature trials were run with algal discs and mayfly grazers. This yielded thermal effects on herbivory in the absence of predators which could alter foraging behavior.

*Algae, Grazers, and Lethal Predators; Herbivory Rate in the Presence of Lethal Predation (Treatment 3)*

Trials at each experimental temperature ran with algal discs, grazers, and lethal predators (stoneflies capable of consuming prey). Ample refuge was provided for grazers in all treatments. Consumption rate of stoneflies was measured by counting surviving grazers and analyzing gut content.

*Algae, Grazers, and Non-Lethal Predators; Herbivory in the Presence of a Non-Lethal Predation (Treatment 4)*

Trials at each experimental temperature 6°C (ambient), 8°C, and 10°C trials were run with algal discs, grazers, and non-lethal predators, i.e. stoneflies that had their mouthparts glued shut (McIntosh and Peckarsky 1999). The use of non-lethal predators eliminates any reductive effect of grazing through direct mortality as compared to lethal predator treatments. Treatment combinations are shown in Figure 6.



**Figure 6. Mesocosm treatments. In each sampling week eight mesocosms ran at each experimental temperature (Ambient, +2°C, and +4°C) with 2 treatment replicates per temperature.**

#### *Video analysis of behaviors*

Two hours into experimentation a single one-minute overhead video of each mesocosm was recorded. Each video was then analyzed for the number of drifting individuals and the number of foraging grazers exposed to predation. Mayflies have been well documented to show drifting behaviors in response to predation and to locate better foraging patches (McIntosh and Peckarsky 1999). They have also been shown to increase their drifting response at elevated temperatures (Scherr et al. 2010). The goal of these videos was to try and understand temperatures effect on grazer behavior and to identify behavioral changes under induced predation pressure.

#### *Statistical analysis*

Changes in algal abundances were calculated as a difference between pre-algal and post-algal chlorophyll  $\alpha$  measurements, a proxy for total standing biomass. These data were analyzed using a regression form of an ANCOVA model in a full factorial design using R software (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for

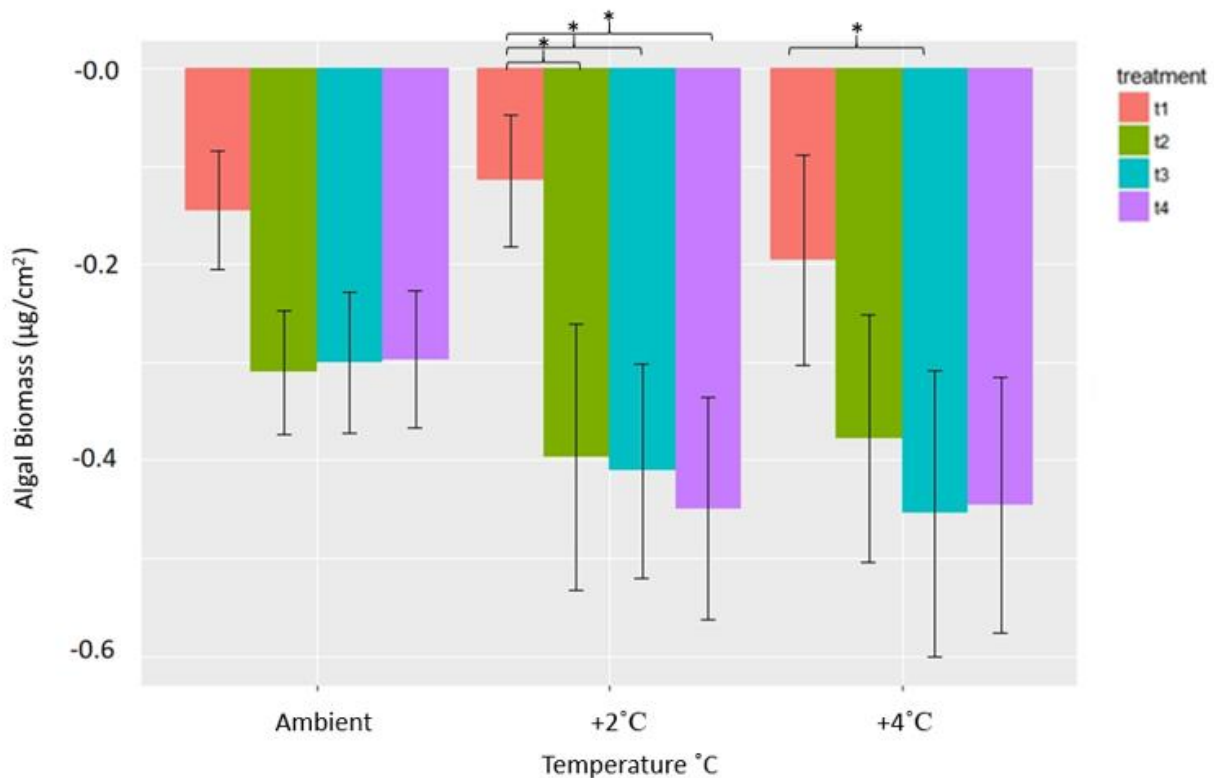
Statistical Computing. Vienna, Austria. URL <https://CRAN.R-project.org/package=lsmmeans>).

Pairwise comparisons of both temperature and treatment means were also analyzed. The variable of “week” (individual experimental runs) was a block in this analysis. Analyses are aimed at determining temperature effects on consumption and the relative effect size of TMIs and DMIs across the temperature regime. Video data were analyzed using a simple linear regression with pairwise comparisons of drifting and foraging behaviors across both temperature and treatments respectively.

## **Results**

### *Grazer consumption along temperature and predatory regimes*

A total of 84 mesocosm replicates were run over a 4-week period. Pairwise comparisons of treatment means showed significant differences of treatments to control, but did not show differences among grazer, non-lethal, and lethal treatments. When averaged across all biotic treatments consumption did yield a significant relationship with temperature, where by consumption increases above ambient temperatures. Pairwise comparisons between biotic treatment means show consumption increasing when comparing ambient temperatures to both 8°C (p-value=0.009) and 10°C (p-value=0.007). However, consumption shows no significant relationship when comparing the two elevated temperatures (p-value=0.998). Treatment by temperature interactions were not found to be significant. Summary of results can be seen in Figure 7.



**Figure 7. Summary of average consumption by grazers (measured as loss of chl- $\alpha$ ) during the 24hr experiments. T1; controls, only algal discs present. T2; unimpacted consumption, fifteen *Baetis* per mesocosm alone. T3; lethal predation, grazing rates under direct predation (DMII). T4; non-lethal predation, (TMII). Pairwise comparisons show no relationship when comparing predator to grazer-alone treatments. Reductions in algal biomass as compared to controls are illustrated by (\*).**

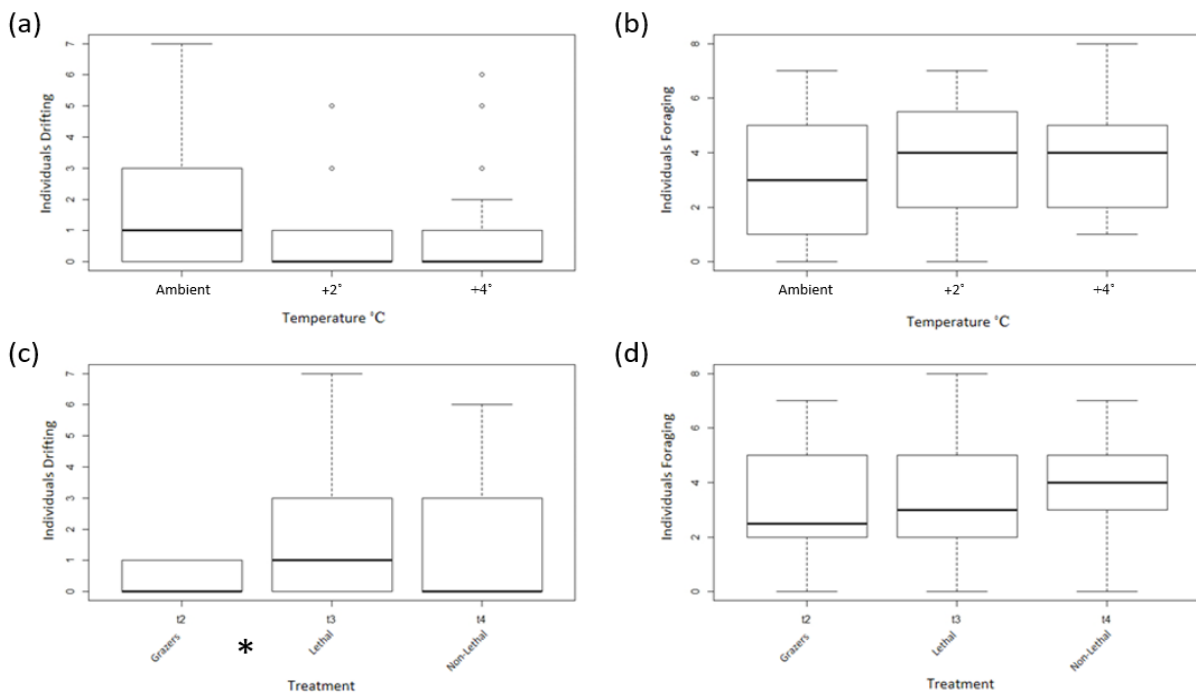
*Influence of lethal and non-lethal predation on algal biomass*

Our analysis shows no effect of predatory regime on algal abundance. At all experimental temperatures herbivores consumed algal resources at similar rates across both predatory regimes as compared to no-predator treatments.

*Behavioral video analysis; drifting and foraging*

Nymphs of *Baetis* were video-recorded for a one minute duration, two hours after experimentation began. The number of individuals drifting within the one minute observation period as well as the number actively foraging on algal discs was analyzed. Mean number of individuals drifting and foraging showed no relationship with increasing temperature. However,

a significant relationship was seen between the number of individuals drifting and predatory regime (p-value=0.001). Pairwise comparisons showed a significant difference between the number of *Baetis* drifting in lethal treatments versus grazer-alone treatments. Non-lethal predators had no significant effects on drifting behavior. No relationship between predatory regime and foraging behavior was observed. Comparable numbers of individuals were observed foraging in all treatment combinations. Results are summarized in Figure 8.



**Figure 8. Video data of drifting and foraging behavior. (a) Number of individuals drifting across temperature treatments shows no significant relationship. (b) Grazers actively foraging showed no relationship with temperature. (c) Drifting is significantly higher in treatments containing lethal predators when compared to no predator treatments. (d) Presence of predators did not influence the number of individuals actively foraging.**

## Discussion

### *Effects of temperature on algal biomass*

Grazer consumption increased significantly beyond ambient temperatures, but grazers did not consume more resources at +4°C treatments as compared to +2°C treatments. The increase in consumption is consistent with predictions suggesting that increased metabolic demands lead to decreases in algal biomass. However, algal response did not change across predatory regimes. Aquatic macroinvertebrates that experience greater than ambient temperatures have been shown to mature at smaller body sizes, reducing fecundity (Rosillon 1988. Scherr et al. 2010). The introduction of predation risk has also been documented to alter mayfly life-history strategies in field settings by reducing time to maturation, leading to smaller body sizes around the time of emergence (Peckarsky et al. 2001). An increase in consumption by predators through increased metabolic demands could further shift optimal body conditions to smaller individuals. As we begin to see average stream temperatures increase, ectotherm growth may decrease, yet consumption could continue to rise with the need for more resources to achieve the same growth rates. This increase in consumption can drive primary producer biomass down leading to a lack of overall resource availability. Thermograph data (Figure 2) show that temperatures of 10°C do occur naturally throughout at least some time during the summer. I conducted pilot experiments designed to determine functioning thermal ranges for both *M. signata* and *B. bicaudatus* and found substantial increases in ambient mortality above 11°C for *Baetis*. Mesocosms held at 11°C and 12 °C caused mortality rates above 70%. The cause of death was most commonly associated with a lack of ability to molt properly and escape from exuvia. Mortality of *Baetis* would occur when nymphs became trapped in partially shed exuvia at these elevated temperatures (personal observations).



### *Algal consumption among predator treatments*

No differences in algal abundances were observed between predator treatments. As long as grazers were present, primary productivity was equal across both predator treatments. As metabolic demands continue to increase along the thermal gradient (Rossillon 1988), we would expect greater algal consumption to meet these requirements. This finding is inconsistent with predictions suggesting TMIIIs and DMIIIs would have different effects on primary productivity depending on the temperature treatment. Consistent grazing between predator and no-predator treatments suggest that *M. signata* has little effect on *Baetis* foraging behavior in this stream system.

In this study, I found no support for cascading effects across all temperature treatments. At ambient temperatures, where non-consumptive effects were predicted to be influential, grazing mayflies consumed at comparable rates with or without predator cues. The inability to utilize *in-situ* algal biomass readings may have contributed to the lack of variability seen between predatory treatments. Average initial chlorophyll  $\alpha$  readings taken from three randomly selected algal discs produced low statistical power for calculating a pre-algal measurement. Treatments designed to separate TMIIIs and DMIIIs concluded similar consumption rates when compared to no-predator treatments. Stonefly predators appeared to have little influence on algal biomass through either consumptive or non-consumptive interactions.

### *Behavioral responses to predation and temperature*

Significant differences were seen in the number of individuals drifting in lethal predator treatments versus grazer alone treatments. *Baetis* individuals in the presence of stoneflies frequently adopt drifting behaviors as an anti-predatory strategy (McIntosh and Peckcarsky

1999). Why grazers did not drift at similar rates in non-lethal treatments as compared to lethal treatments is unknown. Non-lethal predators may have been less active during experimental periods, even though preliminary experiments suggested similar activity levels. From personal observations, non-lethal stoneflies were seen to be just as active as lethal stoneflies. The presence of predators increased drift behavior but did not seem to have impacted the foraging rate of these risk-averse individuals. Grazing rates between low drift and high drift prone individuals were similar. Thus, for individuals under predation pressure, consumption occurs at comparable rates to no-predator treatment grazers.

Video data showed no relationship between the numbers of individuals actively foraging versus temperature or predator presence. I collected nighttime video observations during one week of the experimental period. These videos seemed to indicate greater predator activity at night. *Megarcys signata* often remained hidden in refugia during the day and was far less active as compared to evening behaviors. Predators were observed to be conspicuously foraging in 5/12 mesocosms during night observations while during daytime observations of those same mesocosms revealed only 1/12 individuals actively foraging. Night time *Baetis* grazers did not appear to have distinct differences in foraging behaviors as compared to daytime observations. However, during daytime observations, on average one *Baetis* was observed drifting across all treatment combinations while an average of three grazers were observed drifting at night. Because experimental periods occurred over a full light:dark cycle, these observations are unlikely to have strong impacts on the outcome of this study.

*Alternative trait-mediated interactions:*

From personal observations, *Baetis* densities in Killpecker Creek appear to be elevated as compared to other low order montane streams in the Cache la Poudre and surrounding drainages.

This excess of prey availability may lead to the development of prey behaviors that are indicative of predator swamping. Predator swamping or predator satiation strategies are a form of anti-predatory response whereby individuals periodically come together in large numbers to reduce the chances of any one individual being consumed (Williams, Smith, and Stephen 1993, Linhart 2014). This could be viewed as a trait-mediated interaction just as any anti-predatory behavior. *Baetis* are multivoltine and thus have multiple emerging cohorts throughout the year. The synchronized oviposition of adults leads to large population densities throughout any one individual developmental period. This possible saturation of prey species during development may lead to a lack of strong individual anti-predatory behavior. However, these findings are inconsistent with previous work in similar systems where both strong and weak trophic cascading effects have been documented (McIntosh and Townsend 1996, Carlson and Langkilde 2014). In streams where prey densities are less, it may be more evolutionarily advantageous for individuals to elicit behaviors for avoiding predation.

As streams continue to warm, aquatic macroinvertebrates will begin to experience increases in metabolic demands and taxa are predicted to increase consumption of food resources (Pyne and Poff 2017, Matassa and Trussell 2015, Sanford 2002); increasing the relative influence of consumptive interactions. No evidence was found for top down controls through TMIs or DMIs in a laboratory warming experiment. I show grazers responding to elevated temperatures by increasing rates of grazing but failed to detect differences across predatory regimes.

## Conclusion

Grazing nymphal mayflies play a major role in maintaining primary producer abundance and heterogeneity through direct consumption (Taylor et al. 2002). Stonefly predators have been shown to indirectly affect grazer behavior by introducing a “landscape of fear” which inevitably alters primary producer abundance (Preisser et al. 2005), although these data showed no top-down controls. Ecologists seeking to understand the role climate warming will have on macroinvertebrate trophic interactions must consider environmental factors beyond current ambient conditions. By expanding thermal breadth in controlled laboratory experiments, we could simulate climate warming and examine changes in trophic dynamics of stream dwelling ectotherms. Developing a predictable framework for understanding the role of warming on trophic interactions will be critical as stream temperatures increase globally in the future.

Artificial stream mesocosms allow for a controlled medium on which species interactions can play out. Trophic cascades are innately complex to understand as both direct and indirect effects play major roles in controlling these processes. By physically manipulating predator mouthparts, we could create treatments designed to indirectly measure the influence that trait-mediated interactions have on primary producer abundance. Further looking at how these interactions play out along an increasing thermal gradient could help to illustrate future food web dynamics as global averages increase. Increasing temperatures lead to increased metabolisms requiring greater energy demands of ectotherms our data show algal biomass to decrease beyond ambient temperatures. However, that decrease in biomass is not continued but rather sustained across the thermal gradient. No trends were seen among TMII or DMII treatments, suggesting that predator presence has little influence on primary production for this macroinvertebrate assemblage.

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