

**Trade-offs Associated with Food Availability and
Predator Avoidance Behavior of a Stream Mayfly**

Student: Stephanie Hernandez

Mentor: Bobbi Peckarsky, PhD

Advanced Independent Research Program
2011 REU

Abstract:

We studied the effects of food availability and predator cues on mayflies at the Rocky Mountain Biological Laboratory (RMBL) in Gothic, CO. Organisms in a wide variety of ecological systems must deal with trade-offs between functions necessary to sustain their populations. Specifically, we were interested in how food availability in the presence of predators induces changes in predator avoidance behavior, growth rate, and grazer-algal impact. We manipulated the environment of mayflies (*Baetis bicaudatus*) using treatments with or without chemical cues from brook trout (*Salvelinus fontinalis*) and different levels of periphyton resources in order to test for trade-offs between food availability and predator avoidance behavior. We found that trade-offs do in fact exist between food availability and predator avoidance behavior. Mayflies only exhibited daytime drift, which is a food search behavior that increases the risk of predation, when there were not only no predator cues present but also when food was limited. However, no trade-offs were observed for mayfly growth rates and grazer-algal impact responses to our manipulation of food availability and predator cues. Mayfly growth rates and grazer-algal impact were significantly affected only by food availability, and not by the presence of predator cues; and predator cues did not affect the influence of food levels on mayfly growth or impacts on algae. Results of this and previous experiments suggest that the nonconsumptive effects (NCE) of trout do not cost *Baetis* reduced growth rate but rather costs *Baetis* reduced fecundity due to accelerated development. In the presence of trout, *Baetis* do not grow more slowly, but incur a size-fecundity trade-off by maturing at smaller sizes. We conclude that trade-offs play important roles in the behavior and fitness of organisms such as *Baetis* and are key to understanding food web interactions.

Introduction:

Organisms living in a wide variety of ecological systems often experience trade-offs between functions necessary to sustain their populations (Abrahams 2000, Lack 1947). For example, behaviors necessary for finding food often increase exposure of consumers to predators (Abrahams 2000). As a result, when predators are present, prey often change the locations where they graze or their grazing behavior to be less conspicuous to predators (Krivan and Sirot 2002, Resetarits et al. 2005, Abrams et al. 2007). Trade-offs occur because changes in foraging technique or location that diminish the risk of mortality can be costly to prey through reduction in foraging efficiency. For instance, fox squirrels are at a greater risk of predation when foraging in open areas away from trees where less cover is available to hide from predators. When grazing in these “risky habitats” fox squirrels will more readily give up foraging and move to a new patch than in less risky habitats. Thus, fox squirrels are presented with a foraging trade-off sacrificing potentially good patches in order to avoid predators (Brown et al. 1992). In pond systems, zooplankton have also been shown to change foraging behavior in response to

predators. In the presence of predator cues, zooplankton such as *Daphnia* exhibit diel vertical migration, swimming up or down toward or away from light, in an attempt to be less conspicuous to visual predators (fish) (Dodson 1990, Dodson et al. 1997). In ponds with such predators, *Daphnia* have been shown to migrate to greater depths where less light is present and predators are less likely to see them. This behavior presents *Daphnia* with a trade-off; they are less likely to be eaten but food is scarcer at greater depths (Boersma et al. 1998). Hence, predator induced trade-offs are a common phenomena observed in many different environments including both aquatic and terrestrial systems.

Given the widespread existence of trade-offs between foraging and predator avoidance behavior in ecological systems, many investigators have tested the fitness consequences of such trade-offs for the foragers. For example, experiments carried out in freshwater systems have shown that while small size potentially associated with reduced foraging in the presence of predators can reduce the likelihood of aquatic invertebrates being eaten by visual predators, it can also result in a reduction in fecundity (Allan 1981). In this instance, a life history strategy whereby small size is used as a predator avoidance strategy reducing mortality also results a trade-off for a reduction in the number of offspring contributed to the next generation (Manca et al. 2008, Peckarsky et al. 2001). Similarly, it has been suggested that in the classic predator-prey cycle involving the lynx and hare that lynx effect hare by not only consumption but also through a reduction in hare body size. Hare are able to evade and hide from the lynx more easily when smaller in size however, they pay for this size reduction in fecundity (Hik 1995).

In many terrestrial and aquatic systems (Peckarsky et al. 2008, Schmitz et al 2008), such nonconsumptive effects (NCE) of predators, whereby predators induce costly changes to prey behavior, life history or morphology (e.g., Boersma et al. 1998, Crowl and Covich 1990, Dodson et al.1994, Arnqvist and Johansson 1998), have been shown to have greater impacts on prey population growth than consumptive effects. For example, studies have shown that NCE of predators on bullfrog tadpoles cause changes in tadpole foraging location. These changes in foraging habitat have more widespread effects on tadpole populations than does consumption (Peacor and Werner 2001). In another example, snails raised with crab cues present have been shown to produce thicker shells as a defense mechanism. Although, this mechanism offers greater protection it also leads to a smaller overall body size resulting in a reduction in fecundity (Trussell and Smith 2000). Hence, NCE of predators on prey can have widespread and varying effects on prey that are important to take into account when studying the dynamics of predator-prey interactions.

We explored questions involving the costs and consequences of such trade-offs between food acquisition behavior and predator avoidance behaviors in high elevation streams found in the mountainous regions near The Rocky Mountain Biological Laboratory (RMBL). We specifically tested the NCE of predatory brook trout (*Salvelinus fontinalis*) on a highly abundant mayfly species *Baetis bicaudatus* (Ephemeroptera: Baetidae). *Baetis* is an important species in these high elevation stream ecosystems. It is the favorite prey item for not only brook trout but

also predatory stoneflies (Allan 1983, Peckarsky and Penton 1989). *Baetis* are extremely efficient periphyton grazers, which have a greater impact on periphyton (algae) food resources than any other grazer in the system (Álvarez and Peckarsky 2005). Because they are highly abundant and also influence food webs disproportionately to their abundance, they can be considered “strong interactors” in these stream ecosystems (Power et al. 1996).

To find periphyton *Baetis* mayflies use a foraging behavior known as drift. *Baetis* actively enter the water column and drift from rock to rock searching for food (Kohler and McPeck 1989). If food is scarce then they continue drifting until they find a rock with sufficient food (Peckarsky 1996). However, this grazing technique is risky for *Baetis* as it leaves them vulnerable, exposing them to predation by brook trout. Brook trout are visual feeders that lurk in stream waters waiting to consume drifting invertebrates. The risk associated with predation by brook trout presents *Baetis* with a trade-off between finding the best food resources and avoiding predation.

The objective of this study was to explore this trade-off experimentally by measuring the costs to *Baetis* in terms of lost foraging effort while avoiding getting eaten by brook trout. Past experiments have shown that NCE can lead to a greater reduction in *Baetis* population growth than actual consumption by predators (McPeck and Peckarsky 1998). This effect is due to size reduction of mature *Baetis* in the presence of brook trout (Peckarsky et al. 2002), which leads to a reduction in fecundity. In the presence of brook trout, *Baetis* also show a reduction in daytime feeding activity feeding (drifting), and drift predominately at night when they are less likely to be seen by brook trout (Peckarsky and McIntosh 1998). However, it is not known whether food availability or predator presence plays a larger role on *Baetis* behavior, which factor has greater consequences to *Baetis* performance, or whether there are interactions between those two factors.

To address those unknowns, we manipulated the environment of these mayflies using treatments with or without chemical cues from brook trout and different levels of periphyton resources in order to determine test for trade-offs between food availability and predator avoidance behavior. The experiment was motivated by an earlier one with a similar design carried out in mesocosms in summer 2001, during which only the behavior of *Baetis bicaudatus* was measured. In the 2011 microcosm experiment, we also tested the separate and interactive effects of food and predator risk on *Baetis* growth rates and their impact on the periphyton resource levels.

Methods

Both the 2001 mesocosm experiment and the 2011 microcosm experiment took place at the Rocky Mountain Biological Laboratory (RMBL) in Gunnison County, Colorado. In both

experiments water for the experimental units was filtered to remove invertebrates, but not algal propagules, and then gravity fed from a headwater fishless tributary (Upper Benthette 8: B8) near RMBL.

Preliminary 2001 Mesocosm Experiment:

In 2001 a preliminary experiment was completed that motivated the 2011 experiment. The experiment was designed to test the behavioral response of *Baetis bicaudatus* to variation in food resources and risk of predation, with the goal of determining whether their behavior reflected a greater response to resource acquisition or predator avoidance. Sixteen total cattle tanks (0.82 m² surface area) modified into flow-through experimental units were used including four treatments of four replicates each. *Baetis* obtained from Marmot Creek (a fishless stream located north of RMBL) were placed in 16 cattle tanks at a density of 350 per m². To simulate low food availability, 16 scrubbed and dried rocks were placed in eight of the cattle tanks. Sixteen rocks taken from the East River were placed in the other eight cattle tanks in order to simulate high food availability. A spectrophotometer was used to estimate chlorophyll *a* in order to confirm initial differences between low and high food treatments. The experiment also tested *Baetis* response to those high and low food levels (periphyton on rocks) in the presence or absence of water containing chemical cues from predatory brook trout. Water from a fishless stream (B8) was used as a no-fish predator control; and the same source of fishless water was delivered to a 110 L container containing two brook trout (*Salvelinus fontinalis*) and then dripped into half of each of the high and low food channels. Thus, the experiment included four treatments in a completely cross classified design: fish water/high food, fish water/low food, fishless water/high food, and fishless water/low food.

The behavioral response of *Baetis* to treatments was observed as drift. Observers counted the number of *Baetis* that drifted past a specific transect in each channel for five minutes at 1700, 2100, 0100, 0500, 0900, 1300, and 1700 Mountain Daylight Savings Time on 25-26 July 2001. Total numbers drifting during the seven observation periods were compared among treatments. All light samples and all dark samples were combined in order to calculate nocturnicity (night drift/total drift). A two-way MANOVA was used to test for fish and food effects on the two interdependent response variables, followed by two-way ANOVAs to test for main effects and interactions between food and fish cues on the individual response variables.

2011 Microcosm Experiment:

The 2011 experiment had a similar design to the 2001 experiment; however, it was carried out in smaller experimental units (0.016m² surface area), which enabled us to increase the replication from N = 4 to N = 15, giving the experiment much greater statistical power. For the 2011 experiment we used a streamside system of 60 circular flow-through chambers made of plexiglass (described and illustrated in Peckarsky and Cowan 1991). All chambers were shaded

by a white portable greenhouse (Hansen WeatherPORT, Delta, CO), which allowed enough light for plant growth on substrates and had normal diel light-dark cycles. The treatments measured *Baetis* response to high and low food levels (periphyton on tiles) in the presence or absence of water containing chemical cues from predatory brook trout. This experiment included the same four treatments as the 2001 mesocosm experiment in a completely cross classified design: fish water/high food, fish water/low food, fishless water/high food, and fishless water/low food. All treatments were randomly allocated throughout the channels in the portable green house. In this 2011 experiment three general types of response variables were measured: 1) *Baetis* predator avoidance and foraging behavior measured as total drift (day + night) and the ratio of night drift to total drift (nocturnicity), 2) costs of resource acquisition and predator avoidance measured as change in mg DW of *Baetis* throughout the experiment (growth rate), and 3) the impact of *Baetis* grazers on algae (change in chlorophyll biomass on tile substrates during the experiment). The experiment lasted for two weeks from 28 June to 12 July 2011.

To establish the high and low food treatments, the floor of each chamber was lined with six square unglazed ceramic tiles of approximately 2.25 cm² top surface area each. Low food treatments contained six tiles that were laid out in the sun to dry for two weeks. High food treatments contained six tiles that were incubated in cattle tanks located in the sun and covered with natural stream water continuously delivered from a first order fishless tributary (B8) to simulate natural stream conditions and deliver algal propagules. Plant fertilizer containing NH₄ and PO₄ (Continuous Release Bloom Booster, Scotts Miracle-Gro Company, Marysville, OH, USA) was added to the high food treatment cattle tanks on during the second week to stimulate algal growth. Periphyton was allowed to accumulate for two weeks on the high food tiles before the start of the experiment. Fifteen sets of six extra tiles were left in the sun and fifteen sets of six extra tiles were also allowed to grow periphyton during the same time period; those tiles were used to obtain a baseline chlorophyll *a* estimate at the beginning of the experiment. Standard procedures for extraction of chlorophyll in ethanol were used to estimate chlorophyll *a* on tiles at the beginning and end of the experiment (Nusch 1980). Sets of six tiles from each channel (end of the experiment) and sets of six tiles at the beginning of the experiment were extracted in 90% ethanol for 12 - 24 hours. A narrow bandwidth spectrophotometer was used to measure absorbance. Chlorophyll *a* was calculated using the standard equation for 90% ethanol extractions and expressed as µg chlorophyll *a*/m² (Nusch 1980).

At the end of the experiment we also scrubbed the stream channels with a soft bristle toothbrush to remove periphyton that had grown on the channel walls, floor and screen during the experiment. We estimated chlorophyll *a* that had accumulated on all of the channels using the same process that was used for the tiles to determine whether it varied among treatments.

Six early stage III *Baetis* larvae obtained from another headwater fishless stream near RMBL, Upper Benthette 3 (B3), were placed in each circular flow-through chamber. Early stage

III *Baetis* have begun their reproductive maturation, but were unlikely to emerge as adults during the two week experiment. Initial sizes of *Baetis* used in the experiment were estimated by preserving randomly selected individuals from the same samples as those included in the channels. Fifteen sets of six *Baetis* were dry weighed to obtain an average initial biomass per individual per channel. We brought them back to the lab in stream water and then placed them in a drying oven for 24 hours at 60°C. *Baetis* were then weighed to 0.01 mg accuracy using a Sartorius balance. Throughout the experiment some *Baetis* mortality was observed; however, *Baetis* were not replaced so that growth measurements were made on the original individuals. At the conclusion of the experiment all remaining *Baetis* were taken back to the lab in stream water. They were then dried for 24 hours at 60°C, and similarly bulk weighed in sets of six (or fewer if any mortality occurred) from each channel to obtain the biomass per individual *Baetis* remaining in each channel and average *Baetis* biomass per treatment.

Two brook trout (*Salvelinus fontinalis*) were captured by electrofishing from the East River near Avery picnic ground. At the conclusion of the experiment they weighed 93.2 g and 67.6 g and were 195 mm and 185 mm long (total length), respectively. They were put into a 110-L holding tank which received the water from the same fishless stream as the plexiglass flow-through chambers (B8). The brook trout were fed a diet of 50 prey (including *Baetis* and other mayflies collected from the East River) per trout every other day. Water from the trout holding tank was dripped into all the fish water treatments via tygon tubing at a rate of 3.39 mL/s. As a control, fishless water was dripped directly from the fishless stream at a rate 2.49 mL/s.

We used a Nixon micro-propeller current meter (Charlton Kings Industrial Estate, Cheltenham, Gloucestershire, UK) to measure current velocity in all 60 channels and compared the average current velocities among treatments using 2-way ANOVA. The overall average current velocity was 6.32 cm per second; and there were no significant difference among treatments ($p=0.3269$). Therefore, differences in current velocities could not account for variation of response variables among treatments.

Water temperature was recorded hourly using Onset HOB0 Pendant data loggers (Pocasset, MA, USA) placed in four locations of the Weatherport. The average water temperature was 5.8 °C 1.3. The maximum temperature throughout the two weeks of the experiment was 14.3 °C and the minimum temperature was 3.8 °C.

On day 2 of the experiment we measured behavioral responses of *Baetis* to predator and food treatments most closely approximating the initial chlorophyll levels, but after allowing *Baetis* to acclimate to the channels. Drift behavior was observed once in the daytime (at 1000) and once after dark (at 2200) Mountain Daylight Savings Time. Drift was measured by four observers each counting the number of mayflies drifting past a designated transect in 15 of the

stream channels for one minute. At night head lamps covered with red filters were used as red light has been shown to not affect *Baetis* behavior (Peckarsky and Cowan 1995). *Baetis* of this size class have been previously shown to become more nocturnal and to reduce their total drift behavior in the presence of chemical cues from brook trout (Peckarsky and McIntosh 1998, McIntosh et al. 1999, 2002). Therefore, we divided the number of individuals observed drifting in the night by the total number drifting (night drift/total drift) in each channel to obtain an estimate of drift nocturnicity to compare among treatments. *Baetis* have also been shown to use drift as a food search behavior, abandoning substrates with lower food levels (Peckarsky 1996). Therefore, estimates of total numbers drifting were compared among treatments.

At the end of the experiment we again measured drift because in past experiments drift has been observed to change periodicity and become more nocturnal at the end of experiments of this duration (Álvarez and Peckarsky unpublished data). It has been speculated that those trends may be caused by the high and low food treatments becoming more similar as both algal accrual and *Baetis* grazing occur. Therefore, we repeated the drift observations on the last day and night before the experiment ended. We then conducted a 2-way MANOVA on the second round of drift data (total drift and nocturnicity as interdependent response variables), followed by 2-way ANOVAs to test for significant main treatment and interactive effects of food and predator cues on each response variable separately. Also, by comparing the final chlorophyll *a* values among treatments (including the tiles and the periphyton from the channel walls) we were able to test whether initial differences in resource levels were maintained throughout the experiment.

The average initial weight of *Baetis* used in the experiment was $0.34 \text{ mg} \pm 0.02 \text{ SE}$. *Baetis* growth rates were calculated by dividing the total dry weight of the *Baetis* in each chamber by the number of *Baetis* retrieved at the end of the experiment, and then subtracting the initial average size of *Baetis* (0.34 mg) from the final size. This number was then divided by 14 to calculate the mg of growth per *Baetis* per day.

Chlorophyll *a* accrual was calculated by subtracting the average initial chlorophyll *a* measured on the tiles for each treatment from the final chlorophyll *a* for each channel. This number was then added to the amount of chlorophyll *a* measured on the channel walls to obtain the total amount of accrual per channel. Total algal accrual was then divided by 14 to obtain the $\mu\text{g}/\text{m}^2$ chlorophyll *a* accrual per day. Finally, we standardized algal accrual by the biomass of *Baetis* grazing in each channel as a measure of the ability of *Baetis* to suppress algal growth in each treatment.

Effects of the treatments on response variables were analyzed using a Multivariate Analysis of Variance (MANOVA) and subsequent 2-way ANOVAs to test for effects of main effects and interactions between trout cues and food availability on multiple interdependent response variables: 1) *Baetis* predator avoidance behavior on Day 2 of the experiment, measured

as both total drift and drift nocturnicity (ratio of night drift to total drift) , 2) costs of food acquisition under different predation risks measured as average change in per capita *Baetis* biomass (*Baetis* growth), and 3) treatment effects on the change in periphyton resource levels measured as change in chlorophyll *a* per day per unit *Baetis* biomass retrieved at the end of the experiment. This third response variable measures the impact of grazing *Baetis*, and is a measure of the performance of *Baetis* in terms of their ability to suppress algae growth on the tiles. The interaction between the food and predator treatments enabled us to test how food levels affected predator avoidance behavior, and the associated trade-offs in growth rates and grazing impact. All analyses were performed in [JMP] (SAS, North Carolina) using a significance threshold of 0.05.

Results:

Preliminary 2001 Mesocosm Experiment:

Results from the mesocosm experiment show clearly that total *Baetis* drift was significantly reduced in the high food treatments, not affected by trout cues, and that presence of trout cues did not significantly affect the response of *Baetis* to variability in food availability (Figure 1). While the 2-way MANOVA indicated marginally non-significant effects of trout presence and food availability on *Baetis* behaviors (Wilk's lambda=0.386, $p=0.0780$), subsequent 2-way ANOVAs showed that there was no trout cue effect on total drift, but a significant food effect and no trout cue $\times$ food interaction (trout cue: $p=0.6333$; food availability: $p=0.0038$; trout cue $\times$ food availability interaction: $p=0.9877$).

While there was a trend toward greater *Baetis* drift nocturnicity in the fish than in the fishless treatments (Figure 2), the 2-way ANOVA showed no significant effects of trout cues or food levels on nocturnicity of *Baetis* drift (trout cue effect, $p=0.2609$; food availability effect, $p=0.0740$; trout cue $\times$ food availability interaction, $p=0.9675$).

2011 Microcosm Experiment:

In the microcosm experiment the MANOVA Model testing for trout cue effects, food availability effects, and trout cue $\times$ food availability interactions on total *Baetis* drift, drift nocturnicity, *Baetis* growth, and grazer impact, was highly significant (Wilk's Lambda=0.103, $p<0.0001$). Neither trout cues nor food availability had significant effects; however, there was highly significant trout cue $\times$ food availability interaction indicating that the effect of food availability varied significantly with trout cue presence (MANOVA; trout cue effect, $p=0.1074$; food availability effect, $p=0.0721$; trout cue $\times$ food availability interaction, $p<0.0001$).

Individual 2-way ANOVAs were then performed to test for treatment effects on the response variables individually.

Behavioral Responses

Total drift was significantly higher in treatments with low food than in treatments with high food, and higher in no-fish than fish treatments (Figure 3). Thus, the 2-way ANOVA testing for effects on total drift showed that both trout cues and food availability significantly affected *Baetis* drift behavior with no significant interaction (trout cue effect, $p=0.0210$; food availability effect, $p<0.0001$; trout cue $\times$ food availability interaction, $p=0.7526$).

In contrast to the treatment effects on total drift, drift was strongly nocturnal in all treatments except when there was low food and no fish cues present (Figures 4 and 5). In that treatment, drift was aperiodic occurring similarly in both day and night. The 2-way ANOVA performed on drift nocturnicity showed that there was no trout cue effect or food availability effect but that there was a significant interaction between the two (trout cue effect, $p=0.1713$; food availability effect, $p=0.1595$; trout cue $\times$ food availability interaction, $p=0.0142$). Therefore the effect of trout cues varied significantly with food levels.

As in observations at the beginning of the experiment total drift near the end of the experiment was significantly higher in the low food treatments, but unlike the first observations, it was not affected by fish cues (Figure 6), which had a more subtle, yet significant effect early in the experiment (Figure 3). Thus, total drift responses to treatments near the end of the experiment were different from those of the earlier drift observations. The 2-way ANOVA showed no trout cue effect or trout cue $\times$ food availability interaction, but a significant food availability effect (trout cue effect, $p=0.2808$; food availability effect, $p=0.0174$; trout cue $\times$ food availability interaction, $p=0.5883$).

Also in contrast to the first set of drift observations, drift of *Baetis* in all treatments became primarily nocturnal near the end of the experiment (Figures 7 and 8). The 2-way ANOVA on nocturnicity showed no significant effects, (trout cue effect, $p=0.1216$; food availability effect, $p=0.9161$; trout cue $\times$ food availability interaction, $p=0.9161$).

Baetis Growth

Growth rates of *Baetis* were not affected by trout cues, but were significantly reduced in the low food treatments (Figure 9), and trout cues did not affect the influence of food on *Baetis* growth. The 2-way ANOVA showed no significant effect of trout cues on *Baetis* growth rates, a highly significant food availability effect but no trout cue $\times$ food availability interaction (trout

cue effect, $p=0.809$; food availability effect, $p<0.0001$; trout cue $\times$ food availability interaction, $p=0.1311$).

Grazer Impact

The algae on the high food treatments was examined at the beginning of the experiment and the dominant species were diatoms (Bacillariophyta) (identified by M. A. Penton). Specific diatoms observed on the tiles were *Achnantheidium*, *Caloneis*, *Diatoma*, *Fragilaria*, *Gomphonema*, *Meridian*, *Pinnularia*, *Cymbelloid*, and naviculoid diatoms. There were also some green flagellates, tiny flagellates, and coccoid golden algae (Chrysophyta). Past experiments have shown that *Baetis* prefers to eat diatoms, especially *Achnantheidium*, *Diatoma*, *Gomphonema* *Meridian* and naviculoids (Alvarez M, and M. A. Penton, unpublished data.)

There was a significant differences among high and low treatments in the amount of algae that accrued on the channel walls ($p<0.0001$; Mean difference= $0.014\mu\text{m}^2$). This could be attributed to easier propagation of cells from starting colonies (Sousa 1984, because there was no significant difference between fish and fishless treatments ($p=0.6189$). We included the chlorophyll *a* from the channel walls in the total chlorophyll as it provided another surface for grazing. Comparison of the initial to the final raw chlorophyll levels among treatments shows that high and low food treatments remained significantly different at the end of the treatment (Figure 10), but that chlorophyll *a* accrued at a faster rate in both of the low food treatments. Analyses of the impact of *Baetis* on chlorophyll *a* accrual showed that they were significantly more effective in suppressing algal accumulation in the high food treatments than in the low food treatments (Figure 11). The 2-way ANOVA on the change in chlorophyll *a* per day per unit *Baetis* biomass showed no trout cue effect but a highly significant food availability effect. There was no trout cue $\times$ food availability interaction (trout cue effect, $p=0.5283$; food availability effect, $p<0.0001$; trout cue $\times$ food availability interaction, $p=0.5122$).

Discussion

Both the 2001 mesocosm experiment and 2011 microcosm experiment demonstrate that trade-offs exist between resource acquisition and predator avoidance behavior of *Baetis bicaudatus*. While the 2001 experiment suggested a potential effect of both food and predator cues on drift behavior, in the 2011 experiment, which was better replicated, *Baetis* only exhibited daytime drift when there were not only no predator cues present but also when food was limited (Figure 3). Under all other conditions *Baetis* did not risk drifting during the day, potentially because the cost outweighed the benefit. Interestingly, by the end of the experiment when food levels had increased in the low food treatments, *Baetis* became completely nocturnal no longer taking the risk to drift during the day in the low food-no predator cue treatments. Similar to previous experiments, total drift was also reduced when trout cues were present (e.g. Peckarsky

and McIntosh 1998), but in this case, total drift was also suppressed when food levels were high; and trout cues did not affect the total drift response to low food levels (Figure 4). These results suggest that when resources are scarce *Baetis* drift more to optimize foraging efficiency, but they do so primarily at night when the risk of predation by visually feeding brook trout is lowest. Therefore, the behavioral responses of *Baetis* indicate trade-offs between foraging behavior and predator avoidance. However, no trade-offs were observed in the growth rates and grazer-algal impact responses to our manipulation of food availability and predator cues. In both cases *Baetis* growth rate and grazer-algal impact were significantly affected only by food availability, and not by the presence of predator cues. Furthermore, predator cues did not affect the influence of food levels on *Baetis* growth or impacts on algae.

The experiment focused on non-consumptive effects (NCE) of predators as they have been shown to not only induce behavior changes in prey, but also to exceed consumptive effects on prey in terms of population growth (McPeck and Peckarsky 1998). Specifically, it has been shown that trout affect *Baetis* through mechanisms other than just consumption by inducing them to change their drift behavior (Douglas et. al 1994, Forrester 1994). Thus, our experiment demonstrates the importance of studying the potential fitness costs of behavioral responses (NCE), which in this case showed that detection of chemical cues from predators cause *Baetis* to change their foraging behavior especially when resources are low. Other studies have shown similar behavioral response of prey to both predators and food (Kohler and McPeck 1989, Brown et al. 1992). Kohler and McPeck (1989) have shown that when fish predators are present *Baetis* spends less time drifting to new stones and more time foraging on the same stones. They further showed that when *Baetis* were hungry they exhibited riskier behavior and grazed on the surface of tiles more often where they are more vulnerable to predation (Kohler and McPeck 1989). However, that study did not measure the costs of such behavioral responses in terms of individual fitness.

The results of this study demonstrate that while *Baetis* grew significantly larger in high food than low food treatments, predator cues did not affect *Baetis* growth rates. *Baetis* grew at the same rate regardless of whether predator cues were present (Figure 9). This is fascinating as other studies have shown that a restriction in food availability in the presence of predators does affect prey growth rate of larval anurans (Niecieza 2000). Other studies have shown that increased food and decreased predation causes an increase in growth rate of male prairie voles (Desy and Batzli 2011). However, we observed no resource availability vs. predator avoidance trade-off on *Baetis* growth rates, in contrast to other studies that have shown such trade-offs.

In fact, a multi-year survey of many streams in the Upper East River drainage basin, some of which contain brook trout and others of which are completely fishless showed that *Baetis* growth rates did not differ between fish and fishless streams (Peckarsky et al. 2001).

Nonetheless, observations from that survey and previous experiments have shown that NCE of brook trout do have significant negative effects on *Baetis* fecundity. In streams containing trout, *Baetis* females emerge at smaller sizes and thereby oviposit fewer eggs. This cost cannot be attributed to reduced growth rates but instead to accelerated development rate in the presence of fish. Peckarsky et al. (2001) has shown that *Baetis* in trout streams develop at a significantly faster rate than *Baetis* in streams without trout, growing to the mature larval stage with up to a four-fold size difference between fish and fishless streams. Other studies have reported similar effects showing that pond invertebrates will develop to maturity at smaller sizes when fish are present (Vanni 1987, Manca et al. 2008).

Such effects of detection of trout cues were also induced experimentally in mesocosms (Peckarsky and McIntosh 1998) and in whole streams (Peckarsky et al. 2002). Our results experimentally documented the lack of an effect of trout on *Baetis* growth rates previously observed in the field (Peckarsky et al. 2001), but it was not carried out until *Baetis* were fully mature. Therefore, we did not observe the effects of trout cues on rates of *Baetis* development. Results of this and previous experiments suggest that the NCE of trout does not cost *Baetis* reduced growth rate but rather costs *Baetis* reduced fecundity due to accelerated development. Thus, in the presence of trout, *Baetis* do not grow more slowly, but nonetheless incur a size-fecundity trade-off maturing at smaller sizes in order to avoid predators, resulting in a reduction in overall fitness.

Similar to the lack of an observed food $\times$ predator risk trade-off on *Baetis* growth, we did not observe trade-offs on the impact of *Baetis* on algal accrual. However, a significant food effect indicated that *Baetis* performed better as grazers in high food treatments than low food treatments (Figure 11). Remarkably, *Baetis* are able to compensate for lost daytime feeding and reduced foraging time (lower total drift) and were not only able to grow at the same rate, but also had similar impacts on the accumulation of algae with predator cues and no predator cues. Thus, *Baetis*'s ability to graze algae was also not affected by the presence of predators. *Baetis* are extremely efficient grazers and it has been shown previously that predators do not affect *Baetis* ability as grazers (McIntosh et al. 2001, Álvarez and Peckarsky 2005). These results are in contrast to those of many other studies that have shown decreases in forager impact on their resources in the presence of predator risk. For example, Schmitz and Suttle (2001) showed that spiders changed foraging patterns of grasshoppers leading to an increase in grass growth. Another study showed that when stream invertebrates were exposed to trout algal levels increased (McIntosh and Townsend 1996). Amazingly, unlike other species, *Baetis* exhibit potentially costly predator avoidance behavior and yet are able to avoid resource acquisition costs. .

Despite the lack of observed trade-offs between food availability and predator avoidance behavior, *Baetis* grazing performance was affected by food availability. Interestingly, *Baetis*

were significantly better algal grazers in high food treatments than in low food treatments, performing better by preventing algal accumulation; whereas in low food treatments algae was able to accumulate at higher rates. This observation could be attributed to the differences in the type of food available between the high and low food treatments. High food treatments had well established algal colonies at the beginning of the experiment that *Baetis* could easily graze while low food treatments did not (chlorophyll levels were essentially zero at the beginning of the experiment). Furthermore, the types of diatoms that existed on the high food tiles at the beginning of the experiment were dominated by species of diatoms that have been shown to be preferred via examination gut contents of *Baetis* (*Achnanthes*, *Diatoma*, *Gomphonema* *Meridiana* and naviculoids: Alvarez M, and M. A. Penton, unpublished data.). Unfortunately we did not have enough tiles to examine diatoms on both food treatments at the end of the experiment. Nonetheless, the algae growing on the low food treatments was certainly more sparse and probably in an earlier successional state, which could have been harder for *Baetis* to graze than the algae that had already colonized the high food treatment tiles (Lamberti et al. 1987, Hill and Knight 1988). This speculation could account for the superior performance of *Baetis* grazing in the high food treatments. Other studies have reported similar effects showing that aquatic invertebrates including mayflies have mouth parts that are not effective at grazing understory algae (Lamberti et al. 1987, Hill and Knight 1988, Sumner and McIntire 1982). Those same studies have also suggested that when periphyton reaches greater heights it is more easily dislodged by grazers as they move about on the substrate searching for food (Lamberti et al. 1987, Hill and Knight 1988). We speculate that in the low food treatments certainly early on and possibly late in the experiment predominantly understory algae may have been present making it more difficult for *Baetis* to graze as effectively.

In summary, non-consumptive effects of brook trout on *Baetis* caused behavioral changes that when coupled with low resources resulted in a significant change in *Baetis* foraging behavior. Thus, when predatory brook trout cues were combined with low resources *Baetis* exhibited foraging behavior consistent with trade-offs between predator-avoidance and food acquisition. In order to avoid mortality caused by predators, *Baetis* must either adjust by reducing daytime drift or increase the risk of mortality through predation. The microcosm experiment suggests that given this trade-off *Baetis* will chose to reduce daytime drift becoming more nocturnal when predator cues are present in their environment. Astonishingly, this reduction in overall drift was not observed to cost *Baetis* in terms of growth rate or their ability to perform as algal grazers. Nevertheless, previous studies in which *Baetis* were reared to the mature larval stage demonstrated a size-fecundity trade-off, with accelerated larval development to avoid predation, which results in maturation at a smaller size in more risky environments with brook trout present. Such accelerated development to mature at smaller sizes then results in a reduction in fecundity of female *Baetis*. We conclude that trade-offs play important roles in the behavior and fitness of organisms such as *Baetis* and are key to understanding food web

interactions. Thus, it is vital that trade-offs be taken into account when studying natural communities.

Acknowledgements

I would like to thank Bobbi Peckarsky for agreeing to be my mentor and making this project happen. She has been such an amazing mentor, reviewing numerous drafts of papers, giving great advice, and helping me through this entire process. I would also like to thank Maruxa Álvarez for her preliminary work in 2010 also showing the lack of a predator cue effect on growth of *Baetis*, and the 2001 Benthettes for completing the mesocosm experiment that motivated the 2011 microcosm experiment. I owe a great deal of thanks to Wendy Brown, Marge Penton, Carrie Robbins, Steve Horn, and Tracy Smith for help setting up and taking down the experiment and contribution of ideas. I also thank the National Science Foundation for funding through the Research Experience for Undergraduates Program and the Rocky Mountain Biological Laboratory for hosting me and allowing me to complete this experiment.

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Figures:

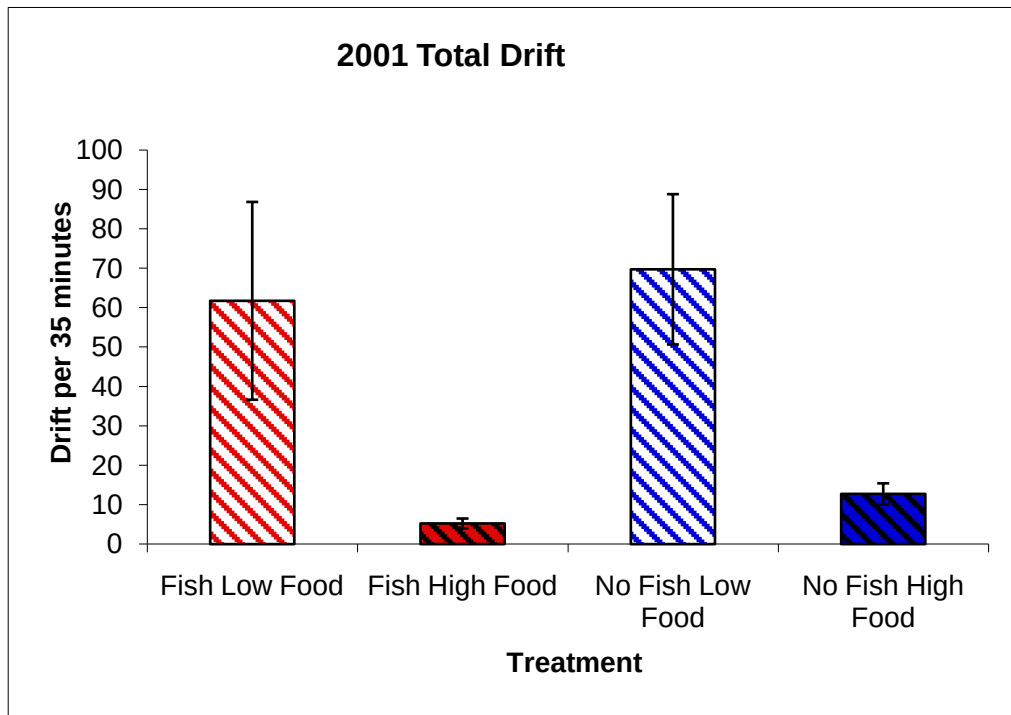


Figure 1 - Mesocosm Experiment 25 – 26 July 2001. Mean number of total *Baetis* drifting for 35 total minutes during 7 observation periods of 5 minutes each per day (Mean $\pm$ SE; N=4). Day drift was measured for five minutes at 1700, 0500, 0900, 1300, and 1700 MDT added to night drift measured for five minutes at 2100 and 0100 MDT.

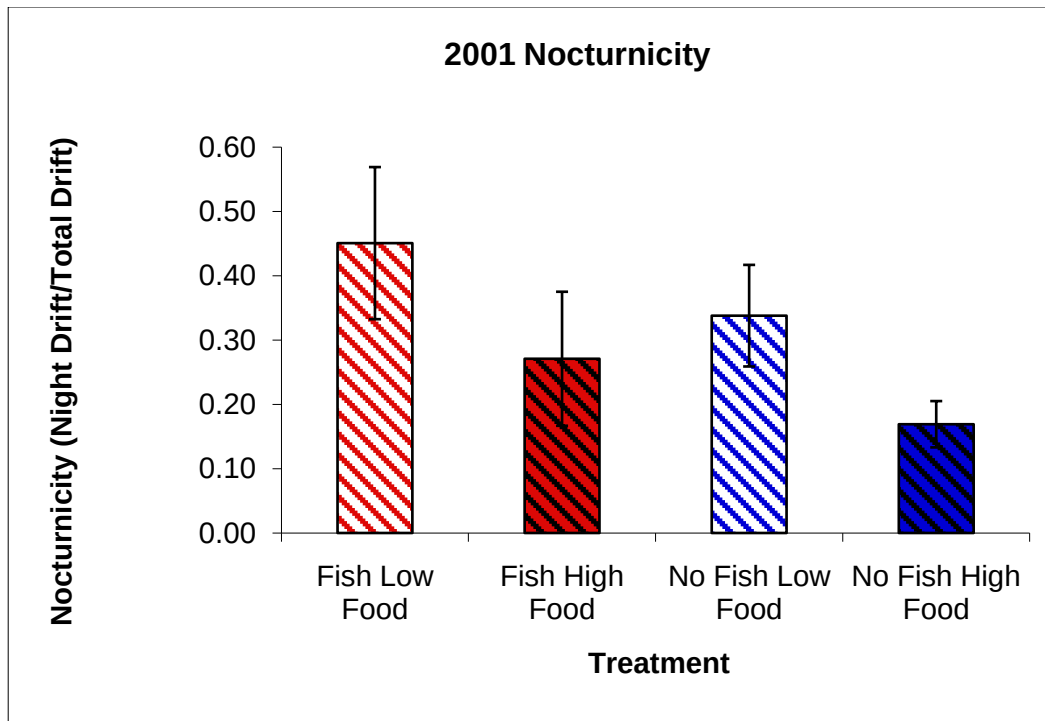


Figure 2 - Mesocosm experiment 25 – 26 July 2001. Mean nocturnicity for each treatment (mean $\pm$ SE; N=15). Nocturnicity was calculated as the number of *Baetis* drifting during the night divided by the number of total *Baetis* drifting.

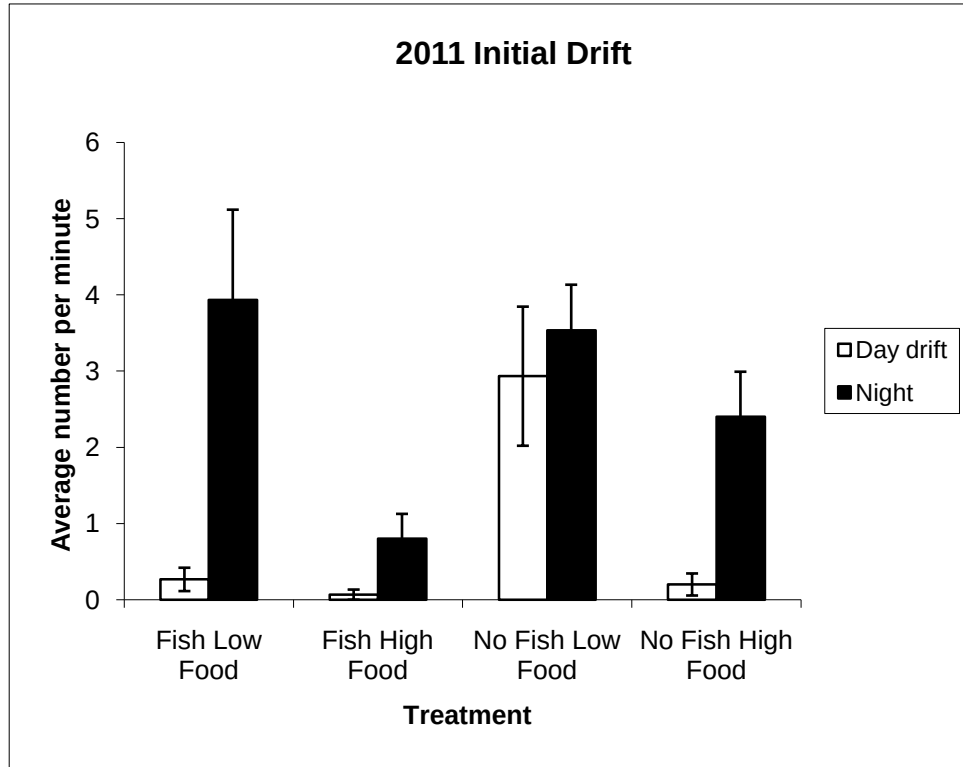


Figure 3 - Microcosm Experiment 30 June 2011. Mean *Baetis* day and night drift (Mean $\pm$ SE; N=15). Day drift measured at 0930 MDT (shown as white bars) and night drift measured at 2130 MDT (shown as dark bars).

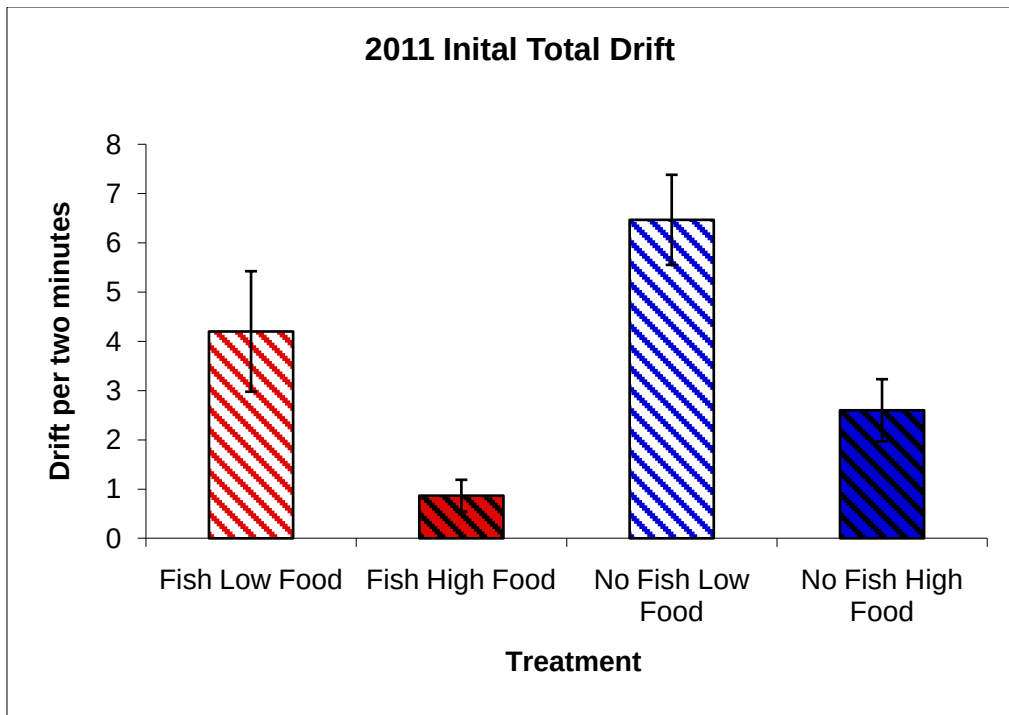


Figure 4 - Microcosm experiment 30 June 2011. Mean number of total *Baetis* drifting for two minutes (Mean $\pm$ SE; N=15). Day drift measured for one minute at 0930 MDT (N=15) added to night drift measured for one minute at 2130 MDT (N=15).

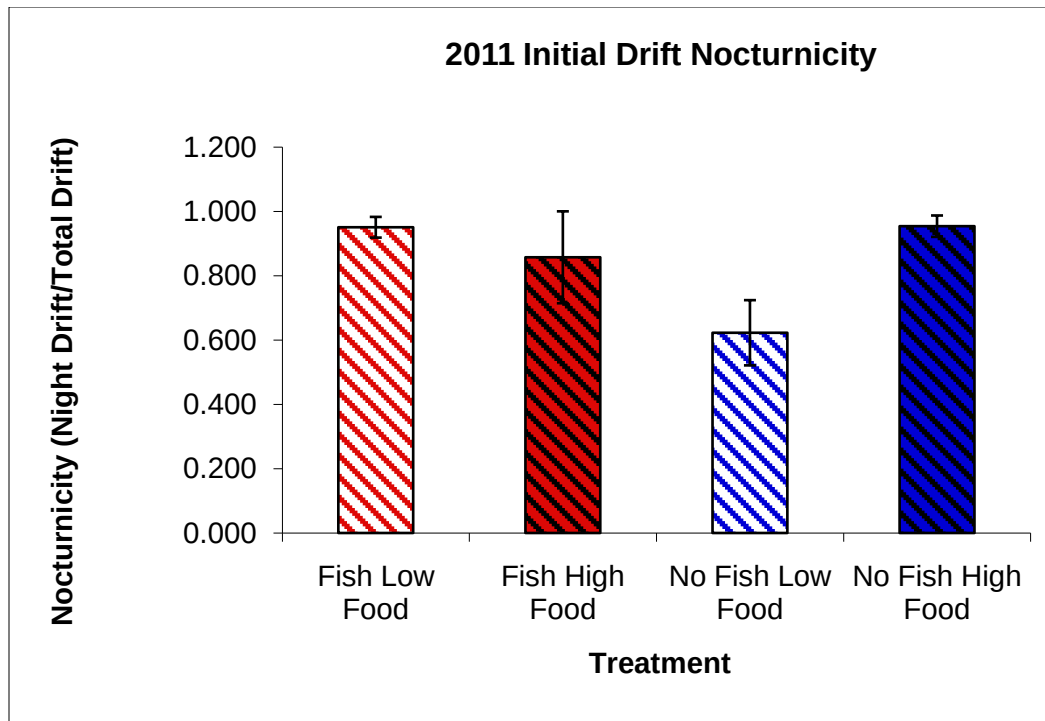


Figure 5 - Microcosm experiment 30 June 2011. Mean nocturnicity for each treatment (Mean $\pm$ SE; N=15). Calculated as the mean number of *Baetis* drifting during the night divided by the mean number of total *Baetis* drifting.

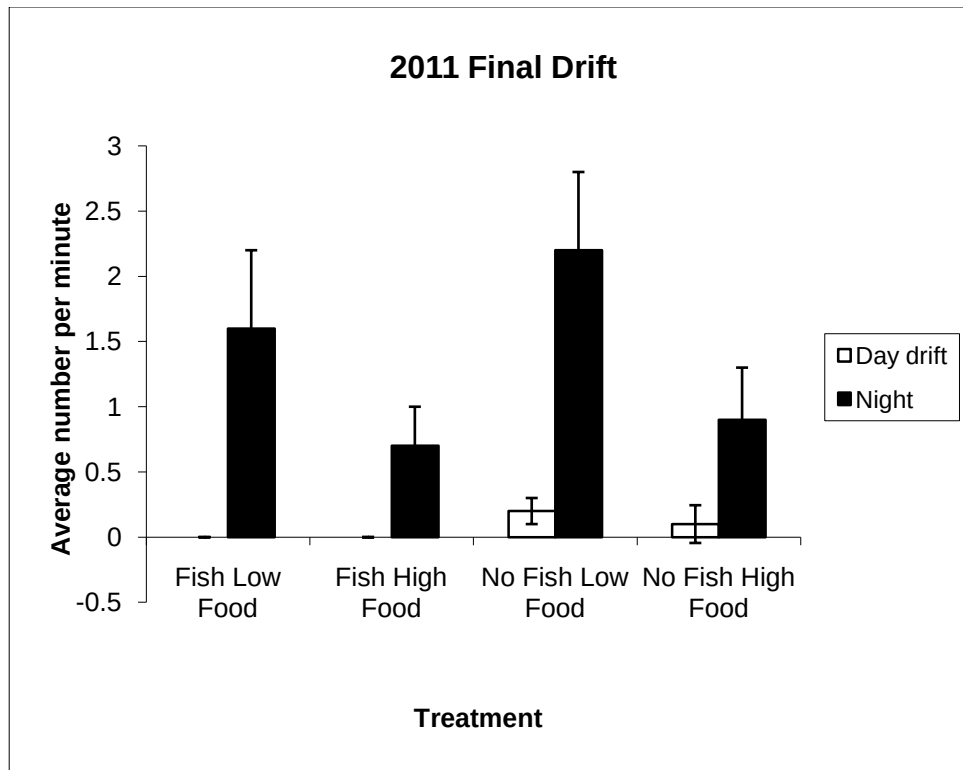


Figure 6 - Mean *Baetis* day and night drift (Mean $\pm$ SE; N=15) taken near the end of the microcosm experiment 11 July 2011. Day drift measured at 0930 MDT (shown as white bars) and night drift measured at 2130 MDT (shown as dark bars).

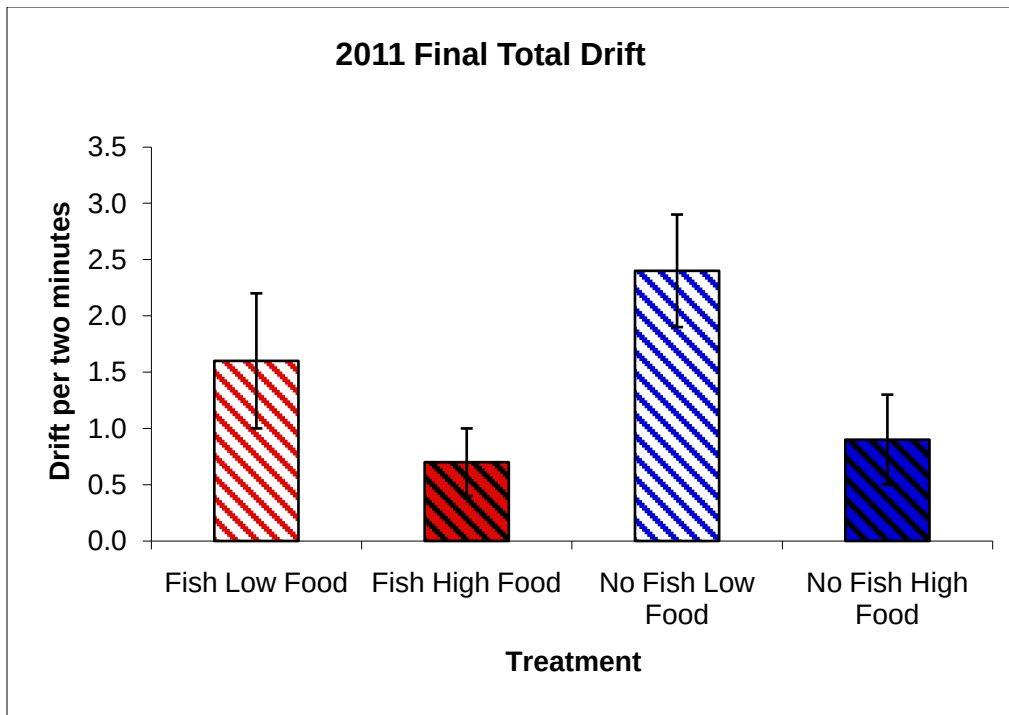


Figure 7 - Mean number of total *Baetis* drifting for two minutes (Mean $\pm$ SE; N=15) near the end of the microcosm experiment (11 July 2011). Day drift measured for one minute at 0930 MDT (N=15) added to night drift measured for one minute at 2130 MDT (N=15).

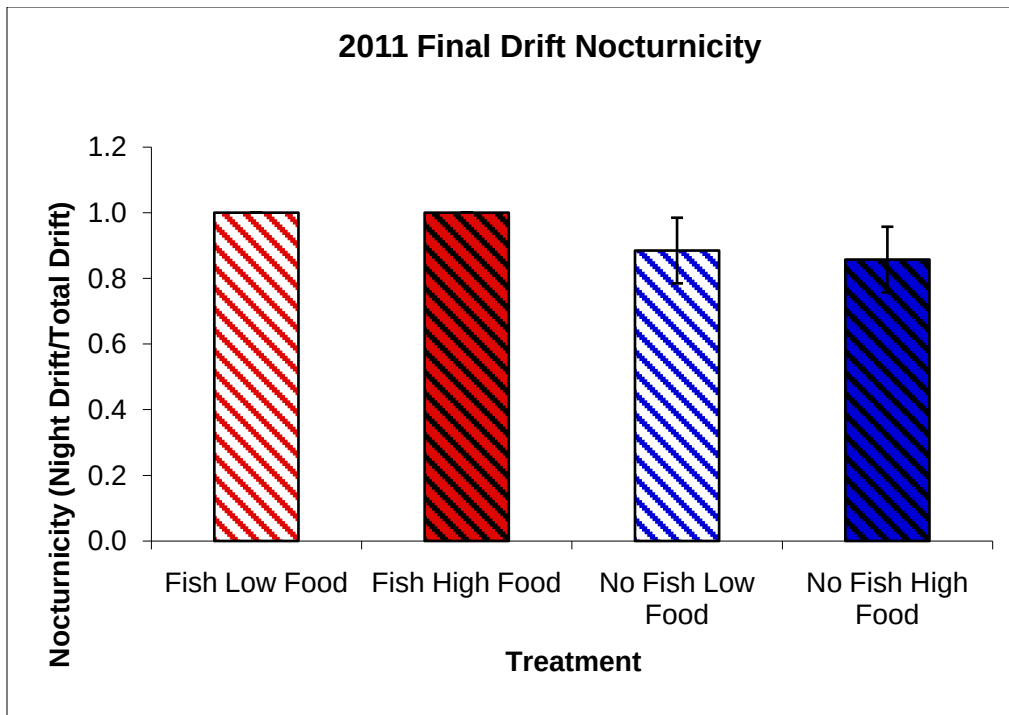


Figure 8 - Mean nocturnicity for each treatment (Mean $\pm$ SE; N=15) measured near the end of the microcosm experiment (11 July 2011). Calculated as the number of *Baetis* drifting during the night divided by the number of total *Baetis* drifting.

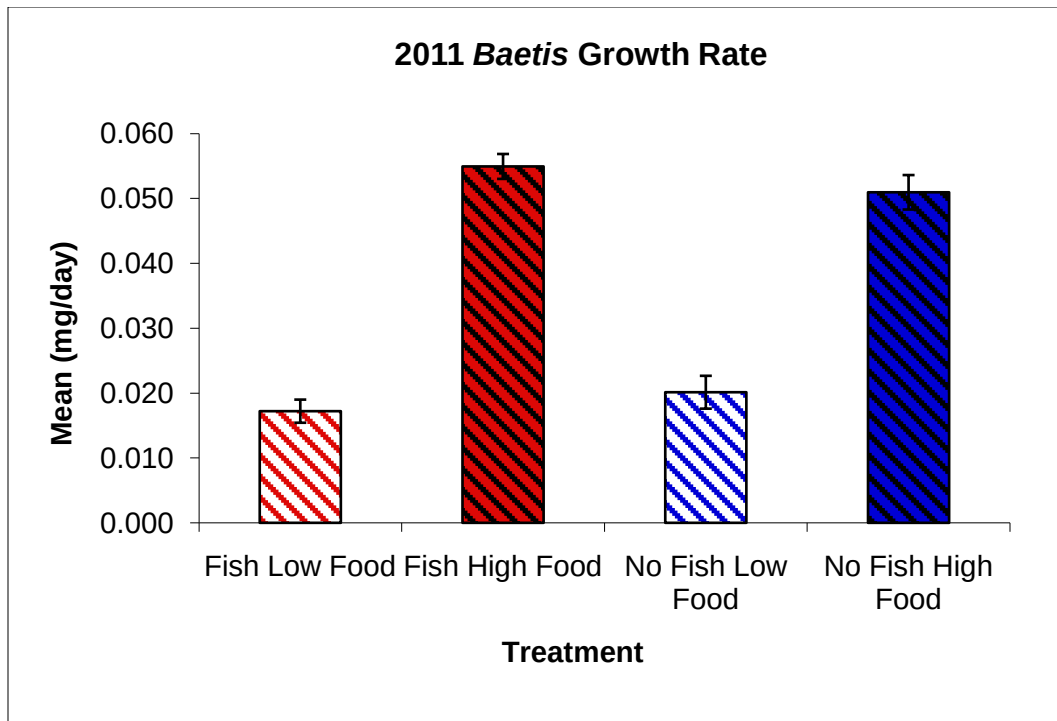


Figure 9 - Mean *Baetis* growth measured as mg of growth per day (Mean $\pm$ SE; N=15). *Baetis* growth rate was calculated by subtracting the mean initial *Baetis* weight from the final *Baetis* weight and dividing by 14 d (the length of the microcosm experiment; 28 June to 12 July 2011).

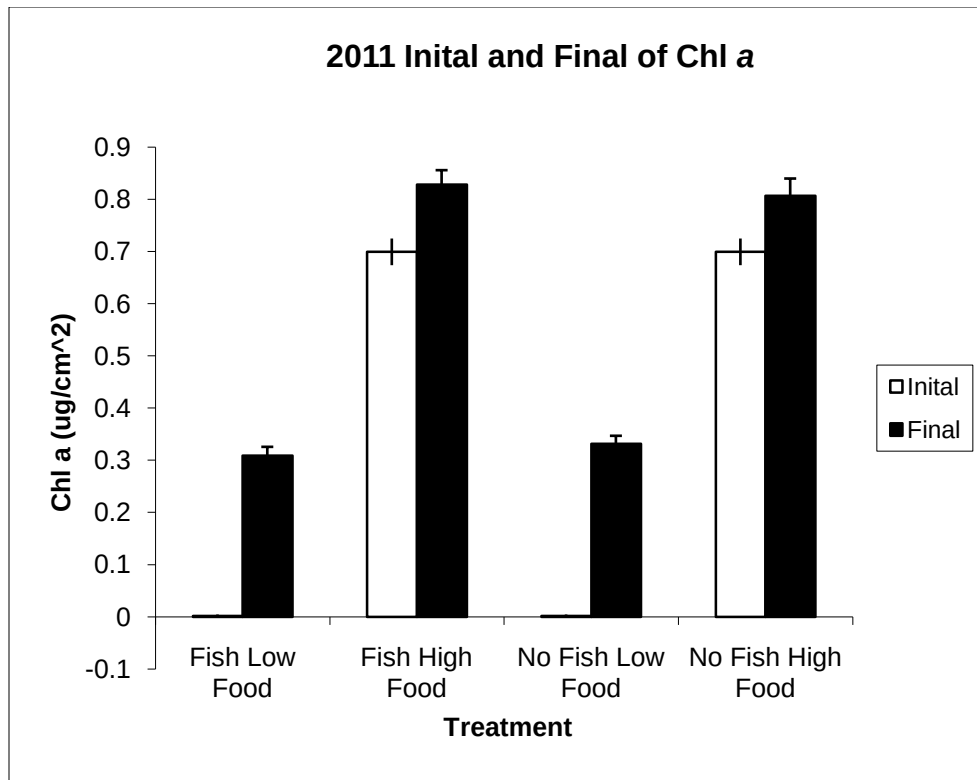


Figure 10 - Mean chlorophyll *a* from the start of the microcosm experiment (initial) to the end of the experiment (final) 28 June to 12 July 2001. Chlorophyll *a* was measured as mg per m². Initial chlorophyll *a* (white bars) and final chlorophyll *a* (dark bars) for each treatment (Mean ± SE; N=15).

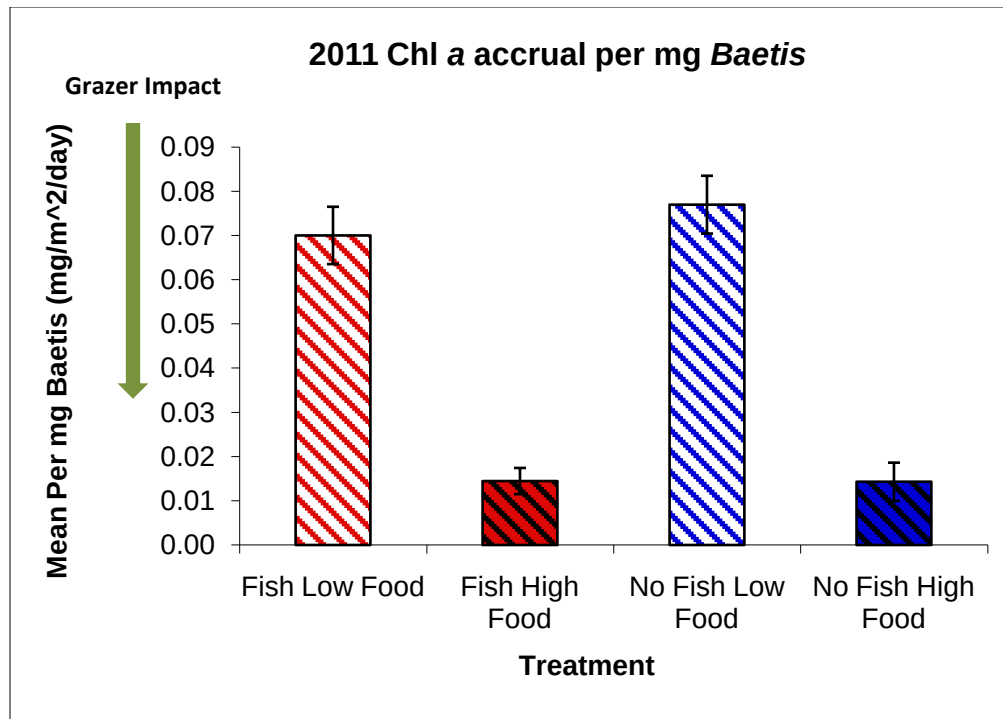


Figure 11 - Chlorophyll *a* accrual per mg *Baetis* per day over the 14 d microcosm experiment 28 June to 12 July 2011. Mean change in chlorophyll *a* (final – initial) was divided by grazer biomass per channel (mg) and 14 days. (Mean ± SE; N=15).