

Herbivore growth responses to nutrient mobilization by detritivores

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Abstract

Past research suggests that the detritus pathway, or brown trophic pathway, is key to the availability of nutrients in nutrient-limited aquatic ecosystems. The interplay between the green and brown paths in aquatic food webs is relatively understudied, yet it is likely that the productivity of nutrient-limited aquatic systems relies on the bottom-up effects of nutrients released during detritus processing. The goal of my study was to clarify the role of detritivorous caddisfly larvae (*Limnephilus externus*), as mobilizers of detritus-bound nutrients in montane ponds? Specifically, I wanted to understand the role that these caddisflies play as joint regulators of the brown (detritus) trophic path (via conversion of detrital to animal production) in these ponds and of the green (algal) trophic path via the mobilization of limiting nutrients for algae? In asking these questions I hypothesized that caddisfly larvae are key to the ecological function of wetland ecosystems because they (1) liberate detritus-bound nutrients that stimulate algal growth, and (2) increase algal growth and stimulate a bottom-up effect on herbivore growth. I tested these hypotheses by manipulating detritivore density and the presence (algal growth responses to nutrient liberation) and absence (control for algal growth responses) in field laboratory microcosms. Results of my experiment revealed (1) a positive correlation between larvae density and herbivore growth rate, suggesting a bottom-up effect on growth rate; (2) that *L. externus* may be key to nutrient availability in nutrient-limited aquatic systems and directly tied to function and productivity, which emphasizes the importance of single-species roles in trophic ecology; and (3) that the green trophic pathway is likely dependent on the brown trophic pathway.

Introduction

Wetland ecosystems are the home to diverse fauna and the unique ecological processes of macroinvertebrates. These processes are implicated in intricate trophic interactions that can act as a microcosm for understanding many different community structures. Aquatic researchers have begun to study wetlands from the community scale in order to generate an accurate knowledge of the complex ecology of such systems. The factors that regulate the composition and structure of wetland environments is relatively understudied, in part because they are physically intermediate between those of deep-water aquatic (ponds and lakes) and terrestrial habitats. Because unique techniques and approaches are needed to study shallow, intermittent habitats like wetlands, the importance of macroinvertebrates has often been overlooked when considering the ecological importance of the wetland as a whole (Batzner & Wissinger 1996). The understanding of wetland processes is incomplete without the knowledge of the role that macroinvertebrate communities play in maintaining the integrity and function of the ecosystem.

The Rocky Mountains of Colorado provide a pristine environment for subalpine wetlands that persist as important systems for vertebrates and invertebrates alike. Our study site is the Mexican Cut Nature Preserve, which includes a subalpine (3400-3500m elevation) wetland/pond complex in the mountains of the White River National Forest. The Mexican Cut Nature Preserve (MCNP) is composed of over 50 small, shallow basins that vary along a permanence gradient from temporary to permanent ponds (Wissinger, 1999b). The ponds are extremely oligotrophic (extremely low nitrogen and phosphorus) and are subject to high levels of UV radiation and fluctuating temperatures. Inputs from the terrestrial environment consisting of needles, twigs, and leaves are the primary food sources for detritivores.

Caddisfly larvae are one of over 100 invertebrate faunas that inhabit both the temporary and permanent ponds of this wetland complex (Wissinger, 1999b). Because the majority of aquatic insects are beneficial generalist consumers, macroinvertebrates play a major role in determining the trophic ecology of the systems they inhabit (Cummins, 1973). Caddisflies in the MCNP have been the interest of many studies that attempt to understand the ecology of benthic invertebrates and their role in nutrient cycling and energy flow in subalpine wetlands (Wissinger et al 2006).

The caddisfly communities of MCNP are made up of several species of the order Trichoptera, family Limnephilidae. Limnephilid caddisflies have complex life histories during which they inhabit aquatic habitats as larvae and terrestrial habitats as metamorphosed adults. Limnephilid larvae are case-building detritivores that feed on allochthonous inputs of vascular plant detritus and often supplement their diet with algae or animal material, as growth rates are limited by quality of food (Jannot, 2008). The consumed detritus is laden with bacteria and fungi, which provide the necessary nutrients for larval development (Wissinger, 1999a).

Previous work at the MCNP suggests that the presence of caddisfly communities in wetland ecosystems is vital to the processing and accessibility of nutrients for lentic systems (Klemmer, et al. 2012). As part of the dominant detrital food chain, caddisfly larvae play an important role in the transport of allochthonous nutrients across the terrestrial system to the wetland, by the CPOM shredder-FPOM collector pathway (Wissinger, 1999a). It is likely that the processing of detritus not only benefits the caddisflies (and their predators), but also provides nutrients for autotrophs inhabiting the same areas. In various studies, the shredder role of the caddisfly larvae in lentic food webs is directly correlated with specific ecosystem functions, such as nutrient availability and productivity (Klemmer, et al. 2012; Thornton and Wissinger, ms.).

Primary production and photosynthesis rely on the processes of detrital food chains to provide significant amounts of carbon inputs, as well as other organic materials that are extracted from the detrital processing (Cummins, 1973).

In the case that algal primary producers, namely benthic substrates, are benefitting from the presence of macroinvertebrates and their ability to provide accessible nutrients through excretion of processed detritus, such macroinvertebrates become directly correlated to the productivity of the ponds. In this way, the success of the green (algal) trophic pathway in lentic systems could be dependent on the brown (detritus) trophic pathway. Furthermore, caddisflies who later graze the primary production of autotrophs are benefitting twice from the processing of detritus; first, when the caddisfly consumed the detritus, and second, when the caddisfly consumed the primary production that profited from caddisfly nutrient excretions (Thornton & Wissinger, ms.). This conclusion would illustrate the importance of caddisfly communities as a permanent link in nutrient cycling of lentic systems.

Specifically, I hypothesize that the nutrients liberated by detritus-processing caddisflies should stimulate algal growth. I also hypothesize that this increased algal growth should result in a bottom up effect that stimulates herbivore growth. This nutrient cross connection between the brown trophic path (detritus) and the green trophic path (algae) in aquatic food webs is poorly studied (Vanni et al., 2002; Webster et al., 2009).

Methods

This study was conducted at Rocky Mountain Biological Lab in an experimental WeatherPort (Global Shelters, Gunnison, CO) that housed 36 experimental microcosms (0.25m² plastic containers) to simulate natural pond systems. The 12 treatment combinations (6 caddisfly

densities [0, 16, 32, 64, 128, 256], light vs. dark) x light vs. dark were assigned in a stratified random pattern to minimize the effects of light and temperature gradients in the WeatherPort. The range of densities mimics that observed over the past 25 years in natural ponds near the RMBL (Klemmer et al. 2012). This design allowed me to determine herbivore growth response to algal biomass stimulated by nutrient mobilization. The dark treatment microcosms were made opaque by covering all sides with duct tape and placing a lid, slightly elevated for air flow, over the top. All microcosms were divided in half (two 0.125m² sides) with coarse-grained mesh (5mm opening), allowing nutrients released during detritus processing by caddisflies on one side to pass through and stimulate algal growth and herbivores (snails) on the other side. Because the caddisflies also graze periphyton (Klemmer et al. 2012), I also tested the hypothesis that the algae stimulated by their detritus processing provided them with a secondary nutritional benefit.

The microcosms were filled with well water and ~25g of detritus was added to one side of each five days before the experiment began. The microcosms were inoculated with 240ml of inoculum (120ml each side) five days prior to the start date in order to microbially condition the detritus, making it a viable food source for caddisflies. The inoculum was biofilm gathered from mesocosms located near the study site, which consisted of a starter culture of bacteria, fungi, and algae obtained by scraping the sides of the mesocosms. The detritus was a mixture of grasses and sedges gathered from a dried fen in the Mexican Cut Nature Preserve. The detritus was dried for three days, and then weighed and ~25g was added to the microcosms.

To determine the effect of nutrient mobilization by caddisfly larvae on herbivore growth, caddisflies (*Limnephilus externus*) and snails (*Stagnicola elodes*) were added to respective sides of each microcosm. Caddisfly larvae were added according to six historic densities (0, 16, 32, 64, 128, 256 m⁻²) and randomly assigned treatments with three replicates of each (186 larvae total).

Ten snails were added to the other side of each microcosm. Snails were blotted dry and weighed to the nearest 0.0001g and then added so that each microcosm had a total herbivore biomass between 2.4000-2.6000g. Snails were reweighed at the end of the experiment to determine changes in body mass. Caddisflies and snails were harvested from mesocosms near the study site at RMBL.

Ammonium and phosphorus levels of each microcosm were analyzed weekly by water sampling and processing, beginning with an initial sample (week 0) to establish a baseline measure of well water nutrient levels. Ammonium sampling took place on each Friday for three weeks. 40ml of water was sampled from the detritivore side of each microcosm and filtered into 60ml opaque bottles. In the lab, ten ml of working reagent (Taylor, et al. 2007) were added to each bottle. After allowing the samples to react for five to six hours, each sample was poured into a cuvette and inserted into a fluorometer (Turner Designs Triology Laboratory, model 7200, Sunnyvale, CA) to obtain a fluorescence value.

Phosphorus samples (filtered 50ml water samples from each microcosm) we collected on each Friday for three weeks. In the lab, 0.4g of potassium persulfate were added to each and the vials were boiled in a pressure cooker for 50 minutes and allowed to cool. I then added 5ml of mixed reagent (Strickland & Parsons, 1968) to each vial and absorbance values at 885nm for each sample were read by a spectrophotometer (Thermo Scientific, Genesys 10S UV-Vis, Waltham, MA).

To measure algal biomass, six periphyton tiles were added to each side of each microcosm. There were three grazed and three ungrazed; grazing was prevented by mesh enclosures. The periphyton tiles were inoculated for seven days prior to the experiment with inoculum in the same manner as the microcosms.

For chlorophyll-*a* analysis, one grazed periphyton tile and one ungrazed periphyton tile were gathered from both the caddisfly and the snail sides of each microcosm each week, beginning with week 2, in order to measure changes in algal biomass. The tiles were placed in separate Whirl-Paks (Nasco, Fort Atkinson, WI) and ten ml of 90% buffered ethanol were added to each. Chlorophyll-*a* was extracted for six to twelve hours. Three ml of each sample were placed in a cuvette and the fluorescence value was obtained using a fluorometer (Turner Designs Trilogy Laboratory, model 7200, Sunnyvale, CA). Following an initial reading, the samples were acidified with 30uL of 0.1N HCl for two minutes and measured again to adjust for the presence of pheophytin (after Nusch, 1980).

A two-way repeated-measures MANOVA followed by individual ANOVAs on multiple interdependent response variables tested for the effects of week, caddisfly density, and light and dark treatments on ammonium concentration, phosphorus concentration, chlorophyll-*a* concentration, and herbivore growth rate.

Results

Detritus analysis

Density had a significant positive effect on quantity of detritus consumed ($F=13.97$, $p<0.0001$; Fig.1). Light treatment had no effect on quantity of detritus consumed ($F=0.53$, $p=0.47$). There was no effect of the interaction between density and light treatment on quantity of detritus consumed ($F=1.05$, $p=0.41$).

Ammonium analysis

Results of ammonium analysis provided unusable data, possibly due to human error in sampling or in pipetting during sample preparation. Because of this ammonium data has been excluded from the results of the experiment.

Phosphorus analysis

Phosphorus concentrations significantly decreased as a function of week (significant time effect: $F=13.42$, $p<0.0001$; Fig.2). However, caddisfly density did not affect phosphorus decrease over time (no significant density $\times$ time interaction: $F=1.03$, $p=0.43$). Phosphorus concentration was greater in the dark than the light treatments (Fig. 3, significant light effect: $F=284.81$, $p<0.0001$), and the change in phosphorus concentration over time differed between light and dark treatments (significant light $\times$ time interaction: $F=17.95$, $p<0.0001$). There was also a significant interaction between phosphorus responses to light treatments and caddisfly densities (Fig. 2, $F=2.21$, $p=0.02$). In light treatments, there was no effect of density (Fig.4), yet in dark treatments there was an effect of density on the change in phosphorus over time. In densities 0, 16, and 32 there was a slight decrease in phosphorus over time, but in densities 64, 128, and 256 there was a significant decrease in phosphorus over time (Fig.5).

Chlorophyll-a analysis

Chlorophyll-*a* concentration significantly increased over time ($F=5.92$, $p=0.004$), but the change over time was not affected by light treatment ($F=0.28$, $p=0.60$) or by caddisfly density ($F=1.44$, $p=0.16$). However, the change in chlorophyll-*a* concentration over time was significantly different between light and dark treatments (significant time $\times$ light interaction:

F=4.34, p=0.02). While chlorophyll-*a* concentration increased over time in the dark, it remained constant in the light (Fig.6). This differential response between light and dark treatments in chlorophyll-*a* concentration was not affected by density (no significant light $\times$ density interaction: F=0.70, p=0.72). Change in chlorophyll-*a* concentration on tiles over time was significantly different among grazing treatments (F=2.40, p=0.03). Tiles grazed by caddisflies had a small increase in chlorophyll-*a* over time, while ungrazed tiles showed a larger increase (Fig.7). However, there were no significant changes in chlorophyll-*a* on tiles grazed by snails or on ungrazed tiles on the side of the microcosms with snails (Fig.8). The response of chlorophyll-*a* among grazing treatments was not affected by caddisfly density (F=.64, p=0.93), light (F=0.42, p=0.87) or among all possible treatment combinations (no significant three-way interactions: F=0.38, p=0.70).

Herbivore growth analysis

Caddisfly density had a significant positive effect on mean herbivore growth rate (F=3.94, p=0.01; Fig.9). Mean herbivore growth rate varied between light and dark treatments (F=39.73, p<0.0001), with higher snail growth rates in the light treatments (Fig.10). However, this light-dark difference in snail growth rates did not vary with caddisfly density (no significant light $\times$ density interaction: F=1.81, p=0.15).

Discussion

The purpose of this study was to investigate the understudied connection between the detrital (brown) trophic pathway and the algal (green) trophic pathway in aquatic ecosystems. Recent work has begun to include detrital dynamics into mapping of trophic interactions in order

to answer questions regarding the re-entry of nutrients into aquatic systems and the structure of aquatic food webs (Moore et al., 2004). Previous work suggests that benthic invertebrates are necessary for the translocation of nutrients from terrestrial ecosystems to aquatic ecosystems, especially in those that are nutrient limited (Vanni, 2002). To this end, detritivorous species such as *Limnephilus externus*, the focal species of my study, play an important role in determining ecosystem productivity. Here I used the cross-connection between the brown and green pathways, specifically herbivore growth responses to nutrient mobilization by detritivores, to further elucidate the role of detritivorous caddisflies in nutrient-limited ponds like those of the MCNP.

Detritus processing and nutrient mobilization

My results revealed a significant positive effect of caddisfly density on the rates of detritus breakdown. In microcosms with the highest density of caddisflies, detritus breakdown was double that of the microcosms containing the lowest density of caddisflies. This result suggests that higher densities of caddisflies will be able to process more detritus and release more detritus-bound nutrients into the surrounding system. In a broader context, factors such as predation or human-impacts that alter the densities of detritivores in natural systems could significantly alter the availability of nutrients and the energy of the trophic web (Klemmer et. al., 2012).

Results of the study also revealed a significant effect of time on water phosphorus concentration and correlated chlorophyll-*a* concentration. Overall, water phosphorus decreased over time and chlorophyll-*a* increased over time, suggesting that in this closed system

phosphorus was rapidly taken up by algal primary producers. This significant decrease in phosphorus concentration over time and density contradicts previous microcosm studies (Thornton 2010, Klemmer 2012), likely because this study has phosphorus uptake by primary producers and by herbivores. This is supported by the significant difference in phosphorus concentration between light and dark treatments; in the light treatments there was less phosphorus present in the water because it was likely immediately taken up by algae for primary production stimulated by available light.

Herbivore growth response

Increased density of caddisflies and the presence of light led to higher growth rates in the herbivore *Stagnicola elodes*. While there was no significant effect of light on chlorophyll-*a* concentration, this may be confounded by the consistent grazing of herbivores, causing lower chlorophyll-*a* presence at the time of sampling. This is likely as the results show a differential response in herbivore growth rate in light and dark treatments; such a result suggests that the light treatment produced more algae, which provided more food for herbivores, stimulating the elevated growth response. The positive correlation of caddisfly density and herbivore growth rate supports the hypothesis that higher densities of caddisflies release more detritus-bound nutrients, which stimulate algal growth and that this increased algal growth results in a bottom-up effect of stimulated herbivore growth.

Conclusions and Implications

The data obtained by this study suggest that caddisflies are key to the ecological function of these subalpine wetland ecosystems and present a model for understanding trophic

interactions and community ecology of lentic areas. Wetlands not only provide important ecosystem services such as water supply and carbon sources, but also interact with nutrient cycling of neighboring terrestrial habitats, making them a vital system for ecosystem function as a whole (Boyer & Polasky, 2004). It is important that invertebrate communities of various wetlands are recognized as unique and deserving of preservation, as many of the taxa found there are not present in terrestrial or aquatic systems (Wissinger, 1999a).

This experiment provides evidence that without such detritivores, it is likely that aquatic systems would experience specific losses in ecosystem function and productivity. By mapping the productivity of the green trophic pathway as a function of the brown trophic pathway, it becomes clear that the two are equally important to the success of the system as a whole. Understanding the relationship between this single-species and ecosystem productivity is vital to maintaining resilient and functional ecosystems, especially those that are utilized for the important services they provide (Klemmer et. al., 2012).

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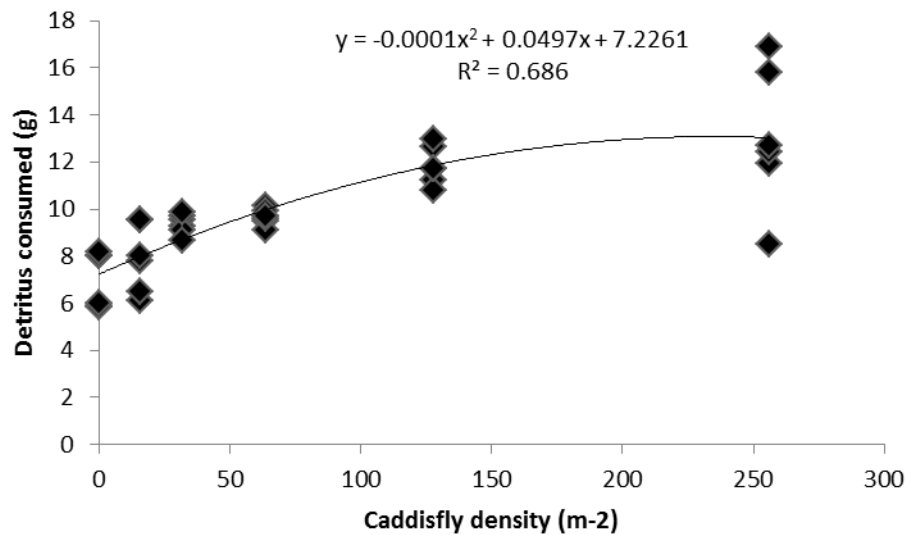


Figure 1: Detritus consumed as a function of caddisfly density.

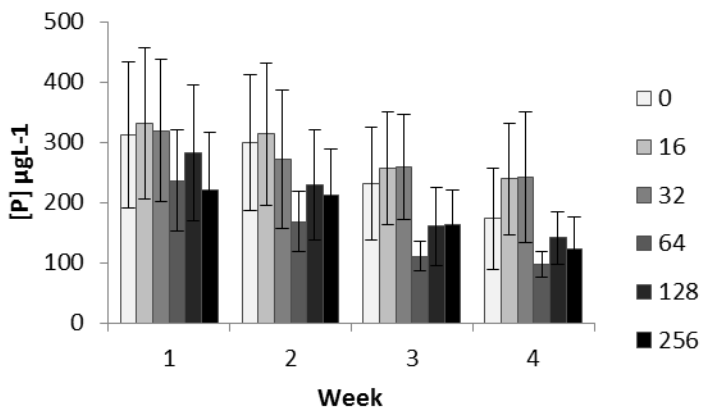


Figure 2: Mean (±SE) phosphorus concentration as a function of week and caddisfly density.

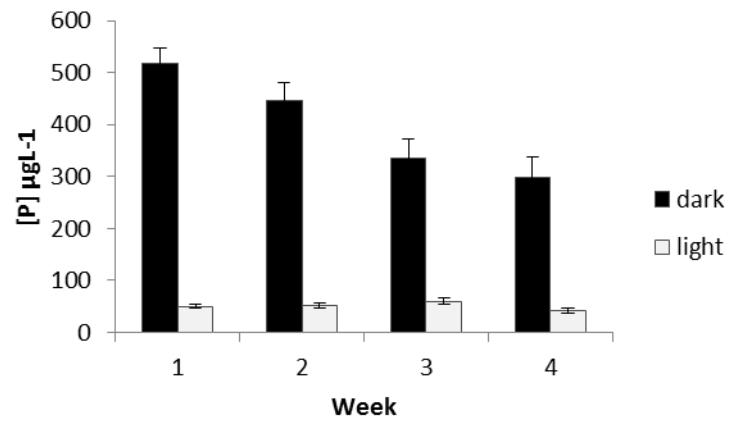


Figure 3: Mean (±SE) phosphorus concentration as a function of week and light treatment.

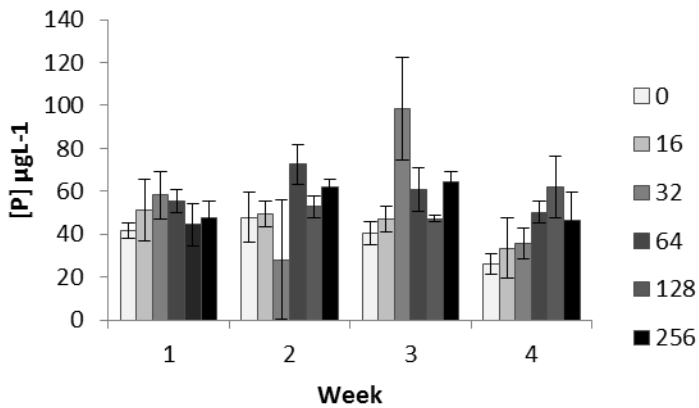


Figure 4: Mean (±SE) phosphorus concentration in light treatment as a function of week and caddisfly density.

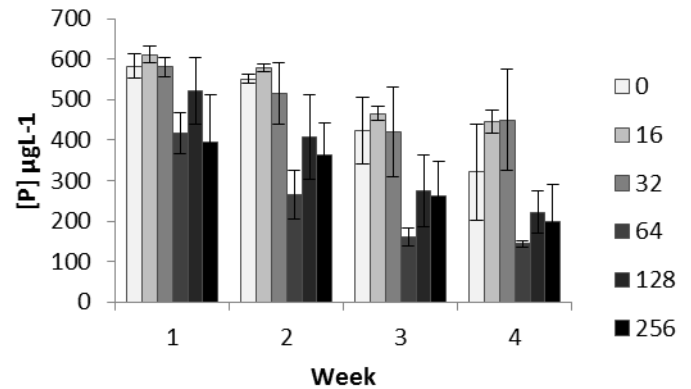


Figure 5: Mean (±SE) phosphorus concentration in dark treatment as a function of week and caddisfly density.

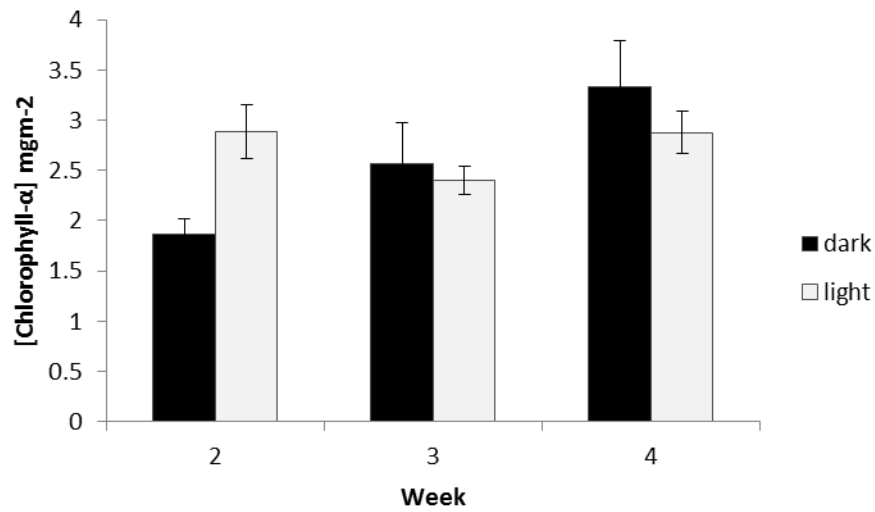


Figure 6: Mean ($\pm$ SE) chlorophyll-*a* concentration as a function of week and light treatment.

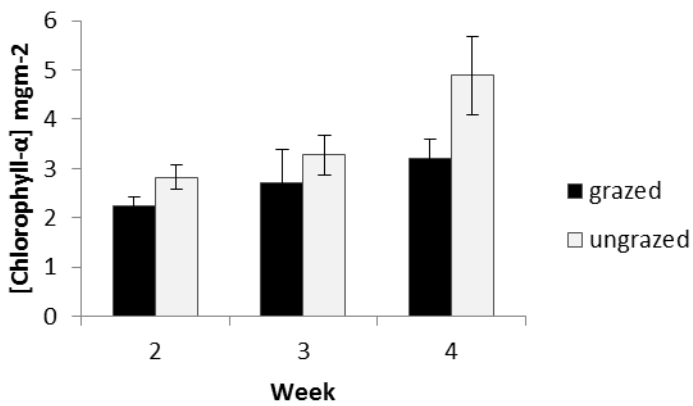


Figure 7: Mean ($\pm$ SE) chlorophyll-*a* concentration caddisfly exposure as a function of week and grazing treatment.

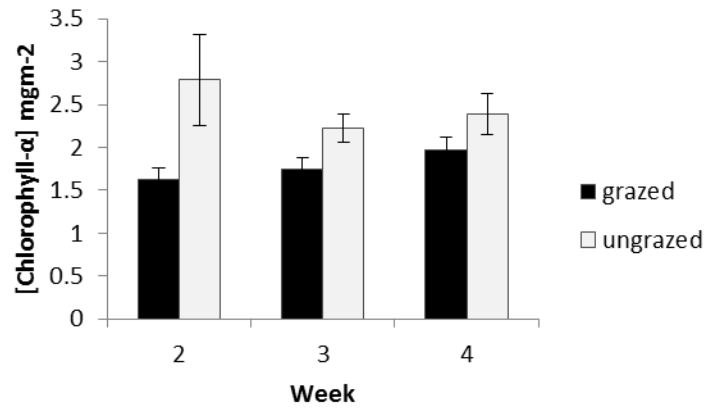


Figure 8: Mean ($\pm$ SE) chlorophyll-*a* concentration herbivore exposure as a function of week and grazing treatment.

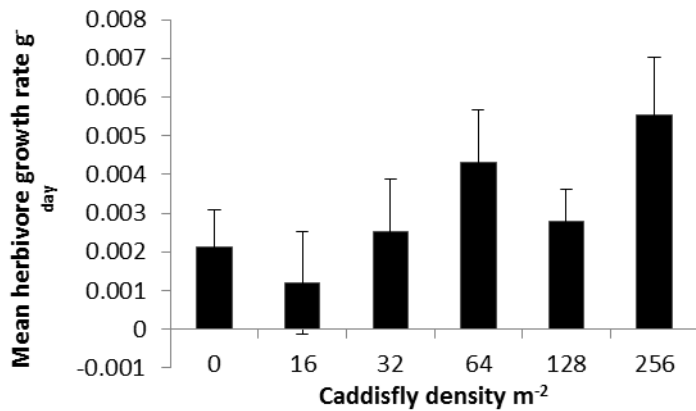


Figure 9: Mean ($\pm$ SE) herbivore growth rate as a function of caddisfly density.

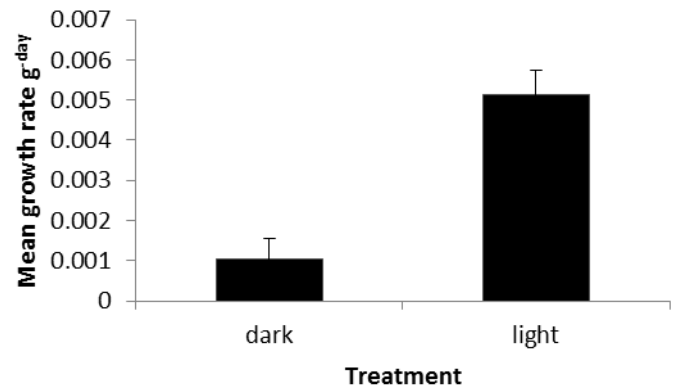


Figure 10: Mean ($\pm$ SE) herbivore growth rate as a function of light treatment.

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