

From brown to green: does caddisfly detritus processing benefit invertebrate algal grazers in subalpine wetlands?

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Abstract

Wetland nutrient cycling occurs through complex feedback mechanisms and dynamic inter- and intra-species interactions. In the shallow, oligotrophic ponds at the Mexican Cut Nature Preserve, near the Rocky Mountain Biological Laboratory in the Elk Mountains of Western Colorado, detritovore caddisfly density is negatively coupled with cyclic fluctuations in tiger salamander populations. The caddisfly *Limnephilus externus* is among the most biomass abundant invertebrates in these ponds and plays a vital role in wetland nutrient cycling by: 1) liberating N and P stored in detritus and 2) stimulating algal growth. In order to test whether the indirect effect of caddisfly detritus processing on algal growth affects species that consume algae, I manipulated caddisfly density in microcosms to create a gradient of detritus processing and compared the effects of nutrient release under ambient light (with stimulation of algal growth) and low light (no stimulation of algal growth). I predicted that caddisfly (detritovore) and mayfly (algal grazer) growth and development would be higher in light vs. low-light conditions, and that the positive feedback on caddisflies to be strongest at high densities. Preliminary results show that: 1) increasing caddisfly density increases the concentration of ammonium available for benthic primary production, 2) chlorophyll-a (proxy for algal biomass) increases along with increased nutrient availability, and 3) caddisfly algae grazing increases at high densities. Further analyses remain to be conducted to determine how consuming algae benefits the growth and development of *Limnephilus* caddisflies and *Callibaetis* mayflies. In a broader context, these results provide evidence that long-term changes in the abundance of a top predator can have cascading and indirect effects on ecosystem processes like nutrient cycling.

Keywords: Mexican Cut Nature Preserve, *Limnephilus externus*, detritus processing, nutrient cycling

Introduction

Ecosystem ecologists study how primary sources of energy move into and through food webs, and how the processing of that energy leads to the cycling of the nutrients that limit energy production. Human perturbations to energy flow and nutrient cycling can reduce ecosystem function, and hence the goods and services that they provide humankind (Srivastava and Vellend 2005, World Resources Institute 2005, Taylor et al. 2006). In shallow, lentic ecosystems, foodwebs depend on the primary production of benthic algae and the mobilization of dead organic plant matter from emergent and terrestrial vegetation; however, a trivial fraction of the emergent and terrestrial vegetation in wetlands is consumed alive (Moore et al. 2006). The energy and nutrients from dead plant material (detritus) enters the food web via microbial decomposers and detritus-feeding animals (detritivores) (Magee 1993). Although detritivores are an important consumer in aquatic food webs, little is known about the relationship of detritivore abundance and nutrient release from detritus (Batzer et al. 2006).

Wetland nutrient cycling occurs through complex feedback mechanisms and dynamic inter- and intra-species interactions. (Brinson et al. 1981). 20 years of research at The Nature Conservancy's Mexican Cut Nature Preserve (MCNP), near the Rocky Mountain Biological Laboratory (RMBL) in the Elk Mountains of Colorado, suggests cyclic fluctuations in salamanders, the top predator in the food webs of permanent ponds (Whiteman and Wissinger 2005, Wissinger et al. 2009). The abundance of some invertebrate prey species in the ponds is negatively coupled with these salamander fluctuations. Cased caddisflies (Trichoptera) represent one group of prey that exhibits negative density-dependence with respect to salamanders (as salamanders increase, caddisflies decrease). These caddisflies account for

a large proportion of biomass among invertebrates and feed primarily on detritus (Wissinger et al. 2003). Research in other aquatic systems, especially streams, suggests that the release of nitrogen and phosphorus by detritivores is critical to maintaining nutrient cycles within and through aquatic ecosystems (Wissinger et al. 1999, Graça 2001, Marshall & Hall 2004, Moore et al. 2006). Although shallow ponds and wetlands are often characterized as systems that are driven by detrital energy, there are surprisingly few studies that document the effects of invertebrates on detrital processing in lentic habitats (reviewed by Wissinger 1999, Batzer et al. 2006, Inkley et al. 2008). Because the Mexican Cut ponds are ultra-oligotrophic, nutrients liberated by this detritus processing could be especially important for stimulating primary production Batzer et al. 1999, Wissinger et al. 1999).

Previous studies suggest that inter-annual changes in the abundance of salamanders should lead to a top-down trophic cascade, which results in variation in detritus processing (Figure 1. right side). These studies have focused on the caddisfly *Limnephilus externus*, which use detritus as a primary food source and as a building material for their protective, portable cases. In 2007, Klemmer and Wissinger (unpub. manuscript) conducted an experiment in cages placed in the littoral zone of ponds and found evidence suggesting that *Limnephilus externus* density has a strong impact on detritus processing. They also found a weak effect on the level of nutrients liberated into the water column. In 2008, Emily Thornton (REU 2008) conducted a similar experiment in laboratory microcosms where she found a strong effect on detritus processing, on the liberation of nutrients, and on algal production. In summary, the results of these experiments suggest that a top-down trophic cascade of detritivore has an indirect effect on algal production via the release of nutrients (Figure 1. solid arrows). In a broader context, these results provide evidence that long-term changes in the

abundance of a top predator can have cascading and indirect effects on ecosystem processes like nutrient cycling (as in Taylor et al. 2006).

The purpose of this research is to determine whether the indirect effect of detritus processing on algal growth affects species that consume algae. In particular, I hypothesize that caddisfly detrital processing will 1) have a positive, secondary feedback effect on the growth and development of caddisflies, which also consume algae, and 2) increase the growth and development of non-caddisfly algal grazers. The positive feedback on caddisflies should be strongest at high densities, where competition for detritus is most intense.

Methods

Experimental Design

I conducted a microcosm experiment, in which I manipulated caddisfly density to create a gradient of detritus processing and compared the effects of nutrient release under ambient light (with stimulation of algal growth) and low light (no stimulation of algal growth). I predicted that if caddisflies increasingly depended on algal production at high densities, their growth and development rates will be higher in light vs. no-light conditions. Similarly, I predicted that the growth and development of other algal grazers will be higher under ambient versus low light conditions. The experiment was designed as a replicated Analysis of Covariance (ANCOVA) (N=3) with five densities of caddisfly larvae (0, 6, 12, 25, and 50) and the presence or absence of light as a covariate.

Microcosm Setup

Microcosms were 0.25m² sweater boxes, randomly arranged in a translucent, portable field laboratory (Weather Port) in front of Willey Laboratory, RMBL (GPS coordinates N 38 57.508 W 106 59.299). In order to impede algal growth in the low light

treatment, the sides of boxes were covered with duct tape and lids were loosely placed on top. Microcosms were filled with 16 L groundwater with low nitrogen ($0.9311 \mu\text{g/L}$) and no chlorine. Detritus from a local sedge (*Carex aquatilis*) served as food and substrate, and was rinsed and dried for four days, before being added 20.0 g to each microcosm. Twenty-four 6.5 cm^2 periphyton tiles were placed in each tank for measuring periphyton biomass. Eight tiles were accessible to caddisflies, while sixteen tiles were divided between two 100 cm^2 enclosures constructed out of 1/8" hardware cloth (Figure 2). All treatments were inoculated with 20 mL of $19.46 \mu\text{g/L}$ algae solution one day before caddisflies were introduced, and again on the fifth day of the experiment, with 20 mL of $1.38 \mu\text{g/L}$ algae solution. Water levels were normalized weekly, and terrestrial insects were removed from microcosms on a daily basis.

Invertebrates

On the first day of the experiment, fourth-instar *Limnephilus exturnus* caddisflies were collected from a local beaver pond (GPS coordinates N 38 39.944 W 107 01.143), allowed to excrete digestive wastes for three hours, and randomly distributed among microcosms, according to treatment. To test the secondary effects of algal biomass (chlorophyll a) on caddisfly growth and on heterospecific grazers, five pond snails with a mean biomass of 1.9436 g were added to the algivore enclosures. Because snail mortality on day three required the removal of snails from each treatment, new snails were collected and added in groups of 5 (mean biomass 1.9371) to the algivore enclosures on day four. Unsuccessful acclimation of the second group of snails to the microcosms necessitated the removal of all snails on day seven. Because of the unforeseen problems with the pond snails, *Callibaetis* mayfly nymphs from a local

beaver pond (GPS coordinates N 38 59.348 W 107 00.669) were used as the invertebrate algivore. A 1/16" fiberglass window screen liner was added to each algivore enclosure, and 6 *Callibaetis* mayfly nymphs were added to the microcosms in the third week of the experiment.

Response Variables

The following response variables were monitored during the experiment: 1) detritus decay rates, 2) caddisfly body and case metrics (growth and allocation of resources to cases), 3) mayfly growth and development, 4) water temperature, light intensity, ammonium, and phosphorus, 5) algal biomass on periphyton tiles, and 6) C:N of the unprocessed detritus, caddisflies, cases, and periphyton.

Water temperature and light intensity were measured hourly using Hobo data-loggers. Weekly ammonium and chlorophyll-a (algal biomass) measurements were conducted fluorometrically with a Turner Designs Trilogy Laboratory Fluorometer at RMBL, following procedures by Taylor et al (2007). Phosphorus samples were also collected weekly and will be analyzed at Allegheny College using a standard colorimetric assay (after Strickland and Parsons 1968).

At the end of the experiment, mayfly growth and development will be evaluated by counting empty cases cast by developing nymphs and emerging adults in each microcosm. Caddisflies will be preserved in 90% ethanol for subsequent analysis of body size (mass and length), development stage (larval instars, pupae), and gut contents. Caddisflies and leftover detritus will be taken back to Allegheny College for C: N analysis using a CNS analyzer (Leco CNS-2000; Sollins et al. 2006).

Statistical Analysis

The effects of caddisfly density and the light treatment (periphyton, no periphyton) on all of the response variables were analyzed as a replicated ANCOVA with 3 replicates of each treatment combination. The chlorophyll-a data was analyzed using a similar model, but with grazed and ungrazed tiles as a nested variable within each treatment combination.

Results

Ammonium

Caddisfly density ($p < 0.0001$), light ($p = 0.0034$), and week ($p < 0.0001$) had statistically significant effects on ammonium concentration ($\mu\text{g/L}$). There was also a significant interaction effect between week and caddisfly density ($p < 0.0001$). Ammonium concentration was highest in the low-light treatments and increased from week 2 to week 3, along a gradient of increasing caddisfly (Figure 3).

Chlorophyll-a (Algal Biomass)

Caddisfly density ($p < 0.0007$) and light ($p < 0.0001$) had statistically significant effects on chlorophyll-a concentration ($\mu\text{g/L}$). The effect of caddisfly grazing ($p = 0.02$) was also significant. In week 3, chlorophyll-a concentrations were highest in the high density caddisfly treatments and higher in the light treatments than in the low-light treatments (Figure 4). Algae was grazed more heavily in the light treatments than in the low light treatments and grazing was more prevalent at higher caddisfly densities.

Discussion

Nutrient Release and Algal Growth

Further analyses are necessary to confirm or refute my hypotheses that caddisfly detrital processing will 1) have a positive, secondary feedback effect on the growth and

development of caddisflies, which also consume algae, and 2) increase the growth and development of non-caddisfly algal grazers. However, preliminary results support evidence from Klemmer and Wissinger (unpub. Manuscript) and Thornton (REU 2008), that: 1) *Limnephilus externus* density has a strong impact on detritus processing, 2) detritus processing increases the liberation of nutrients, and 3) increased nutrient availability stimulates the growth of benthic algae. In this experiment, the light treatment had higher chlorophyll-a concentrations (higher algal biomass) and lower ammonium concentrations than the low-light treatment, since N limits algal growth in the presence of light. Caddisflies grazed benthic algae more heavily in the light treatment, since more algae was available. Increasing caddisfly density created competition for detritus, and it is possible that caddisfly algae grazing in the light treatments may have alleviated some of the effects of competition. Under competitive forces, limnephilid caddisflies have smaller bodies, heavily grazed cases, and resort to cannibalism, however further analyses are necessary to confirm whether or not grazing algae alleviated the effects of competition for detritus.

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Figures

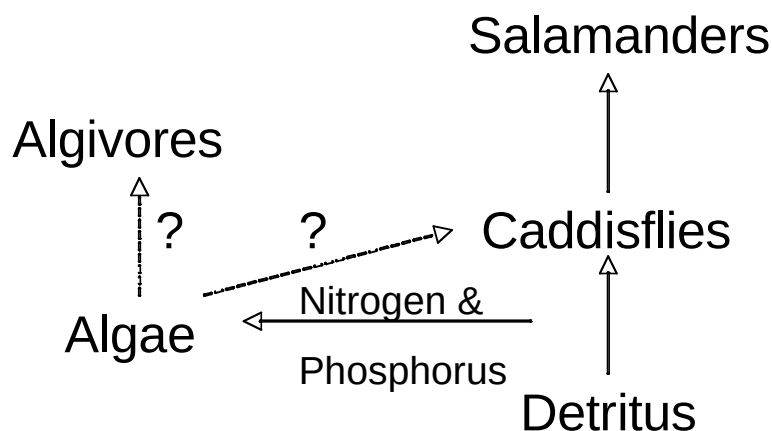


Figure 1. Summary of interactions among salamanders, caddisflies, and detritus processing. Dashed lines indicate the hypothesized indirect effects that are the focus of this study.



Figure 2. Microcosm set-up (light treatment). Caddisflies and detritus remained outside of cages. Cage on the left is algae-only and cage on the right is mayfly-only.

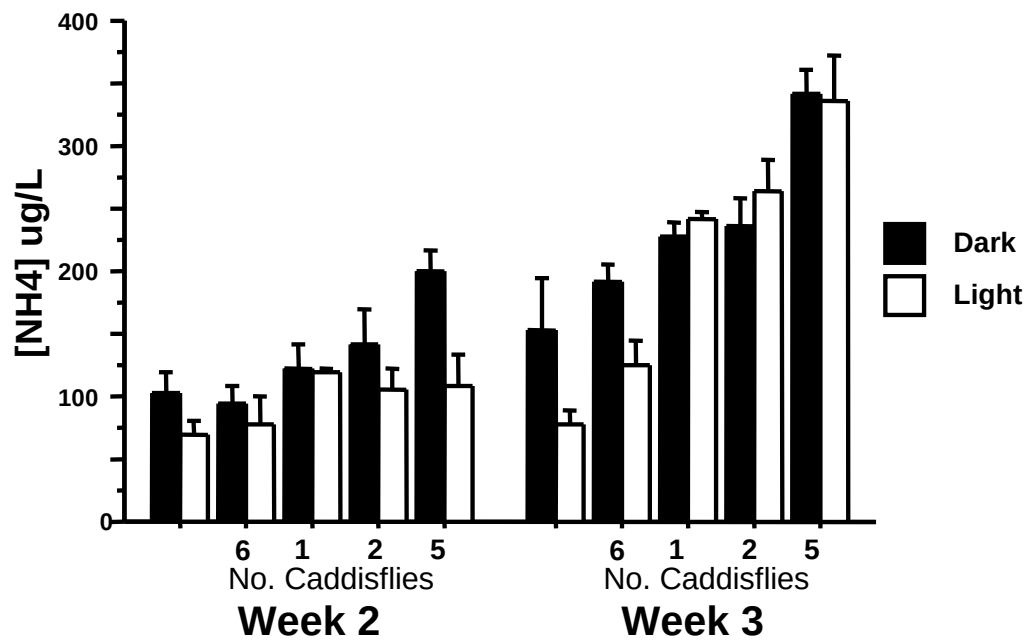


Figure 3. The effect of caddisfly density and light on ammonium concentration (ug/L) in Week 2 and Week 3. Error bars represent standard error.

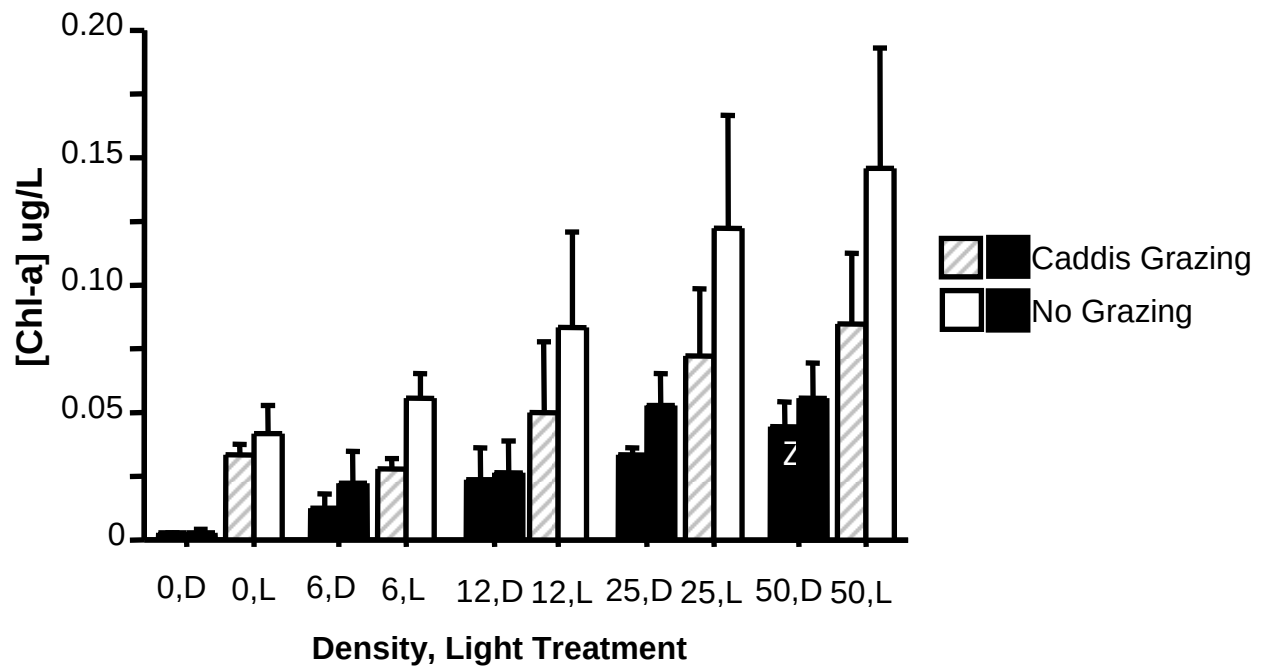


Figure 4. The effect of caddisfly density and light on chlorophyll-a concentration (ug/L) in Week 3. Error bars represent standard error.