

Do Pond Caddisflies Reap a Double Benefit from Detritus Processing?

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Abstract There is a lack of research investigating the double benefit pond caddisflies might gain from detritus processing. To fill the void in the literature, this study tested the hypotheses that as caddisfly density increases, nutrient concentration in the water column increases, algal growth increases, and caddisfly consumption of algae, an alternative food source, increases. Results supported the hypothesis that as density increases, nutrient concentration (phosphorus) in the water column increases. Chlorophyll-*a* (an indicator of algal growth) concentrations also increased as caddisfly density increased. Evidence of increased algal consumption at high densities supported the hypothesis that caddisflies switch to an alternative food source in high-competition environments.

Keywords: *Limnephilus externus*, detritus processing, double benefit, alternative food source

Introduction

Dead organic matter, or detritus, accumulates at the bottom of many aquatic ecosystems and often serves as the dominant source of energy and nutrients (Graça 2001; Moore 2007). This is especially true for shallow lentic ecosystems that are often characterized as having “detritus-based” food webs (Batzner & Sharitz 2006). Detritivores choose to consume detritus conditioned by bacteria and fungi rather than that which is not because of the nutritional benefit (Inkley et al. 2008; Graça 2001). Some invertebrates consume detritus and leave it lower in nitrogen and phosphorus than it originally was (Klemmer et al. unpublished). This suggests that these invertebrates mobilize nutrients in their habitat (Klemmer et al. unpub.). When excreted from the detritivores, nutrients, namely nitrogen and phosphorus, stimulate the growth of primary producers including algae (Klemmer et al. unpub.; Batzner et al. 1999). Primary producers are the base of aquatic food webs, and thus, the release of nutrients by detritivores can create the foundation for the success of entire wetland ecosystems (Marshall & Hall 2004).

The majority of research concerning nutrient release by detritivores has been conducted in stream ecosystems (Inkley et al. 2008; see review by Allan & Castillo 2008). Pond ecosystems are different from streams in that released nutrients remain within them, rather than washing downstream (Inkley et al. 2008). This may allow consumers in ponds to repeatedly benefit from the released nutrients (Klemmer et al. unpub.).

Caddisflies are a specialized group of detritivores. In their larval stage, caddisflies use detritus both as food and as the construction material for their protective cases (Klemmer et al. unpub.; Wissinger et al. 2006). Like other detritivores, caddisflies release nutrients that stimulate plant growth (Klemmer et al. unpub.). However, they are unique because there is evidence that when competition for detritus is high, pond caddisflies switch food preference and begin to consume algae (Klemmer et al. unpub.). They seem to reap a double nutritional benefit from processing detritus; first from the conditioned detritus, and then from the algae (Batzner et al. 1999).

The purpose of this study was to shed light on the relationships between caddisfly population density, detritus processing, nutrient release, algal growth, and algal consumption as an alternative food source. We hypothesized that as caddisfly densities increased, nutrient release levels would increase, causing more algae to grow. As competition for detritus increased, algae would become an alternative food source for the caddisflies.

The results of this experiment are significant because they allow us to better understand the role of caddisflies in liberating these nutrients and because few studies have been done to track the flow of nutrients through lentic habitats (Batzner et al. 1999). Also, no one has yet investigated the potential double benefit to caddisflies of detritus processing. In addition, experimental results may be compared to those of studies on similar ecosystems to increase knowledge of those systems. For example, nutrient release and alternative food sources may be related to salamander-caddisfly population interactions at the Mexican Cut Nature Preserve (Klemmer et al. unpub.).

Materials and Methods

Experimental Setup

Limnephilus externus caddisflies were placed in 0.25m² microcosms to determine how density affected nutrient release, algal growth, and alternative food choice. One week before the experiment began, microcosms were filled with tap water from Willey Laboratory and ~25g (mean: 25.09g) of *Carex aquatilis* detritus, a local sedge species. Three days later, water was replaced to eliminate any variation in the initial amount of nutrients on the detritus. Sixteen periphyton tiles (8 suspended, 8 unsuspended) were hung from a wooden stake above each microcosm with fishing line that allowed them to be submerged at grazing and above grazing level (Figure1). The underside of each tile was taped so that the suspended and unsuspended tiles had equal surface areas. Caddisflies and detritus were collected from cattle tanks located near Gothic Road. On day one of the experiment, caddisflies were sorted into instars and fourth instar individuals were added to microcosms in appropriate densities. To encourage algal growth in the microcosms, we added 20mL of 49µg/L algae solution to each. This algae was obtained from the stock tanks where we collected the caddisflies for the experiment. On day three of the experiment, 16mL of Aquashade algaecide solution (1ppm) were added to three of the six replicates of each density. The algaecide was used to indicate the difference in growth and development between caddisflies that were allowed access to algae as a secondary food source and those that were not.

This experiment was designed as a replicated analysis of covariance (N=3) with six densities of caddisfly larvae (0, 3, 6, 12, 25, 50) and the presence or absence of algaecide as the covariate. All microcosms were housed in a translucent weather port located at the Rocky Mountain Biological Laboratory, Gothic, CO. Placement of the microcosms was random to

ensure that position within the weather port had no effect on the experiment. Microcosms were filled to the 16L mark once a week to replace water lost to evaporation and sample extraction.

Tiles were repaired and debris was removed as necessary throughout the experiment.

Ammonium Analysis

Each week for three weeks, 60mL opaque bottles were rinsed and filled with 40mL water samples from each microcosm. 10mL of working reagent (Taylor et al. 2007) were then added to each bottle and allowed to react for 5-6 hours. 3mL of each sample were then inserted into a test tube that was placed in a Turner Designs Trilogy Laboratory Fluorometer, which determined the concentration of NH_4 in each sample.

Chlorophyll-a Analysis

Each week, two suspended and two unsuspended tiles were harvested from each microcosm. The two types of tiles were placed into separate plastic bags (two bags per microcosm) with 10mL 90% buffered ethanol. The tiles were then extracted in a refrigerator overnight. Once at room temperature and in dim light, 3mL of each sample were placed in a glass test tube and measured with a Turner Designs Trilogy Laboratory Fluorometer. All samples were then acidified with 30 μ L 0.1N HCl and remeasured after 2-5 minutes to account for degraded chlorophyll-*a* in the original reading (after Flecker et al. 2002, see also: Nusch 1980, Welschmeyer 1994).

We conducted a 3-way analysis of variance (ANOVA) for the effects of sample date, caddisfly density, and presence or absence of algaecide on chlorophyll-*a* after testing for assumptions of homoscedasticity and normality. Subsequent protected two-way and one-way ANOVA and post-hoc multiple comparison tests were used to disentangle the interactive effects

of the independent variables and to determine which particular dates and caddisfly densities differed from each other.

Phosphorus Analysis

Water samples (50mL) were filtered and collected from each microcosm each week. We added 0.4 g of potassium persulfate to oxidize each sample and samples were boiled in a pressure cooker for an hour. After cooling, 5mL of reagent (Strickland & Parsons 1968) were added to each sample and allowed to react for 30 minutes. Then samples were analyzed using a spectrophotometer at 885nm (Strickland & Parsons 1968). Statistical analysis for phosphorus concentration was conducted identically to chlorophyll-*a* analysis (see above). Week three data was analyzed using linear regression analysis.

Future Analyses

At the end of the experiment, larvae were preserved in 90% ethanol for subsequent analysis of body size (mass, length) developmental stage (larval instars, pupae), and diet contents. The effects of caddisfly density and the algaecide treatment (presence, absence) on all of the response variables are to be analyzed using a replicated ANCOVA model with three replicates of each treatment combination.

Results

Chlorophyll-a

Chlorophyll-*a* concentration increased as caddisfly density increased ($F_{5,120}=28.998$; $P<0.0001$; Fig.2) and as experiment duration increased ($F_{3,120}=163.852$; $P<0.0001$; Fig. 2). There was also an interaction between week and the number of caddisflies on chlorophyll-*a* concentration ($F_{15,120}=3.186$; $P=0.0002$; Fig.2). There was a slight effect of grazing, resulting in less chlorophyll-*a* on tiles that were unsuspended (grazed) than there were on suspended

(ungrazed) tiles ($F_{1,170}=5.541$; $P=0.0197$; Fig. 3). There was no statistical difference between the amount of chlorophyll-*a* in the algae and no algae treatments ($F_{1,286}=3.355$; $P=0.0680$; Fig. 4).

Phosphorus

Phosphorus was measured each week during the course of the experiment to determine how caddisfly density affected the amount of phosphorus in the water column. Results indicated that phosphorus concentration increased as caddisfly density increased ($F_{5,72}=9.108$; $P<0.0001$; Fig. 5, 6). Phosphorus concentration also increased as the duration of the experiment lengthened ($F_{2,72}=12.621$; $P<0.0001$; Fig. 5).

Discussion

Our hypotheses have been supported by the data analyzed thus far. Phosphorus levels in the water column increase as a result of increasing caddisfly density (Fig. 5). This is probably a result of the phosphorus content of caddisfly excretion, and we predict that the ammonium concentrations in the water column will follow a similar pattern (Klemmer et al. unpub.).

As expected, growth of algae on periphyton tiles also increases with caddisfly density. Chlorophyll-*a* concentration is used as an indicator of algal growth. In Figure 2 we can see that amount of chlorophyll-*a* increased as caddisfly density increased and as the duration of the experiment lengthened. This was expected because more nutrients in the water (phosphorus and (presumably) ammonium) stimulate more algal growth. As mentioned above, more caddisflies excrete more nutrients, thus encouraging more algal growth and higher concentrations of chlorophyll-*a*.

This study also provides evidence that caddisflies switch to an alternative food source when competition for detritus is high. Figure 3 shows that at densities of 6, 12, and 25,

chlorophyll-*a* concentration on grazed tiles is lower than on ungrazed tiles, suggesting that caddisflies have been consuming the difference in algae (Klemmer et al. unpub.). This does not; however, seem to be the case in the 50 caddisfly density treatment, where the difference was expected to be most pronounced. A possible explanation for this is that as caddisflies become desperate for food in high competition situations, they wriggle their bodies to float themselves up to the ungrazed periphyton tiles (Thornton, personal observation). An additional explanation for this may be a design flaw in the ungrazed tiles. Tiles were superglued to fishing line and then suspended from wooden stakes with duct tape (Fig.1). On multiple occasions, the superglue or tape did not hold, dropping ungrazed tiles to the bottom of the tank where they were possibly grazed before repair. In future experiments, a screen may be laid in each microcosm between the ungrazed and grazed tiles. This would allow water and nutrients to reach both types of tiles, but broken tiles could not sink to the bottom of the tank and caddisflies would not be able to float to the ungrazed tiles.

An additional thing to consider in follow-up experiments is algaecide application. In this study, algaecide was applied only once. However, because no difference was detected in the amount of chlorophyll-*a* (algae) in algaecide and no algaecide treatments, one may determine that a single application is ineffective. In future studies, algaecide should be applied at regular intervals to inhibit algal growth.

In summary, although several analyses have yet to be done, results show support for the double benefit hypothesis. Results also support hypotheses that as caddisfly density increases, so do nutrient concentrations in the water column and algal growth. Corrections of experimental design flaws in future experiments will most likely yield even more supportive results.

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Figures and Tables



Figure 1: Microcosm setup with suspended and unsuspended tiles

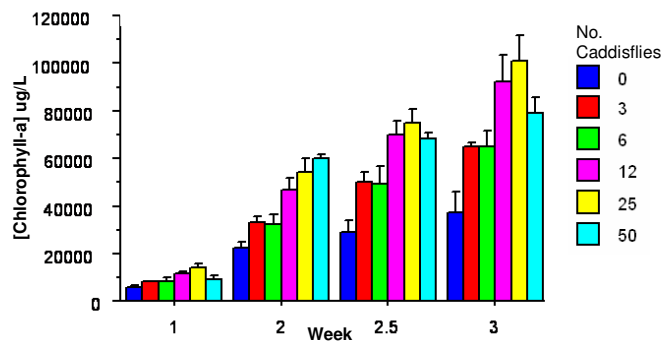


Figure 2: Chlorophyll-*a* concentration in each treatment

Each color bar represents one caddisfly density treatment over the course of the experiment (3 weeks). In this and all subsequent figures, error bars represent standard error.

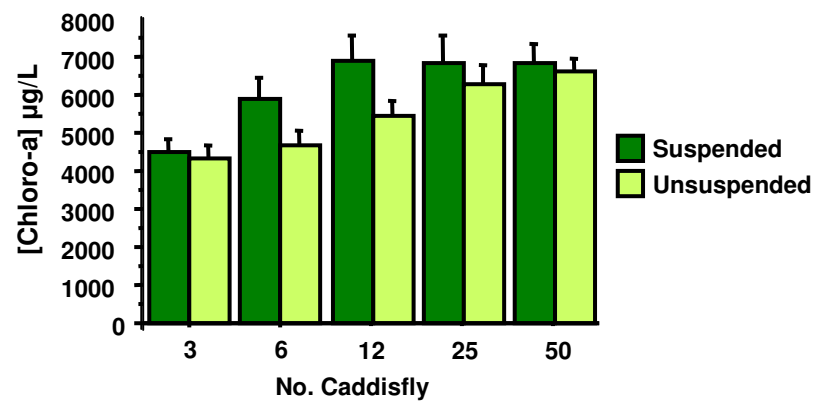


Figure 3: Periphyton on grazed and ungrazed tiles

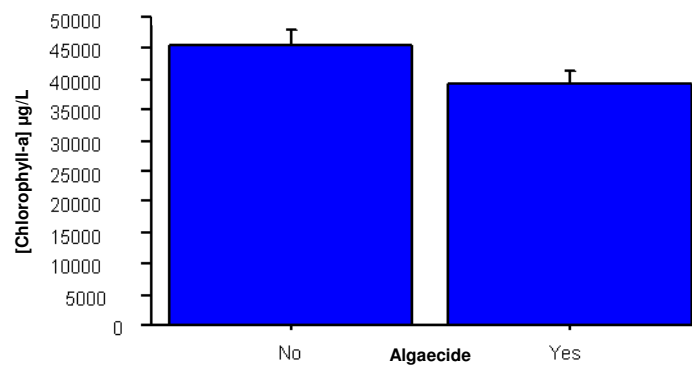


Figure 4: Periphyton in algaecide and no algaecide treatments

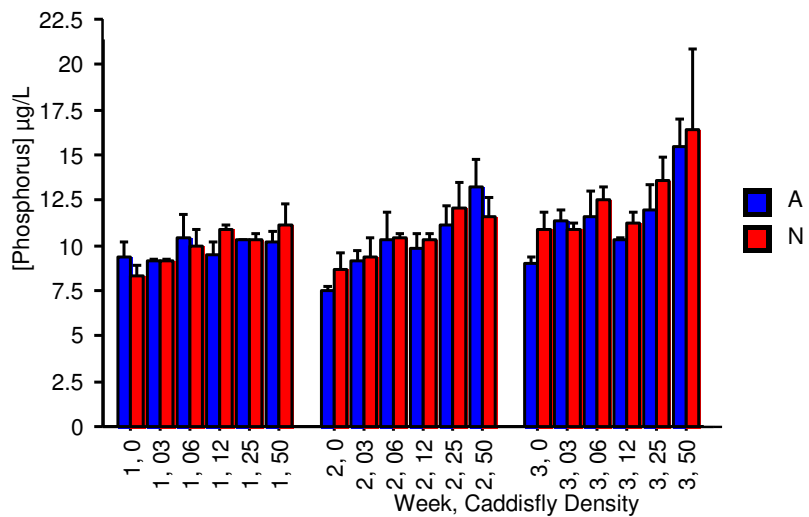


Figure 5: Effects of caddisfly density on water column phosphorus. A represents treatments with algaecide while N represents treatments with no algaecide.

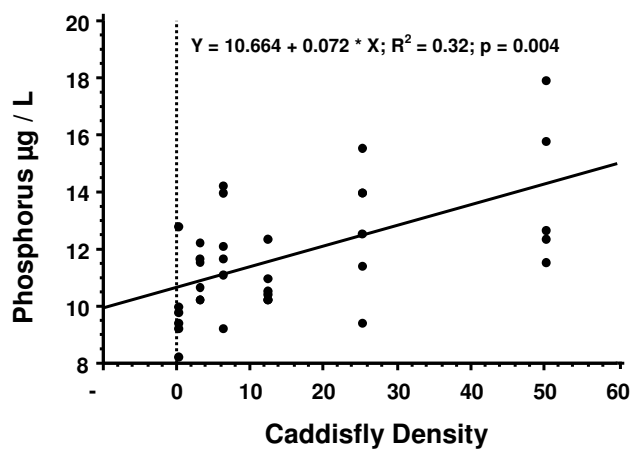


Figure 6: Week 3 Effect of caddisfly density on water column phosphorus