

Limnephilus externus Case Grazing

Abstract:

The subalpine region of Colorado hosts a variety of aquatic habitats. The organisms that inhabit these ecosystems are presented with many ecological adversities and differing predator guilds. *Limnephilus externus* caddisfly larvae native to montane and subalpine ponds of the Elk Mountains of Colorado face predatory interactions as well as seasonal time constraints. They have adapted to these adversities by engineering intricate casings made from sedge and other pond debris held together with silk. Caddisfly case morphology differs among species to accommodate for a variety of environmental and biotic variables. Tradeoffs and the proper allocation of resources are necessary for the survival of caddisfly larvae to adulthood. Field observations have documented *L. externus* individuals grazing on their own casings as well as the cases of conspecifics. In this study we take surveys of pond variables such as *L. externus* densities, predator densities, amount of emergent and marginal sedge cover in percentage and elevation to determine what factors influence *L. externus* case grazing and case size. A controlled sweater box experiment was conducted to understand the extent that sedge availability impacts case size and case grazing. The results showed that case size increased as sedge availability increased and case grazing increased as sedge availability decreased. We expect that case size will positively correlate with predator abundance and amount of sedge, whereas *L. externus* density and elevation will negatively correlate with case size.

Introduction:

The subalpine mountain habitats situated in the Elk Mountains, Colorado, contain organisms with a variety of challenges to overcome during their short growing season. The three to four month growing season in this region falls between long winters with heavy snowfall and short summers with low precipitation (RMBL weather station), limiting resources and constraining developmental ability (Wellborn et al., 1996). For any ecosystem, abiotic conditions and predation are the primary drivers behind evolutionary changes in morphology and life history traits (Helwerda & Schilthuizen, 2014). This is particularly true in this region of Colorado where subalpine ponds provide a large variety of permanent, semipermanent, and temporary aquatic habitats, each with their own host of ecological adversities (S. a. Wissinger et al., 2003). The proper allocation of resources is vital for the survival and continued existence of all organisms that inhabit this competitive ecosystem.

The numerous temporary and permanent wetlands present in this region of Colorado have been integral in understanding larval caddisfly adaptations. Diverse behavioral, physiological, and morphological adaptations are necessary for these caddisflies to finish their development before their larval environments dry out (S. A. Wissinger et al., 2004). Caddisfly larvae are known for engineering intricate casings stitched together with silk produced from specialized

glands (S. A. Wissinger et al., 2006). These cases act as a housing unit for the larvae as they traverse the freshwater environment they inhabit. The shapes and designs of the larvae cases differ between groups depending on the necessary tradeoffs for each environment. For example, *Asynarchus nigriculus* prefers to prioritize an expedited emergence time and focus more of their resources on preparation for pupation (S. a. Wissinger et al., 2003). This allocation of resources can be correlated with their light and mobile casings. In fact, *Limnephilus externus* in particular seem to build cases more for protection than respiration (Williams et al., 1987). These bulkier and heavier casings can serve as a refuge from lethal predators during their relatively longer development time (S. A. Wissinger et al., 2004). The differences in such building strategies can act to help or hinder the defensive abilities and mobility of the caddisfly larvae which demonstrates distinct evolutionary trade-offs (Hansell, 1984).

L. externus is widely distributed across temporary, permanent, and semi-permanent habitats in the sub-alpine region of Colorado. This wide distribution has exposed them to a variety of predators such as salamanders (*Ambystoma tigrinum*) and predaceous diving beetles (*Dytiscus dauricus*) which has driven evolutionary changes in case morphology (S. A. Wissinger et al., 2006). Wissinger et al. verified the defensive ability of *L. externus* casings, in comparison with other caddis from the same region, when exposed to these predators (2006). While the cases provide protection, because of their size there is a trade-off with predator protection and the energy needed to move the case around. At our study site, the larval case of *L. externus* is composed of sedge stem fragments in a stout “hedgehog” like structure. The cases are comparatively short, being only slightly longer than the larval body but proportionately wide. *L. externus* acts as a shredder, relying on decomposing leaves and other detritus for their building material as well as sustenance (Wallace, 2008; S. A. Wissinger et al., 2004). Although food in the form of sedge may appear to be plentiful for extended periods of time in many ponds, this is not always the case (Otto, 1983).

Sedge buildup is the greatest around the margins of ponds (littoral habitat) and as these habitats dry in temporary ponds, the aquatic invertebrates lose access to crucial nutrients (Klemmer et al., 2012). Field observations have documented *L. externus* grazing on their own casings as well as the cases of their neighboring companions (unpublished Klemmer data). Reasons for this case grazing are unknown, but it could be due to partitioning resources on their bodies for later use or due to predatory interactions. My research is aimed towards discovering drivers of case grazing and case size. The goal of this research is to determine which environmental variables and biotic variables affect *Limnephilus externus* case grazing and case size. To discover drivers of case grazing and case size we conducted pond surveys throughout the valley to measure (i) *L. externus* densities and average case sizes, (ii) predator densities, (iii) amount of emergent and marginal sedge cover in percentage and (iiii) elevation. We also randomly selected ten to fifteen *L. externus* to be brought back to the lab to attain average larval dry mass and case dry mass for each pond. Additional *L. externus* were brought back to the lab to perform a controlled sweater box experiment to correlate amount of available sedge to *L. externus* case size. I hypothesize that predator densities and amount of emergent and marginal sedge will have a positive correlation with *L. externus* case size, whereas *L. externus* densities

and elevation will negatively correlate with case size. For the sweater box experiment I expect to see an increase in case size as sedge availability increases.

Methods:

This study was done at a variety of permanent, semipermanent, and temporary aquatic habitats located around Rocky Mountain Biological Laboratory (RMBL) in central Colorado. In this study, we focused on a total of 9 ponds that are known to house approximately 50–60 species of aquatic invertebrates including some caddisfly predators such as diving beetles and salamanders (S. A. Wissinger et al., 1999). *Limnephillus externus* is the primary macroinvertebrate detritivore in these aquatic habitats and typically accounts for 25–45% of total biomass (Klemmer et al., 2012). Limnephilid caddisfly larvae are shredders and rely on sedge for case building material as well as sustenance (Wallace, 2008; S. A. Wissinger et al., 2004). *L. externus* case grazing has been observed on multiple occasions but the reason for which is not well understood. Larval behavior and case morphology reflect evolutionary changes caused by forced adaptations to their environment (S. A. Wissinger et al., 2006). Thus, case grazing should reflect resource availability and predation within the ecosystem they inhabit.

Survey design

To test my hypothesis, I conducted pond surveys to gather data for *L. externus* densities, predator densities, amount of sedge present in ponds and pond elevations. The case survey was done for a total of 9 ponds around Rocky Mountain Biological Lab: two ponds at the Kettle ponds, five ponds at Mid-valley, and two ponds at the Mexican Cut. For each pond, four (North, East, South, West) 1/3 m² D-net sweeps were taken per pond to approximate *L. externus* densities and predator densities. Sedge amounts and elevation in feet were recorded for the ponds in the sample space. Ten to fifteen *L. externus* individuals were randomly selected from each pond and brought back to the lab to get case size, dry larval mass, and dry case mass to give an estimate of case and larval size for the varying locations.

Experimental Design

The experiment was designed to expose the caddisfly larvae to a variety of different detritus levels to determine what extent detritus level in a pond has on case grazing and case size. *L. externus* sedge dependent case size was quantified using eight microcosm experiments conducted in a white plastic tent (WeatherPORT, Hansen WeatherPORT, Delta, CO) at the Rocky Mountain Biological Laboratory. All experiments were conducted during mid-summer (July 7, 2023, to July 21, 2023) and used (58.4 × 41.3 × 15.2 cm) plastic bins for microcosms replicates. For all experiments, sedge detritus (*Carex aquatilis*) was gathered from Fence Line Pond located in the montane wetlands (2928m elevation) between the Gothic townsite and Mt Crested Butte (38°55'17"N 106°57'31"W). Sedge detritus was given a standard squeeze to remove excess water before distributing into the varying treatment groups. Wet sedge was used for the experiment because it would retain its original microbial content. The experiment microcosms had varying levels of detritus: two with no detritus (0 grams), low detritus (20 grams), medium

detritus (60 grams), and high detritus (180 grams) (Table 1). Four, 60-gram wet detritus amounts were set aside to later be used for the wet to dry weight conversion factor.

Next, 4th instar *L. externus* were collected from Fishhook Pond (38°58'27"N 106°59'50"W) and distributed evenly based on case size into half of the microcosms. Initial case length, average case width, and max width (mm) were measured for all invertebrates used in the experiment. The remaining half of the microcosms would contain no *L. externus*, this was the control group to account for any bacterial or fungal decomposition that might occur over the course of the experiment. A sheet of thin plastic mesh was added to all experiment microcosms for the caddisfly larvae to use for tank navigation.

The experiment ran for 14 days. After completion of the experiment, the remaining *L. externus* were removed from the tanks where the number of alive, number of dead, and number of empty cases were counted. Initial case size measurements were repeated on the remaining caddisfly larvae before being placed in a drying oven at 60 degree C for < 48 hr to get average larval mass and case mass for each microcosm. Remaining sedge detritus was washed through a 1mm D-net and placed into a pre-weighted tin with its corresponding label. All detritus used in the experiment was placed into the drying oven to estimate final dry mass. Final experiment data was contrasted with initial case size measurements and survey results to understand the impact of sedge availability on case size reduction through intraspecific case grazing.

Table 1: Number of experimental sweater box treatments. Ten 4th instar LE's per detritus level. Without caddis row serves as the control group.

	No Detritus	Low Detritus	Mid Detritus	High Detritus
With Caddis	x 4	x 4	x 4	x 4
Without Caddis (Control)	x 4	x 4	x 4	x 4

Statistical Analysis

Initial case size measurements and final case size measurements were used to get the growth rate for each treatment group. This was used as the response variable in an ANOVA to test the effect of sedge detritus availability on case length, average case width, and max case width, where sedge detritus availability was the fixed effect, and the experimental block was randomized. The block effect was never statistically significant, but it was still included in the final model to represent how the studies were set up. A Tukey test was run on each data set to indicate significant statistical differences between the sedge detritus treatment variables.

Results:

Our experimental manipulation resulted in the expected changes in case size. For the sweater box experiment I hypothesized that there would an increase in case size as sedge availability increases. There were significant changes in *Limniphilus externus* case size and densities between the varying treatment groups (Fig. 1)(Fig. 2)(Fig. 3)(ANOVA $P < 0.05$, $P < 0.0005$, $P <$

0.0001). The high detritus treatment group results were inconclusive due to excessive amounts of sedge detritus present. This caused the tanks to become anoxic and the *Limnephilus externus* to suffocate prior to the completion of the experiment.

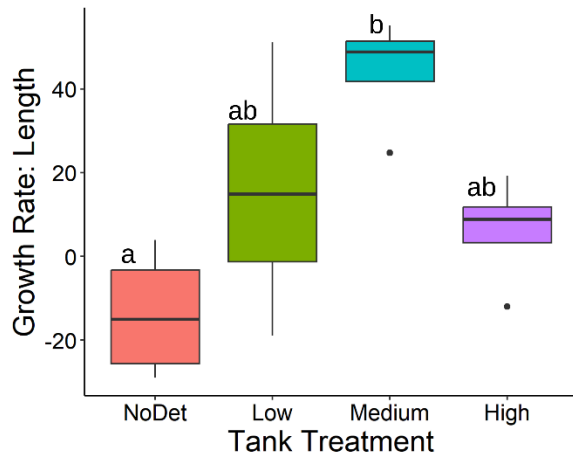


Fig.1 *L. externus* case length growth rate for sedge detritus treatment groups in a 2 week microcosm experiment. Differing letters indicate significant differences between the treatment groups.

For case length, the no detritus treatment group showed a growth rate of negative 13.82 percent, indicating a case length decrease. The low detritus treatment group showed an increase in case length with an average growth rate of 15.45 percent. The medium detritus treatment group demonstrated that case length increased on average 44.33 percent. The change in case length for the high detritus treatment group was inconclusive due to the environment becoming anoxic. An ANOVA test was used to determine that there were statistically significant differences between the sedge detritus treatments ($P < .05$, $P = 0.00821$). A Tukey test was used to indicate the presence of a significant difference between the no detritus and the high detritus treatments. (Fig. 1).

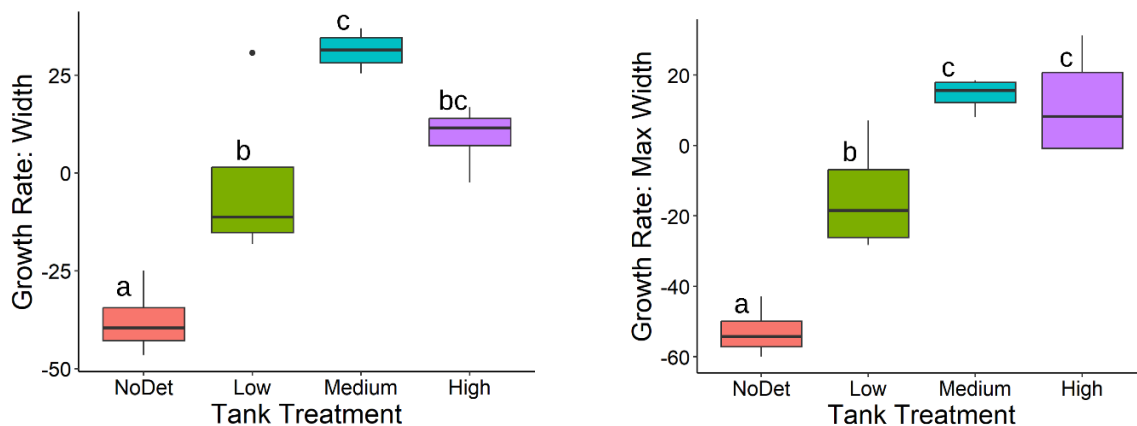


Fig. 2 & 3 *L. externus* case width growth rate and max width growth rate for sedge detritus treatment groups in a 2 week microcosm experiment. Differing letters indicate significant differences between the treatment groups.

Average case width presented more dramatic results, demonstrating a drastic decrease in case width with a growth rate of negative 37.64 percent for the no detritus treatment group. In the low detritus treatment group, case width was seen to decrease only slightly (-2.51%) whereas for the medium treatment group, average case width increased (31.31%). There were significant statistical differences between the differing treatment groups (ANOVA $P < 0.00001$, $P = 0.00000687$), specifically the no detritus, low detritus, and medium detritus treatments (Fig. 2). Max case width in relation to detritus availability presented very similar results to average case width (ANOVA $P < .00005$, $P = 0.0000132$) (Fig. 3). The max width for the no detritus treatment group decreased on average 52.84 percent, the low detritus treatment group decreased approximately 14.51 percent, and max case width for the medium treatment group increased about 14.43 percent. For max case width there were significant differences between the no detritus, low detritus, and medium detritus treatments groups.

L. externus case width and length increased in the presence of higher sedge detritus availability (Fig. 1)(Fig. 2). On multiple occasions, in the no detritus treatment tanks, resident *L. externus* were observed grazing on the cases of a conspecific. This in addition to case size reductions for the nutrient limited treatments infers that intraspecific case grazing had a greater likelihood of occurring in treatments with a limited source of nutrients (Fig. 1)(Fig. 2)(Fig. 3).

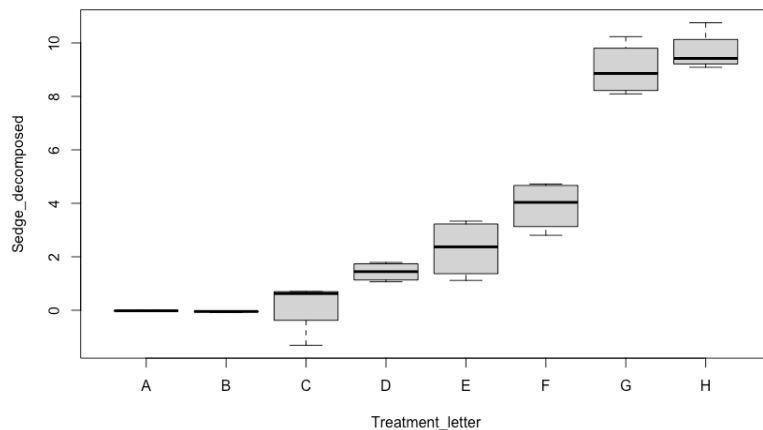


Fig. 4 Sedge detritus decomposed in relation to treatment type. A-no detritus no caddis, B-no detritus caddis present, C-low detritus no caddis, D-low detritus caddis present, E-medium detritus no caddis, F-medium detritus caddis present, G-high detritus no caddis, H-high detritus caddis present.

Decomposition of sedge detritus was significantly higher in the treatment groups containing *L. externus* when compared to treatment groups without (Fig. 4). There was no significant difference when comparing sedge decomposition of the same treatment type.

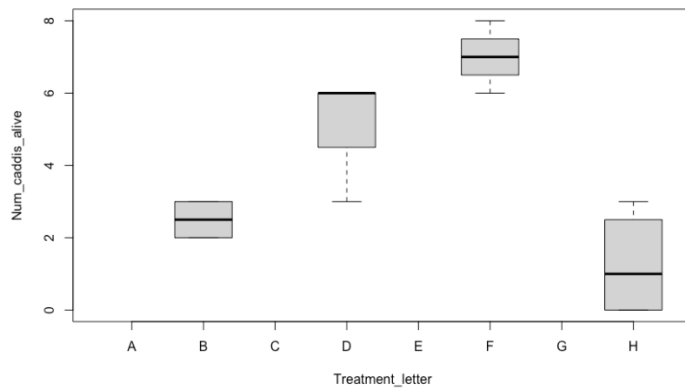


Fig. 5 Number of *L. externus* alive at the end of the experiment in relation to treatment type. A-no detritus no caddis, B-no detritus caddis present, C-low detritus no caddis, D-low detritus caddis present, E-medium detritus no caddis, F-medium detritus caddis present, G-high detritus no caddis, H-high detritus caddis present. ($P = .00006179$)

There was significant correlation between survival rate of the caddisfly larvae used in the experiment and what treatment they were assigned to (Fig. 5) (ANOVA $P < 0.0001$). Survival rate increased and sedge detritus availability increased. The high detritus treatment group became anoxic and caused the caddisfly larvae to suffocate early in the experiment leading to insufficient results for that treatment group.

Discussion

In the Elk Mountains of Colorado, pond permanence has high variability which greatly impacts sedge availability and puts developmental time constraints on invertebrate assemblages (Wellborn et al., 1996). The wide distribution of *L. externus* and a delayed pond emergence time has exposed them to a variety of predators such as salamanders (*Ambystoma tigrinum*) and predaceous diving beetles (*Dytiscus dauricus*) which has driven evolutionary changes in case morphology (S. A. Wissinger et al., 2006). The larval housing unit of *L. externus* has increased defensive abilities when compared to other caddis inhabiting the same region (S. A. Wissinger et al., 2006), and can also serve as a food source when resource become scarce in the later season.

Limnephilus externus relies on sedge detritus for sustenance as well as for case building material (Wallace, 2008; S. A. Wissinger et al., 2004), thus the amount of sedge present in a given pond has a large impact on *L. externus* survival rates. The results of our experiment showed that *L. externus* survival rates were positively correlated with increased sedge detritus availability. This outcome indicates that early utilization of annual biomass senescence could increase survival rates in the later season when decreased sedge availability and developmental time constraints are apparent.

Resource partitioning is an essential concept in community ecology and frequently employed to explain how different species can survive in the presence of competition for the same limited food resource (Schoener, 1974). Inter-, as well as intraspecific competition for

annual biomass senescence limits food availability for detritivores that inhabit the same ecosystem. Early allocation of resources towards case growth can greatly increase the survival rate of *L. externus* during the later season when resources become scarce (Otto, 1983). Food in the form of sedge detritus may appear to be plentiful for extended periods of time in many ponds, but this is not always the case (Otto, 1983). Sedge buildup is the greatest around the margins of ponds (littoral habitat) and as these habitats dry in temporary ponds, the aquatic invertebrates lose access to crucial nutrients (Klemmer et al., 2012). Limited food resources in addition to pond drying are environmental stressors that cause the caddisfly larvae to respond in unique ways. Food shortages have been reported to cause caddisfly larvae to aggregate and feed on their own cases as well as cases of conspecifics (Lund et al., 2016). On multiple occasions over the course of the experiment, resident *L. externus* were observed grazing on the cases of conspecifics. Case grazing was observed the most in tanks with low to no detritus levels indicating that case grazing increases as sedge availability decreases. For the No Detritus treatment group, since there was no sedge detritus present in the tanks, the reduction in case size can only be attributed to case grazing. These results indicate that during periods of reduced food availability *Limnephilus externus* will rely on partitioned food resources from their larval housing unit or the sedge case of conspecifics as a source of nutrients.

The results showed that a sedge detritus availability had less of an impact on case length than it did on case width. This may be explained by the evolutionary changes in *L. externus* morphology. The larval housing unit of *L. externus* is comparatively short, never extending much longer than the larval body, but proportionally wide in comparison to other caddis from the same region (S. A. Wissinger et al., 2006). *L. externus* inhabits lentic ecosystems where growth in the form of length does not benefit the caddisfly larvae and would act to hinder movement, whereas a larger width will aid them in increased protection from predators (S. A. Wissinger et al., 2006).

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