

Effects of a caddisfly range shift on competition and facilitation in high elevation ponds

Abstract:

As temperatures warm, the climate crisis is having a significant influence on species all across the world and altering their distributions. Colorado's high elevation ponds have recently observed a species range shift, traveling to higher altitudes than they have previously inhabited. The Mexican Cut Nature Preserve near the Rocky Mountain Biological Laboratory in Gothic, CO, is home to a prominent caddisfly *Limnephilus externus*, but recently a new range-shifting species, *Nemotaulius hostilis*, has appeared. Caddisflies play an essential role in nutrient cycling and putting energy back into the food web. The range-shifting species is known to excrete high rates of phosphorus (P), and could potentially facilitate the growth of a nutrient dense food source for the resident species. However, the range-shifting species and the resident species have differing life histories and emergence patterns that could increase interspecific competition between the two caddisfly populations. This study aims to understand the ecological impacts that the range-shifting species *Nemotaulius hostilis* is having on facilitating resources or competing for resources with the resident species *Limnephilus externus*.

Introduction:

The climate crisis is greatly impacting species all over the world and changing their distribution as climates warm. Many range shift patterns indicate species are migrating to higher latitudes or altitudes to stay within climates they are best suited within (Chen et al., 2011). However, the consequences and benefits of these range shifts are largely unknown. It is important for scientists to explore the ecological impacts that range shifts are causing and work to understand these implications before they become immeasurable.

Scientists have recently observed a range shift in caddisfly species across high elevation ponds in Colorado; species of caddisflies are moving to higher altitudes than those they have historically inhabited (Balik et al., 2023). It is unknown how the range shift of caddisflies will influence surrounding ecosystems positively or negatively (Balik et al., 2023). Caddisflies are freshwater aquatic macroinvertebrates that are important in nutrient cycling and an integral part of aquatic food webs (Balik et al., 2022). The detritivorous nature of caddisflies allows nutrients in detritus from both aquatic and terrestrial ecosystems to be recycled into food webs (Klemmer et al., 2012). Specifically, caddisflies play an essential role in facilitating nutrient cycling by excreting phosphorus (P) and nitrogen (N) that aid in plant and algal growth (Balik et al., 2018). Thus, the introduction of a new caddisfly species to an aquatic ecosystem may drastically alter nutrient cycling and food web interactions (Hall et al., 2003 and Small et al., 2011.).

Caddisfly populations are present in freshwater ponds along different elevational gradients in the Rocky Mountains. The Mexican Cut is a nature preserve among the Elk Mountain range and is home to a suite of high elevation ponds (11,200-11,400ft). *Limnephilus externus* is a ubiquitous resident caddisfly of the Mexican Cut, however in recent years a new

range-shifting species has arrived, *Nemotaulius hostilis* (Balik et al., 2023). Caddisflies such as *Limnephilus externus*, work to facilitate nutrient cycling through consuming detritus and excreting phosphorus and nitrogen (Balik et al., 2023). However, the life history patterns of *Limnephilus externus* and *Nemotaulius hostilis* differ entirely. Female *Limnephilus externus* lay egg masses in early fall before ponds freeze. Over winter, *Limnephilus externus* develop as eggs until June when the ice melts, when they hatch into their larval form. In late July *Limnephilus externus* begin to pupate and emerge as adults in August leaving the aquatic environment until they come back in the fall to lay their eggs (Wissinger et al., 2003). In contrast, *Nemotaulius hostilis* lay their eggs in early August, overwinter as larvae, and emerge as adults in early to mid July (Figure 1.). The life history differences mean that *Nemotaulius hostilis* is consuming detritus over winter (a period where caddisflies, historically, were not active), and may reduce the detritus availability for the *Limnephilus externus* larvae in spring. Alternatively, the increased excretion of nutrients in the pond caused by *Nemotaulius hostilis*, may increase algal food resources for *Limnephilus externus* that facilitates their growth. *Limnephilus externus* has been observed diet switching to feed on algae when it is abundant and sedge was limiting (Klemmer et al., 2012).

Figure 1.

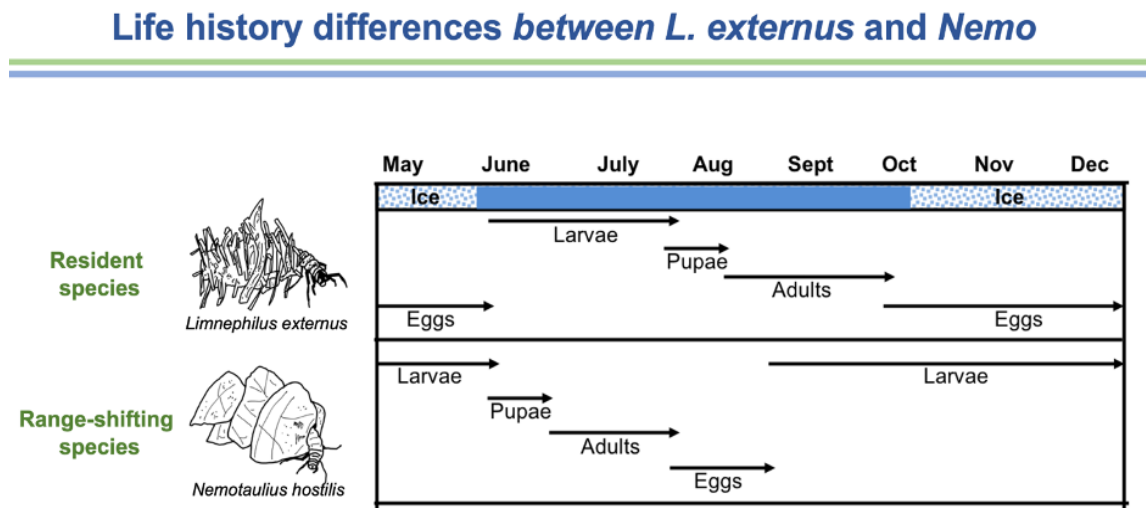


Figure 1. Diagram of *Limnephilus externus* and *Nemotaulius hostilis* life histories. Note major differences between the larval form of *Limnephilus externus* and *Nemotaulius hostilis* (Klemmer, unpublished data).

The purpose of this study is to determine if the range shift of *Nemotaulius hostilis* to high elevation ponds at the Mexican Cut Nature Preserve is causing an increase in competition between *Limnephilus externus* and *Nemotaulius hostilis* and facilitating a more abundant algal food resource for *Limnephilus externus* through N and P nutrient excretion. To test our hypothesis we conducted a controlled lab experiment and a field experiment test for resource competition. The lab experiment allows us to test if limited detritus abundance limits the growth

of *Limnephilus externus*, indicating a response to competition, and if *Limnephilus externus* shifts diet resources to algae, allowing for facilitation to occur. Our field experiment investigates whether or not *Nemotaulius hostilis* is accelerating detritus breakdown within the ponds when *Limnephilus externus* is no longer present. The breakdown of detritus is both an indicator of potential competition with *Limnephilus externus* and the mechanism by which facilitation may occur.

Methods:

The study was conducted at the Mexican Cut Nature Preserve and at the Rocky Mountain Biological Laboratory in Gothic, Colorado. The focus of the study is on competition and facilitation between *Nemotaulius hostilis* and *Limnephilus externus*. Initially we had planned to conduct experiments using the *Nemotaulius hostilis* population at the Mexican Cut Nature Preserve, but low abundances of *Nemotaulius hostilis* larvae meant that this was not possible. Instead, we were able to measure possible responses of *Limnephilus externus* to competition and facilitation in a controlled experiment. In addition, we used a leaf pack experiment to determine if *Nemotaulius hostilis* and *Limnephilus externus* are potentially competing for resources.

Experimental Design 1: Experimental test for resource competition

The experiment was designed to rear *Limnephilus externus* larvae in a variety of detritus abundances to measure caddisfly responses to varying levels and types of resources. The experiment was conducted inside a white plastic tent (WeatherPORT, Hansen WeatherPORT, Delta, CO) at the Rocky Mountain Biological Laboratory. We ran the experiment from July 7, 2023, to July 21, 2023 and used 0.25 m³ (51 × 36 × 13 cm) plastic bins for microcosm replicates. All treatment groups contained one mesh plastic sheet for caddisfly mobility, one round mesh cage (10cm²), twelve 2.54cm² unglazed ceramic tiles, and varying levels of detritus.

For all treatment groups, sedge detritus (*Carex aquatilis*) was gathered from Fence Line Pond located in the montane wetlands (2928m) 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 weighing and distributing into the varying treatment groups. Wet sedge was used for the experiment to retain its original microbial content (following Balik et al 2022). The treatment groups consisted of four different sedge amounts (no detritus, 0g; low detritus, 20g; medium detritus, 60g; high detritus, 180g). These four detritus treatments were crossed by the presence and absence of caddisflies, for a total of eight different treatment groups each replicated four times.

Half of the treatment groups containing no caddisflies acted as controls for the corresponding treatments containing caddisflies. Fourth instar *Limnephilus externus* were collected from Fishhook Pond (38°58'27"N 106°59'50"W) and distributed evenly by case size into the treatments. Ten similar caddisflies were added to each treatment group containing caddisflies.

Lastly, twelve 2.54cm² unglazed ceramic tiles were added to all treatment groups to grow and measure algae biomass, an important supplemental food for *Limnephilus externus* (Klemmer et al 2012). Six tiles were added to all of the microcosms and placed beside the mesh cages. The other six tiles were added to each cage within the microcosms to act as a control of non-grazed tiles.

The experiment ran for fourteen days. After the experiment was completed caddisflies were removed and we recorded the number of living caddisflies, dead caddisflies, and empty caddisfly cases. Then tiles were removed from each treatment group and placed in whirl paks. Each set of tiles in each treatment were placed in separate whirl paks, and those within the cage were separated from those outside the cage. The whirl paks were then placed in the freezer to preserve algae and chlorophyll levels. Next, 15mL of buffered ethanol was added to each whirl pak and placed in the fridge for 18 hours to extract chl-a pigments. After 18 hours the solution was distributed across five, 5mL vials. Each vial contained approximately 3mL of the solution. Individually each vial was put into the fluorometer for a chlorophyll A reading. All measurements were recorded, and measurements from each whirl pak sample were added together to calculate a whole sample measurement.

Experimental Design 2: Field test of resource competition

The experiment was designed to determine if *Nemotaulius hostilis* is feeding on sedge detritus (*Carex aquatilis*) in high elevation ponds over the fall and winter. The experiment was conducted at the Mexican Cut Nature Preserve (3500m) in its high elevation ponds (1, 3,5,6,8,9,12). The experiment was conducted twice per year starting with one leaf pack being put in the ponds during mid summer (28 July 2022) and was removed in fall (23 September 2022), and the second distributed in early fall (24 September 2022) and collected in mid summer (20 July 2023).

Seven ponds at the Mexican Cut Nature were a part of the experiment. Each pond received six leaf pack pairs placed on the north, south, east, and west sides of the ponds. Each leaf pack pair consisted of two different leaf packs. The first leaf pack acted as the control for microbial and micro-detritivore breakdown and was made by sewing together fine mesh (1mm²) into a small bag (20cm²). The second leaf pack was made by sewing together one side of fine mesh and one side of coarse mesh (2.54cm²) that enabled all caddisfly species to access and consume detritus. All leaf packs contained about 5g of air-dried sedge collected from a dried pond at the Mexican Cut Nature Preserve and a label indicating date, pond number, and location within the pond. The two leaf packs were zip tied at one corner and oriented side by side. The leaf pack pairs were placed on the bottom at the edge of each pond location and secured using three bamboo poles. Each leaf pack pair was collected and placed in its own plastic bag. After leaf packs were collected they were placed in the freezer until they could be processed.

To process leaf packs they were first defrosted in their plastic bags. Then we removed the pair from the bag, separated the packs, rinsed them, and carefully cut each pack open. After the pack was open the contents were transferred into a 1mm sieve and rinsed again. In the sieve we

picked out caddisflies and placed them into labeled vials, and any other contents that were not part of the original added sedge. Then the sedge was transferred to a pre-weighed aluminum pan. Once all packs were emptied to weigh pans, all weigh pans went into the drying oven at fifty degrees celsius for at least forty-eight hours, after which pans were removed and weighed. To the nearest 0.0001g. Using the data of the pre-weighed pan, we calculated the dry mass of the remaining sedge by subtracting the pre-weighed pan weight with the dry mass and pan weight data. The result was the dry mass weight of the remaining sedge at the end of the experiment.

Data analysis:

Detritus remaining (g dry mass) in the leaf bags in fall and winter were each analyzed with separate linear models containing pond identity and mesh size (coarse vs fine) and their interaction as fixed factors.

The biomass of algae on tiles in the resource competition experiment was analyzed with a 3-way ANOVA with detritus abundance, caddisfly presence, and caged vs uncaged tiles as fixed factors, with all possible two-and three-way interactions also included in the model.

Results:

Fall and winter detritus breakdown

Detritus breakdown in the fall leaf packs was generally greater than that of the winter leaf packs (i.e., less detritus remaining), especially in the coarse mesh bags. The statistical analysis for the fall leaf packs indicates that there is a significant difference in detritus breakdown between ponds ($P = 0.028$). For example, less detritus remained in pond 8 compared to pond 12 (Figure 2.A). Similarly, coarse leaf packs had significantly more detritus breakdown than the fine leaf packs ($P < 0.0001$) and there was significant interaction between mesh size and pond number ($P = 0.025$). For example, detritus breakdown showed little difference between mesh sizes in pond 5 and in pond 12, whereas ponds 1, 8 and 9 showed a much larger effect of mesh size on detritus breakdown.

Detritus breakdown in the winter leaf packs showed some significant interactions, but was statistically weaker than those of the fall leaf packs. The statistical analysis of the winter leaf packs indicates that there is a significant difference in detritus breakdown between ponds ($P < 0.0001$) and detritus breakdown between mesh sizes ($P = 0.0008$). However, there was no significant correlation between mesh size and pond number ($P = 0.187$). Little to no correlation between mesh size and pond number was displayed, however Pond 3 indicated a stronger correlation than the rest of the ponds (Figure 2.B).

Figure 2.

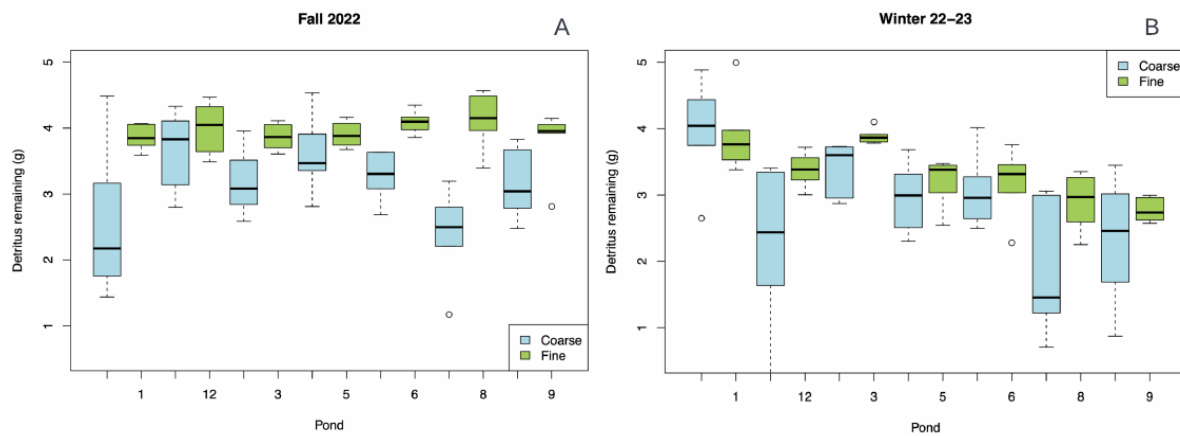


Figure 2. Detritus breakdown of Fall 2022 (A) and Winter 2022-23 (B) leaf packs in 7 different ponds at the Mexican Cut Nature Preserve. Values are expressed as (g) of detritus remaining in each type of mesh leaf pack for each pond.

Chlorophyll A concentrations in resource competition experiment

Chlorophyll A concentrations on the tiles within the experiment were not significantly correlated with caddisflies within the treatment groups ($P = 0.32$; Figure 2.A), and whether the tiles were caged or uncaged ($P = 0.19$; Figure 2.B). However, there was a significant effect of detritus abundance on the concentration of chlorophyll A ($P < 0.0001$). Interactions between these three variables were all not significant, although there is also a possibility that there is a weak interaction between the presence of caddisflies and whether or not the tiles were caged or uncaged ($P = 0.09$).

Figure 3.

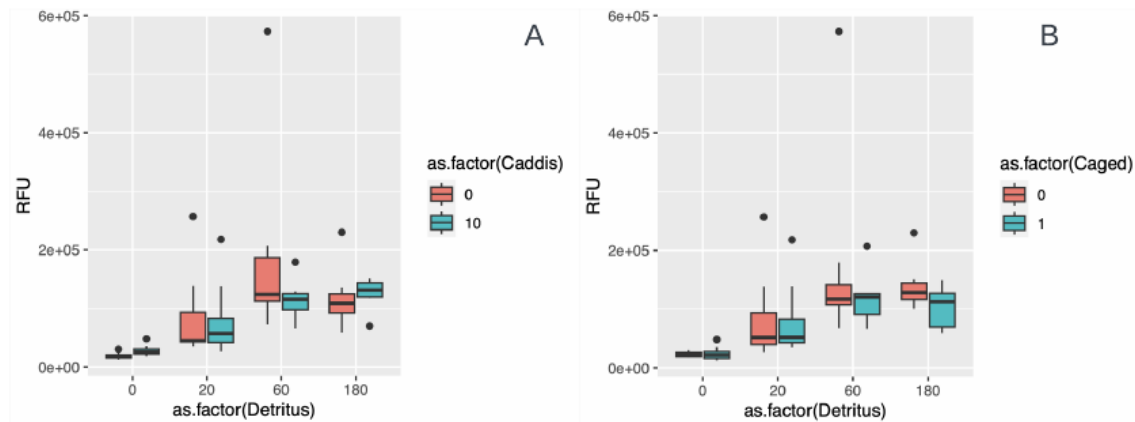


Figure 3. Chlorophyll A concentrations taken from tiles in the resource competition experiment, 2023. Chlorophyll was measured in RFU (relative fluorescence unit). Figure 3.A, 0 indicates treatment with no caddisflies, and 10 indicates treatment with 10 caddisflies added. Figure 3.B, 0 indicates uncaged tiles, and 1 indicates caged tiles.

Discussion:

Fall and winter detritus breakdown

To determine what variables were causing detritus breakdown we analyzed both the fall and winter leaf pack data separately. For the Fall 2022 leaf packs, it was found that detritus breakdown differed between ponds, and was significantly higher in coarse mesh leaf packs over the fine mesh leaf packs. It can be interpreted that this was significant due to an overall effect of detritivores on detritus breakdown, and more specifically by *Nemotaulius hostilis* on detritus breakdown. It was also found that the interaction between mesh size and pond number was significant, meaning that *Nemotaulius hostilis* likely had a greater impact on detritus breakdown in some ponds than others. For example, detritus breakdown was greater in pond 1 than pond 12, this is likely correlated with the high *Nemotaulius hostilis* densities in pond 1 recorded from the fall of 2022. The relationship between the effect of mesh size and pond number may be influenced by differences in caddisfly density among each pond, and we will test this in the future by comparing the data from the leaf packs with current and past caddisfly density surveys of the corresponding ponds. Comparing the two data sets will help to give us a more holistic view of *Nemotaulius hostilis* impacts on detritus breakdown.

For the Winter 2022-23 leaf packs, it was determined that the effects of mesh size and pond number on detritus breakdown were significant, however there was no significant relationship between mesh size and pond number. Strong and significant effects of pond number on detritus breakdown means that there were great differences in detritus breakdown amongst all seven ponds. A significant, but weaker p-value in mesh size significance describes a weaker correlation between the size of the mesh and amount of detritus breakdown. Ultimately, the lack

of correlation between mesh size and pond number means that there likely was a weak caddisfly effect across all ponds. The discrepancies between the two periods and significance of variables may be due to a number of factors that have not been researched yet such as temperature, the time leaf packs were deployed, *Nemotaulius hostilis* life stage, and possible dormancy.

Overall, the data analyzed supports that *Limnephilus externus* and *Nemotaulius hostilis* could compete for detritus as a resource within the ponds. Interestingly, the analysis shows great differences between detritus breakdown in the fall and winter periods which may also correspond to differences in the potential for exploitation competition across these two seasons; specifically, *Nemotaulius hostilis* are not consuming as much detritus in the winter period than the fall period. During the fall period *Nemotaulius hostilis* were in their third and fourth instars and working to get to their later instars that they become in the winter period. In their winter period *Nemotaulius hostilis* should be at or progressing to their fifth instars. It is possible that *Nemotaulius hostilis* does not consume as much detritus as a late instar, but it may also be that they enter a dormancy in the winter period when the ponds freeze that has not yet been discovered. Using caddisfly density data may also help us to determine if this lack of winter period breakdown was due to the harsh 2022-23 winter that may have dropped *Nemotaulius hostilis* survival rates. However, if *Nemotaulius hostilis* are consuming detritus over the fall then there will be less detritus remaining for *Limnephilus externus* in the spring. We know that all of the detritus comes from senesced and dead sedge and fronds in fall, so the abundance of detritus in the ponds in the fall and winter is also what *Limnephilus externus* has for the following spring and summer. If *Nemotaulius hostilis* consumes all of the detritus there will be no more detritus left for *Limnephilus externus*. In conclusion, we have found significant results that support resource competition between *Limnephilus externus* and *Nemotaulius hostilis*.

Chlorophyll A concentrations in resource competition experiment

The statistical analysis of the chlorophyll A measurements across caged versus uncaged tiles, and the presence of *Limnephilus externus* proved to be statistically insignificant. However, the relationship between chlorophyll A concentration and detritus abundance was strong and significant. The results of the analysis may indicate issues within the experimental design that may not have allowed us to effectively measure the ability for resource switching to occur. This may be the case because we did not effectively facilitate the growth of algae within all treatment groups. The statistical significance of the detritus abundance shows that algal growth is dependent on detritus abundance, meaning that in the treatments of zero and low detritus there would be very little algae available to the caddisflies for grazing. Algal growth may be increased in the higher detritus treatments due to leaching of nitrogen and phosphorus into the water column, and may also bring more algal cells to the tanks which settle and colonize the tiles. Alternatively, we would want the algae availability to be in high abundance to truly test if *Limnephilus externus* are switching resources under stress of competition and in the absence of detritus. The ability for *Nemotaulius hostilis* to facilitate an increase in algal growth through phosphorus excretion may provide a more nutrient rich food resource for *Limnephilus externus*.

We now know that *Limnephilus externus* and *Nemotaulius hostilis* are competing for detritus, but there is also potential that the range-shift of *Nemotaulius hostilis* may facilitate an increase in resource quality for *Limnephilus externus*. More research is needed to determine whether this holds true, but shows the potential for both negative and positive impacts that rang-shifts might have on an ecosystem.

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