Pycnopsyche (Trichoptera: Limnephilidae) larvae influence on *Orconectes Propinquus* grazing

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Abstract

Crayfish and caddisflies both play important roles as detritivores in streams. However, crayfish occupy a complex trophic role as omnivores, consuming both plant material and other herbivores. Their relationship with caddisflies exemplifies the crayfish's complex trophic role. Crayfish may either reduce plant material by feeding or increase plant material via top-down control on caddisfly herbivory. It is unknown whether their role as predator or their role as herbivore is more impactful in stream communities. I hypothesized that in the presence of both caddisflies and crayfish, less vegetation would be consumed than in situations where crayfish alone were present as the crayfish would eat caddisflies instead of plant material. Crayfish predation on caddisflies would reduce the herbivory of both the crayfish and the caddisflies. Cages were stocked with plant matter and either no organisms, crayfish only, caddisflies only, or both crayfish and caddisflies. The cages with crayfish had more plant matter consumed than those without, and the presence of caddisflies had no significant impact in any treatment. No caddisflies were consumed by crayfish. Overall, the presence of caddisflies was not shown to reduce crayfish foraging on vegetation. Further study is needed to explore the interaction between crayfish and caddisflies.

Introduction

In streams, many macroinvertebrates keep the water clear and free of rotting organic matter by consuming leaf litter, periphyton, aquatic plants, and various detritus. Northern caddisfly larva (*Pycnopsyche spp.*) and northern clearwater crayfish (*Orconectes propinquus*) are two macroinvertebrates common in Midwestern United States streams. *Pycnopsyche* are important detritivores in cool, woodland streams (Wiggins 1997), consuming allochthonous carbon mostly in the form of fallen leaves (Hutchens et. al 1997). Northern clearwater crayfish inhabit a complex trophic role as omnivores and, in addition to acting as detritivores and herbivores, act as predators on smaller macroinvertebrates. However, it is not well known whether crayfish play a more significant role as herbivores, reducing leaf litter, or as predators, controlling populations of herbivores and detritivores and therefore preserving leaf litter (Lodge et. al 1994).

Caddisfly larvae are part of the widely-used EPT (Ephemeroptera Plecoptera Trichoptera) Index used for water quality and their presence generally indicates cleaner water (Masese and Raburu 2017). It is important to understand other impacts on caddisfly presence, such as predation, in order to better understand the extent of their presence as a metric of water quality. For example, if crayfish prey on caddisflies heavily, then a difference in Trichoptera abundance in two streams may be due to the presence of crayfish rather than a disparity in water quality. This study explores the caddisflies' role in the ecosystem, both as consumers and as prey.

Orconectes Propinquus are regarded as ecosystem engineers in forested, headwater streams for their reduction in the abundance of fine particulate matter, which opens habitat for Heptageniid mayfly larvae, and their influence on rate of detrivory in the stream, which influences the benthic habitat by removing decomposing material (Creed 2004). *O. propinquus* have been found to be important "gardeners" in stream ecosystems, their foraging determining the presence or absence of *Cladophora* on stream beds (Hart 1992). They scavenge detritus and a variety of other macroinvertebrates including other crayfish (Capelli 1980). Omnivorous crayfish have been found to be key in breaking down leaf litter in their streams (Zhang et. al 2004), an important part of incorporating allochthonous carbon into a stream ecosystem. It is becoming more important to study the role of *O. propinquus* as the invasive rusty crayfish (*Orconectes rusticus*) spreads throughout Michigan and Wisconsin and outcompetes *O. propinquus* (Capelli and Munjal 1982). This study seeks to investigate whether the availability of caddisflies as prey for crayfish impacts their role as grazers in Tenderfoot Creek. This information would shed light on the mechanisms by which crayfish engineer their ecosystems.

I predicted that the presence of caddisflies would decrease the amount of leaf material that crayfish eat because they would prey on caddisflies, satiating the crayfish in addition to controlling the amount the caddisflies eat. The statistical null hypothesis is that groups of crayfish alone, caddisflies alone, crayfish and caddisflies together, and a control group with neither crayfish nor caddisflies will all see the same amount of leaf material consumed over a six-day period. The biological null hypothesis is that the group with crayfish and caddisflies will consume the same amount of leaf material as the sum of the crayfish only group and the caddisfly only group.

Methods

Study site

The portion of Tenderfoot Creek used is located on the University of Notre Dame's Environmental Research Center, a facility in Michigan's upper peninsula that is largely reserved for research purposes and is free from significant human development. Tenderfoot Creek is a first order stream flowing out of Tenderfoot Lake and a tributary of the Ontonagon River Watershed. This study took place in early July 2019.

Study organisms

We collected all study organisms in the Ontonagon River Watershed. We collected the caddisfly specimens from the stream by hand under rocks, on sticks, and on the skeleton of a white-tailed deer (*Odocoileus virginianus*), which suggests that they feed on a variety of detritus along with their main diet of leaf litter. We also found and collected crayfish by hand in rocky areas of shallow streambed.

Experimental apparatus

We placed twenty 15 cm by 15 cm unglazed clay tiles in fine wire mesh cages that excluded all but the smallest macroinvertebrates. We haphazardly placed them in a semi-shaded riffle in Tenderfoot Creek. We placed one leaf of romaine lettuce (*Lactuca sativa*) cut to the same length as the tile in each cage and weighed them down with a small, dry, clean rock from the shoreline. We chose lettuce for its ease of acquisition and because of its large leaves, which were easy to measure and to prevent from floating in the cages. We weighed each lettuce piece before placing it in its cage.

Estimating initial dry weight of experimental lettuce

We weighed another, similar group of lettuce pieces and dried them at 80˚C until they reached a constant weight after one day, then weighed them again. We used a linear regression between these initial weights and dry weights to estimate what the initial dry weight of the experimental lettuce used in the cages would have been had it been dried (Figure 1). We used this estimation to avoid drying the lettuce before putting it in the stream, where it would likely have disintegrated and would not have imitated the fresh vegetation that foragers generally find in streams.

Experimental design and procedure

We randomly assigned each experimental cage one of four treatments: control, caddisflies only, crayfish only, or both caddisflies and crayfish. The control group had only the lettuce, the tile, and the rock in the cages with no other organisms added. The caddisflies only group had two caddisflies placed inside the cage in addition to the control setup. The crayfish only group had one crayfish added in addition to the control setup, and the caddisflies and crayfish group had two caddisflies and one crayfish added in addition to the control setup. The crayfish used had an average carapace length of 18.06 ± 1.36 mm.

We checked the cages every two days and moved them closer to the center of the creek as water level dropped in order to keep the bottoms of the cages in approximately 5 cm of water. One crayfish died in the course of the experiment and was replaced. Two dead caddisflies in the caddisfly only experimental group were replaced in a similar manner. If a caddisfly had died in the cages with both crayfish and caddisflies, we would not have replaced them as they would have been assumed to have been predated by the crayfish. The cages remained in the stream for six days. After retrieval from the stream, we lightly rinsed the lettuce to remove silt while maintaining structural integrity, dried it at 80˚C until it reached a constant weight after one day, and weighed it.

The difference between initial estimated dry weight of the lettuce and the final dry weight of the lettuce from each cage was considered the mass of lettuce consumed. We conducted a one-way ANOVA to determine if there was a significant difference between the mass of lettuce consumed between the treatments using RStudio (3.5.2, RStudio, Boston, Massachusetts).

Results

After confirming the normality of the mass of lettuce consumed data for all 20 cages with a Shapiro-Wilk normality test (p=0.6625), a one-way ANOVA showed that there was a significant difference between the mass of lettuce consumed in different experimental groups (mean mass of lettuce consumed ± standard deviation; control = 0.19 ± 0.08 g; crayfish only = 0.40 ± .08 g; caddisflies only = 0.20 ± 0.10 g; both crayfish and caddisflies = 0.37 ± 0.17 g; $F_{3,16}=4.866$, $p=0.0157$, Figure 2).

A Tukey's Honestly Significant Difference Test showed that the crayfish only group and the group with both caddisflies and crayfish did not differ significantly (p=0.9753). The control group did not differ significantly from the group with only caddisflies (p=1.0000). The crayfish only group significantly differed from the caddisfly only group (p=0.0520), as well as from the control group (p=0.0520). The group with both caddisflies and crayfish differed from the caddisfly only group (p=0.1118) and from the control group (p=0.1118), if only marginally significantly.

On average, the cages that contained a crayfish had 0.39 ± 0.13 g of lettuce consumed, while the cages that did not contain crayfish had 0.19 ± 0.09 g of lettuce consumed.

Discussion

This study sought to investigate the impact of the presence of caddisflies on the trophic role of crayfish. The results of this study indicate minimal interaction between crayfish and caddisflies, at least as regards predation. Also, the caddisflies ate a negligible amount of vegetation compared to the crayfish. As for the EPT index, it appears as though predation by *O. propinquus* does not have an impact on the presence of caddisflies in the genus *Pycnopsyche.* These caddisflies do not appear to be a component, much less an important one, of the crayfish diet. Because no crayfish ate caddisflies, it is impossible to tell whether giving the crayfish prey to feed on would decrease the amount of vegetation eaten in the system. This limits the scope of this study to the impact of caddisfly larvae presence on crayfish herbivory, which appears to be none. Our statistical null hypothesis has been rejected, but the biological null hypothesis has not. While there may be secondary interactions between the two species as crayfish engineer their environments, this study shows no direct interaction.

Unsurprisingly, this study saw that cages with crayfish differed from those without. However, the caddisfly group did not differ from the control. Caddisflies did not significantly reduce lettuce mass through feeding. In the field, I observed small holes in the lettuce of the cages with only caddisflies, which I believe to be evidence of herbivory. However, it is possible these holes were the result of microbial decomposition. I recommend further studies with greater numbers of caddisflies to investigate whether they consume a significant amount of leaf material. It could also be the case that caddisflies do not eat lettuce specifically but would eat another source of leaf material. Although the caddisflies did not significantly diminish the leaf material provided for them, they do play a role as consumers of leaf litter in their ecosystems (Tornwall and Creed 2016), and I expect I would have seen evidence of this had the experiment been run for longer or with more caddisflies.

An unexpected result was that there was no predation of caddisflies by crayfish throughout the duration of the study. Given that crayfish thrive on a diet with both macroinvertebrates and vegetation or macroinvertebrates only over a vegetation only diet, I expected that crayfish would prey on the available macroinvertebrates (Hills et. al 1993). There are several possible explanations for this lack of predation. While the crayfish are opportunistic scavengers that feed on aquatic insects, I could not find record of them specifically feeding on Trichoptera larvae (Capelli 1980). Perhaps caddisflies are too well defended by their cases for the small crayfish to extract them. It is also possible that the crayfish were satiated by the lettuce, which was easy to access, and did not feel pressure to exert themselves by hunting the caddisflies. It is possible that if the crayfish had run out of lettuce before the end of the experiment, they would have preyed on the caddisflies. A future study could place crayfish and caddisflies together with no alternate food source for the crayfish to see if crayfish prey upon caddisflies when they are their only option for food.

Additionally, the caddisflies sometimes wedged themselves between the tile and the cage wall, which could be an effective hiding technique. However, crayfish would also wedge themselves in this area of the cage, so I find it unlikely that the caddisflies would have been able to evade the crayfish had they decided to attack except by receding into their cases. The cases of the caddisflies not only provide a physical shield from attack, but probably also allow the caddisfly to elude the attention of crayfish due to their nondescript appearance (Duffield et. al 1977). The caddisflies' defenses appear to have served them well as they evaded predation in a small enclosure with a crayfish for six days. Another study could present crayfish with caddisflies with and without their cases to test the efficacy of the case in deterring crayfish predation.

Another possible explanation is that the crayfish worried more about being eaten themselves than about eating other macroinvertebrates. This study used small crayfish, which are predated by native fish (Didonato and Lodge 1993). Schools of small fish did occasionally swim in the vicinity of the experimental cages during this study. Perhaps crayfish were more cautious and hid under the lettuce to avoid fish instead of actively hunting. Another study should use different sizes of crayfish to see if there is a relationship between size and predation on caddisflies.

This experiment focused solely on the interaction of crayfish and caddisflies on the grazing potential of both species. However, this is a limited view of the whole stream ecosystem. Further observational studies of areas with and without crayfish and caddisflies are needed to fully understand how this relationship functions in the context of the stream. Other avenues of potential future research include offering the crayfish different prey to get a clearer picture of how hunting impacts crayfish herbivory and comparing the behavior of different species of crayfish. Specifically, I recommend mayfly larvae as the offered prey because they have been found in the stomachs of *O. propinquus* (Capelli 1980).

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Figures

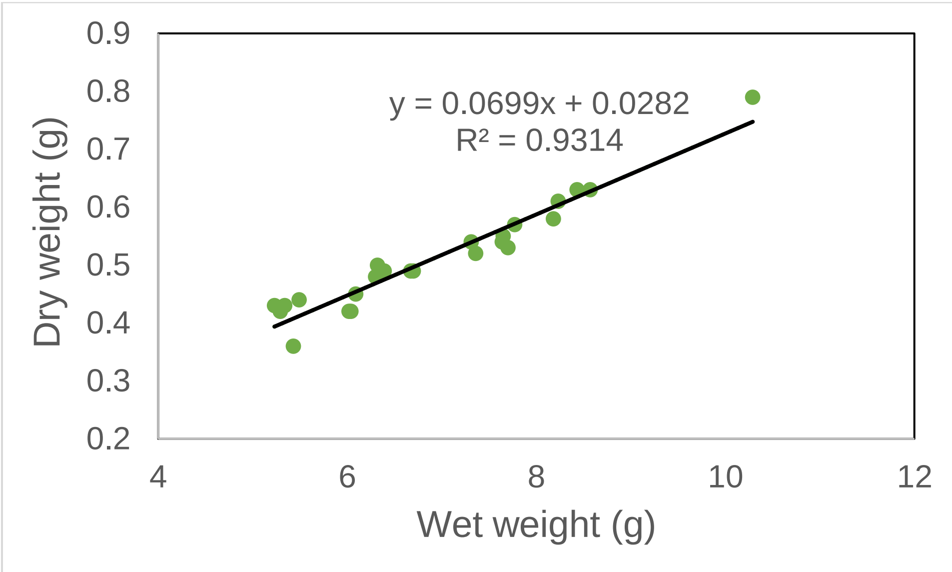

Figure 1. Linear regression shows the relationship between the initial wet weight of a group of romaine lettuce leaves and their dry weight after being dried at 80˚C until reaching a constant weight after one day. This expression (Dry weight = 0.0699 * Wet weight + 0.0282) was used to estimate the initial dry weight of the lettuce used in the experiment.

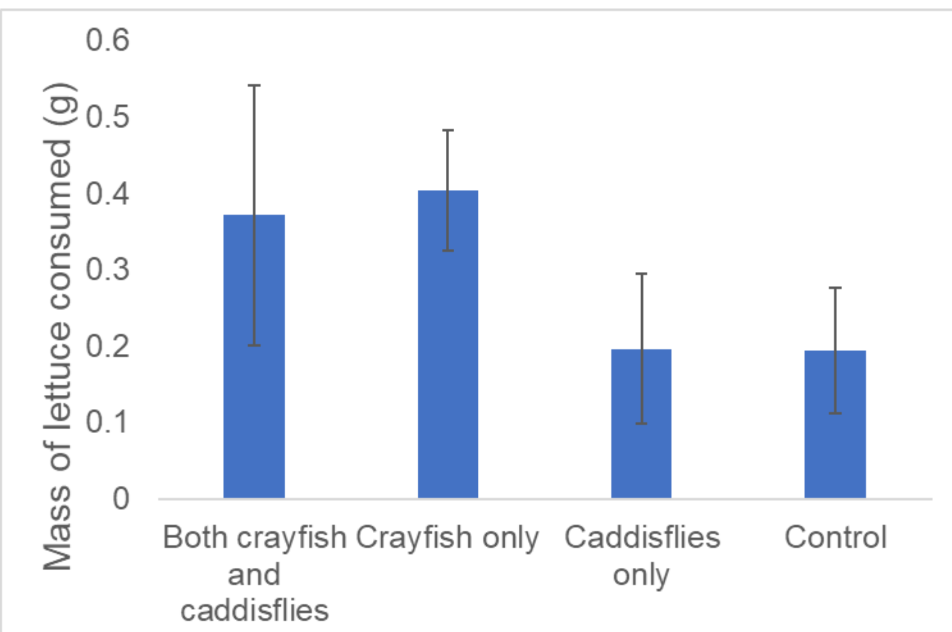

Figure 2. Bar graph of mass of lettuce consumed (g) for each experimental group. The error bars indicate standard deviation. The two groups with crayfish consumed significantly more lettuce than those without crayfish.

Appendix

Cage Number	Treatment	Initial Wet Weight (g)	Initial Dry Weight (g)	Final Wet Weight (g)	Final Dry Weight (g)	Mass Consumed (g)
1	BOTH	10.55	0.77	3.36	0.23	0.54
2	CRAY	9.65	0.70	2.29	0.18	0.52
3	CONTROL	8.54	0.63	5.1	0.36	0.27
4	CONTROL	8.55	0.63	7.86	0.38	0.25
5	CAD	10.97	0.80	11.54	0.7	0.10
6	CONTROL	8.3	0.61	11	0.55	0.06
7	CAD	8.48	0.62	8.08	0.49	0.13
8	CONTROL	9.65	0.70	8.74	0.52	0.18
9	BOTH	9.56	0.70	3.84	0.23	0.47
10	CAD	8.81	0.64	5.18	0.38	0.26
11	BOTH	9.52	0.69	9.17	0.59	0.10
12	CONTROL	8.7	0.64	7.2	0.42	0.22
13	CAD	8.74	0.64	7.94	0.48	0.16
14	CRAY	8.23	0.60	3.78	0.23	0.37
15	BOTH	9.05	0.66	5.91	0.35	0.31
16	CRAY	8.7	0.64	3.77	0.23	0.41
17	BOTH	10.31	0.75	6.09	0.31	0.44
18	CAD	9.49	0.69	8.95	0.36	0.33
19	CRAY	9.1	0.66	5.44	0.25	0.41
20	CRAY	8.24	0.60	5.45	0.3	0.30

Appendix 1. This table contains the raw data collected in this experiment. The treatments are BOTH (both crayfish and caddisflies), CRAY (crayfish only), CAD (caddisflies only), and CONTROL (control).