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ANALYSIS OF *PHYSELLA ACUTA* BEHAVIOR FROM POND AND STREAM POPULATIONS

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ABSTRACT

This study explores the behavioral ecology of Physella acuta from two different populations when exposed to a crayfish predator. One population, the stream population, has been exposed to this crayfish in the wild, whereas the other population, the pond population, is naïve to this particular predator. The goal of this project was to identify if the predator naïve population recognized the crayfish as a threat, and if so if they would behave differently. We found that neither population recognized this particular crayfish as a threat, likely because of the fact that it was a juvenile. The p-value of the population as a source of variance was .907, far above the typical alpha. However, we did find that snails from the pond population were significantly more likely to float at the surface of the water. The p-value of the source population as a source of variance in float data was .00047. This likely means that aquatic snails from pond ecosystems rely on their float response more often than those in systems with running water.

Keywords: Physella acuta, Cambarus, predator naïve, behavioral ecology, antipredator response

INTRODUCTION

Predation is known to affect behavior in prey species, and previous exposure of prey species has been shown to be a factor in the behavior of members of the same species collected from different sites (Sih et al. 2010). This is also observed in introduced species (Rehage 2009). Naïveté in prey species in some cases is shown to decrease predator recognition in naïve individuals (Martin 2014), but in other cases it does not, and naïve individuals exhibit antipredator behavior as readily as experienced individuals(SHAVE et al. 1994)(Kovalenko et al. 2010).

Aquatic snails have been used as model species for many ecological studies exploring anti predatory behaviors and adaptations. Previous study has determined that aquatic snails prefer moving to the water surface in the presence of crayfish, and moving under cover in the presence of fish predators. (Rehage 2009) Morphological studies have shown that larger size and smaller shell aperture occurs in snails developing in conjunction with predators (Dewitt et al. 1999)

A recent study identified three avoidance behaviors in *P. acuta*: leave the water, bury under cover, or float to the top (Amponsah 2019). A study from 2000 found that the antipredator responses of a few snails in the genus *Physella* increased with the increased risk of predation. "Physella increased its avoidance behaviour as risk increased. Crayfish cue elicited a significantly greater response than from controls. Cues from injured conspecifics elicited the strongest response. Physella exhibited several types of avoidance behaviour, including burial into the substratum, moving to the water surface, and crawling out of the water. The type of cue present influenced response type. Cues from crayfish reduced burial and increased movement to the water surface or out of the water. Cues from injured-conspecifics significantly increased crawling completely out of the water." (McCarthy and Fisher 2000) Crawling out of the water completely is a risk for aquatic snails because it exposes them to the risk of desiccation, and so when exposed to a lower risk predation source, there is lower pressure to expose itself to that risk of desiccation.

Physella acuta is a species of aquatic bladder snail in the taxon *Physid*, which contains many freshwater aquatic snails often used as model organisms in ecology and behavior studies. This snail breeds readily and is found in streams and ponds worldwide. *P. acuta* is considered an invasive species in South America, but its invasive status in other areas is not well known because it is unclear exactly where this species originally evolved (Dillon et al. 2002). It is likely, however, that *P. acuta* is native to North America, because although it was first described in France by Draparnaud in 1805, this species is genetically most similar to North American species. This species is a detritivore and an herbivore, feeding primarily on decaying vegetation and some fresh vegetation and algae. Behavioral studies of *P. acuta* focus on behavior in the presence of a predator, of which *P. acuta* has several, including crayfish and freshwater fish.

Crayfish are heterophytes. They feed on detritus and decaying matter, as well as living plant matter and living animal matter. Crayfish forage from multiple trophic levels, making them omnivores, and unique in the average stream system in terms of the variety of prey crayfish forage. They are not generally prey selective, although they do prefer live animal prey, especially as juveniles (Momot 2008). Crayfish are habitat selective and cannot thrive in habitats with low water quality and low dissolved oxygen. Crayfish are valuable not only due to their trophic roles, but because they are a relatively large decapod invertebrate that resides its entire life cycle aquatically in the same habitat. Because of this, habitat usage of the crayfish in its various life stages is important to habitat selection. Crayfish require a variety of refuge sizes to accommodate sizes and needs at different life stages(Reynolds et al. 2013). Crayfish are often used as indicator species for water quality(Trouilhé et al. 2007)(Gouin et al. 2011). Crayfish do predate on living aquatic snails (Nyström and Pérez 1998). Crayfish are notoriously difficult to identify, particularly as juveniles, therefore the crayfish used in this study could only be narrowed down to either a member of the *Cambarus 'acuminatus'* complex, or *Cambarus latimanus*. *C. latimanus* is a small stream crayfish that from a variety of trophic levels. Its life history is distinguished from other stream crayfish in that it likely buries its eggs under substrate after deposition, rather the female than carrying them under its tail (Bouchard 1978). The *acuminatus* complex is a poorly known crayfish complex, that appears to prefer streams containing higher proportions of cobble (Lieb et al. 2008).

In this study the antipredator behavior of *Physella acuta* from a site where crayfish are present is compared to that of *P. acuta* from a site where crayfish are absent. This study also explores the behavior in general of *P. acuta* from both sites, as the habitat differs beyond simply the presence of different predators. This study will determine if there is a conclusive difference in the behavior of *P. acuta* from two different, but related ecosystems. The hypothesis is that there will be a difference in antipredator behavior between these populations.

FIELD SITE

The two collection sites in this study are two parts of the same water system, a system of two suburban ponds connected by a stream called Long Branch Creek that runs under the road and through a lightly wooded urban area. The smaller pond is approximately 9.4 thousand square meters (estimated from "maps.raleighnc.gov" iMaps), which is where all pond snails were collected. The smaller pond is upstream of the collection site for the stream snails, and it is likely that there is gene flow between the pond and the stream. There are crayfish, small fish, *Lithobates pipiens,* and *Eurycea bislineata* present at the stream collection site. *P. acuta* was the only aquatic snail identified in the stream during collection. The species richness in the small pond is higher, with a variety of fish and frogs. Great Blue Heron, Yellow Bellied Slider, and American Bullfrog are some of the macrofauna present at this site. There are also several other aquatic snails including the pond snail and ramshorn snail.

Table 1: waterway characteristics

	Pond	Stream
Average Depth	12cm	7cm
Average Width	1.21m	3.05m
Substrate	100% Silt	90% Cobble 5% Gravel 5% Boulder

Macrophyte Coverage	70% algae	10% algae, 3% Japanese knotweed
Waterside Vegetation	Cultivated meadow and riparian buffer: Dahlia, red oak, loblolly pine, riparian buffer species	Cultivated meadow on one side, heavily forested on the other. Japanese knotweed, White oak, azalea, maple, loblolly pine, white pine, sweet gum, riparian buffer species

Table 2: water quality of collection sites

	Pond	Stream
Ammonia	.4ppm	.3ppm
Nitrite	0ppm	0ppm
Nitrate	0ppm	0ppm
pH	6.6	7.0
Temperature (C)	26	23.5

Figure 1: Highlighted in purple above are the two collection sites in Cary, North Carolina

METHODS AND MATERIALS

Collection methods

Snails were collected opportunistically from each site, with 330 snails each used from the pond or the stream, and 660 snails tested in total. Snails were collected from an area of 12 square meters in the pond, and an area of 55 square meters in the stream. Only one juvenile crayfish was collected, after a variety of fishing methods failed, the crayfish was collected in a net within the same area where snails were collected from the stream.

Rearing methods

The subjects were reared in 3 independent enclosures, in filtered water taken from their collection sites. Each tank was filtered, and the two snail tanks had slightly modified filtration systems. The crayfish tank contained an air stone to improve dissolved oxygen. The snail tanks required sealed mesh lids, or the snails would escape and desiccate on the dry surfaces surrounding the tank. Snails were fed fresh leaves from near the collection site, and crayfish was fed earthworms, algae collected from the collection site, and fresh vegetation.

Modified filtration system for the stream snails such that the snails cannot block the mesh covering the intake on the filter. Drilling holes in the PVC and adding a narrower fitting to the end of the PVC allows water to be taken up from above the mesh rather than just through the mesh lid.

(Figure 2: Original stream filtration set up which could be obstructed by snails or snail eggs.)

(Figure 3: Modification of filter intake)

(Figure 4: Pond tank before equipping a larger filter medium)

Data Collection

For each run, there are 3 tests:

The control, in which five snails from the same habitat are placed in a small clear tub with water and a piece of folded felt to act as cover. Test 1, in which five snails from the same habitat are placed in a small clear tub with water and a piece of folded felt to act as cover, and a plastic toy crayfish of similar size to the live crayfish is placed in the container. And test 2, in which five snails from the same habitat are placed in a small clear tub with water and a piece of folded felt to act as cover, and a live crayfish is placed in the container (the crayfish is too young to successfully predate on any snails, but it does aggress towards them) In the data collection these are referred to as Control, Toy, and Real, respectively.

The position of each snail was evaluated at time 0, and then again, every 60 seconds, staggered by 30 seconds per test. The position of each snail is recorded according to these parameters:

Floating: the foot of the snail is not touching any surface, and the snail is not fully retracted into its shell

Hiding: the shell of the snail is more than 50% covered by the felt cover

Ground: the snail is completely visible from above and is either on the bottom of the container or the top of the felt

Wall: any part of the snail's foot is attached to the wall of the container

Escaped: the snail has left the water, and is either attached to the wall above the waterline or has fallen out of the container all together

Data was collected using the data sheet as shown below, Toy refers to Test 1, containing the plastic crayfish, and Real refers to Test 2, containing the live crayfish. The numbers zero through five indicate the time at which the data point was taken. Zero is the initial behavior, as soon as the snail is dropped in, and each increment is one minute.

Control	Float	Hide	Wall	Ground	Escape
0					
1					
2					
3					
4					
5					
Toy	Float	Hide	Wall	Ground	Escape
0					
1					
2					
3					
4					
5					
Crayfish	Float	Hide	Wall	Ground	Escape
0					
1					
2					
3					
4					
5					

Analysis Methods

Two factor ANOVA in Excel was used to determine results.

RESULTS

There was no significant difference between the different tests for snail behavior. Snails from both sites did not significantly change in behavior when exposed to a live crayfish vs a plastic crayfish vs the control. That said, snails collected from the pond population were significantly more likely to float than those from the stream. In an ANOVA, location of collection site as a source of variance had p-value of 0.000471. The p-value for control versus live crayfish as a source of variance in behavior across both sites was .907, indicating that the variance in behavior in snails exposed to a live crayfish compared with those that were exposed to nothing at all is not significant.

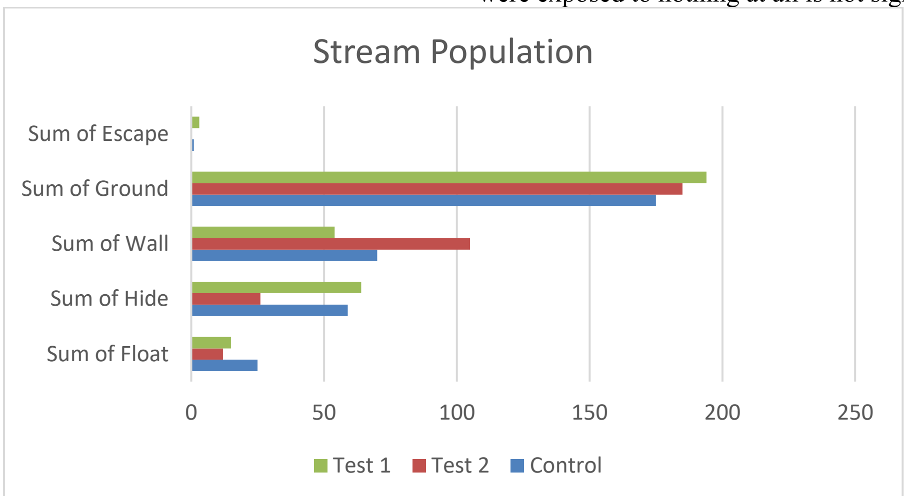

Figure 5: Sum of behaviors in stream population

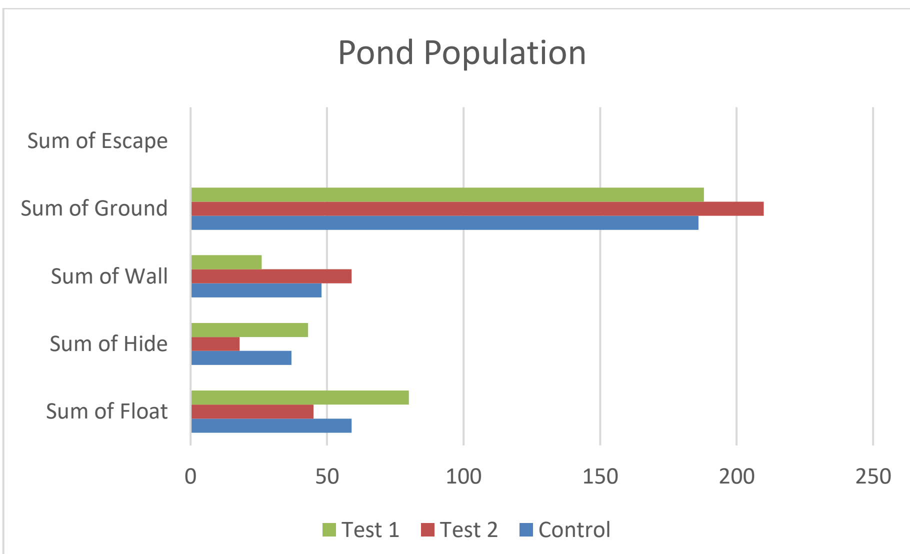

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Figure 6: Sum of behaviors in pond population

Table 3: percent of observed snails in all observations at each time exhibiting each behavior, by source location

	Float	Hide	Wall	Ground	Escape
Pond	18.5%	9.89%	13.4%	58.9%	0%
Stream	5.25%	15.05%	23.1%	55.9%	0.004%
Total	11.9%	12.5%	18.3%	57.5%	0.002%

DOI

DISCUSSION

The snails in general from either site didn't particularly respond to the presence of a predator in their environment except cases in which the crayfish aggressed toward them directly. In those cases the snails retracted fully into their shells without fail. In one case, with a snail from the stream, a snail floated to the surface when aggressed. However, there was no clear change in foraging and general behavior from either type of snail when exposed to the live crayfish. In fact, in one case with snails from the pond population, two snails exhibited mating behavior. There was also no predator recognition of the plastic model crayfish, snails regarded it as additional cover and hid under it or rested on it, in subjects from both sites. This result runs counter to the majority of previous research in predator recognition in this species, *Physella acuta* tend to respond to predator cues from crayfish and exhibit hiding behavior or substratum avoidance (floating or climbing to the water surface). Escape was the least common behavior of all the snails, likely because the snails were not in the test container for very long. Snails kept in a container for more than an hour regularly leave the water and often leave the container altogether. The most likely reason these subjects did not exhibit that behavior more often with the live test is that the crayfish collected was a juvenile and could not predate upon these snails despite its efforts. It is also possible there are crayfish in the pond environment, although it is unlikely, the crayfish found in this area are stream selective and require higher dissolved oxygen than is provided in a pond like this one.

It was found that *P. acuta* from the pond site float more often than those from the stream site. This was also recorded anecdotally while returning snails from the pond population to the pre-release tank, approximately 50-60% of the pond sourced snails floated when dropped in water, whereas less than 10% of the snails sourced from the stream site floated when dropped into water. It's possible snails in the pond population are more likely to float as a response to being introduced to water, and as a behavior in general because it is safer to float in a still pond than in a stream with a current. If stream snails floated, then they would likely either be crushed against stones or float down to the next pond. However, floating is a more effective predator response to crayfish than hiding under cover. Crayfish can't swim very effectively, and so can't reach snails that are floating on the water surface. Crayfish do however, crawl in the substrate and burrow under cover, and as such can easily find snails under cover. It is unlikely that there was undue stress on the snails due to their captivity. Snails in both enclosures bred a great deal, and after release there were more than 20 clutches of eggs in each tank, which indicates that the snails were not much disturbed by their new surroundings.

Future areas of study in this system could include repeating this test with more, larger crayfish, and introducing the cues of predated upon snails (crushed snails), or scent cues from the large crayfish. One could also explore subjects from the large pond as well and compare the behavior of snails from all three sites. This study could be repeated as well with different predators such as bullfrogs or small fish. Another study that could illuminate these differences could be a metabolic study, looking at metabolic responses when exposed to predator cues. The pond system has more predators of this species than the stream, because although fish and frogs are present in the stream, they are too small to predate upon this species successfully, and there are no turtles present in the stream habitat. A morphological study of these snails might be informative as well, although there may be little difference because there is quite a bit of emigration from the pond to the stream system possible.

ACKNOWLEDGEMENTS

I'd like to thank Mindy McNeely and Dr. Stephen Blackwelder for their company while collecting subjects.

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