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EFFECTS OF PREDATION ON THE BEHAVIOR OF *GAMMARUS MINUS*

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ABSTRACT

Prey change their behaviors in the presence of predator cues, in both visual and olfactory senses. The freshwater shrimp *Gammarus minus* faces various levels of predation in its natural habitats. One of its most common predators is the sculpin (*Cottus cognatus*). We studied the effects that predator familiarity has on the hiding behavior of *Gammarus minus* in the presence and absence of visual and olfactory predator cues. Amphipods from a spring with no predators, one with few predators, and one with abundant predators were exposed to environments having predator scent, a predator, or no predator. We observed the number of amphipods visible in each situation. The results demonstrated that regardless of predator cues, if amphipods are accustomed to predators they will exert anti-predator behavior. However, if the amphipods are unfamiliar with sculpins, they will exert more hiding in the presence of a predator. The effects of predator scent are inconclusive and require further testing.

Keywords: behavior, Gammarus minus, *predation*, *sculpin*, *spring ecosystem*

INTRODUCTION

Chemical cues released from predators have been shown to influence the behavior of prey species in various experimental studies. Prey species that detect the presence of a predator by scent or sight are likely to exhibit anti-predator behavior including avoidance behaviors such as reduced feeding and movement and use of refuges. Dahl and Greenberg (1996) found that a relative of *Gammarus minus, Gammarus pulex* prefers a coarser substrate when predators are present, which is hypothesized to be due to the fact that coarse habitat provides more physical shelter for the amphipods (Dahl 1996). Prey will undertake these anti-predator behaviors after detecting the scent of a predator in order to lower their vulnerability to predation (Downes 2002). The predator avoidance behavior studied in this experiment was the use of shelter by *Gammarus minus* (Figure 1) to avoid predation.

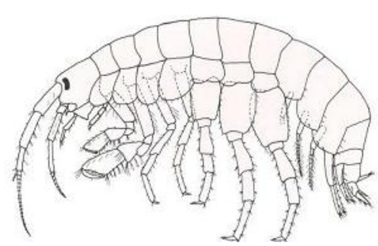

Figure 1. Sketch of Gammarus minus

The species *Gammarus minus* is mainly restricted to freshwater springs and cave systems in much of the Appalachians, south to Missouri and Arkansas, and west to Illinois. This study focuses on *Gammarus minus* inhabiting spring ecosystems. Due to the small, isolated, stable ecosystems of freshwater springs, *G. minus* is exposed to relatively the same level of predation from year to year. Numerous studies have shown that evidence of vertebrate predators in an aquatic ecosystem will cause a reduction in the activity of *G. minus* (Huang 1990). We hypothesized that the degree of predation on *G. minus* in a spring should be reflected in the extent to which they exhibit hiding behavior. One of the chief predators in these springs is the sculpin *Cottus cognatus* (Figure 2).

Figure 2. Photograph of a sculpin Cottus cognatus

MATERIALS AND METHODS

Field Sites

During March and April 2004, *Gammarus minus* were collected from three freshwater springs in Huntingdon County, Pennsylvania. Petersburg Spring had no sculpin predators, whereas Blue Spring had a medium abundance of small sculpins and Williamsburg Spring had a great abundance of larger sculpins. The springs also varied in both abiotic and biotic qualities, as well as in amphipod qualities (Table 1). The amphipods in Williamsburg were the smallest and most difficult to find. The Petersburg amphipods were the easiest to find, the largest, and had the most amplexed pairs.

Table 1. Some abiotic and biotic features of the three study springs. Differences in some amphipod characteristics are also indicated.

Spring Characteristics		**Williamsburg Spring**	**Blue Spring**	**Petersburg Spring**
Abiotic Qualities	**Sediment**	high	low	low
	Substrate	rocky (spring house)	rocky	rocky
	Water	cloudy; very slow moving (spring house)	Clean; swift moving	Clean; moderately fast
Biotic Qualities	**Fish**	numerous (large)	Less numerous (small)	none
	Other	algae, pond scum, salamanders, crayfish	many isopods and caddis flies	nematodes and salamanders
Amphipod Qualities	**Amplexed Pairs**	few	many	many
	Ease of Finding	difficult	easy	very easy
	Size	small	large	large

Procedures

At each of the three springs, qualitative observations, such as how easily the amphipods were caught and the surrounding environmental conditions, were recorded (Table 1). In order to set up this experiment, nine rectangular plastic containers with lids were obtained. Each of the nine containers were ¾ filled with water from Petersburg spring, which contained no predators. The containers were also filled with rocks on one side, giving the amphipods objects to hide underneath. They were placed in a cold storage room for the duration of the experiment, which simulated the approximate water temperatures amphipods would have experienced in their natural springs. The water was stirred every day at the surface to prevent a film from developing, thus facilitating surface to air oxygen exchange.

Three containers had amphipods from Petersburg Spring, three containers had amphipods from Blue Spring, and three containers had amphipods from Williamsburg Spring. In one control container, for each of the springs, no predator was placed in the water. In another container, for each of the springs, the water was made so that it contained a "predator scent." In order to do this, sculpins obtained from Williamsburg Spring were left in water for two days, after which they were removed and the amphipods were placed into the containers. In the last container, for each of the springs, amphipods were subject to predation. In order to set up these containers, fish were first left in the water for two days, as was done previously with the "fish scent" containers. The fish were then taken out and amphipods were placed into the containers. The amphipods were allowed a three day period to adjust to the "scent" and the experimental surroundings, after which time the fish were placed back into the containers. For a summary of the conditions of each container see Table 2.

Table 2. Descriptions of the experimental containers

Container	Conditions
1	Petersburg Spring – no predator
2	Petersburg Spring – predator scent
3	Petersburg Spring – predator
4	Blue Spring – no predator
5	Blue Spring – predator scent
6	Blue Spring – predator
7	Williamsburg Spring – no predator
8	Williamsburg Spring – predator scent
9	Williamsburg Spring – predator

The day after fish were placed back into containers subject to predation, observations were made on all the containers. Lids were removed and only those amphipods that were visible to the naked eye were counted. Two other counts were done three days apart. After the last count was completed, fish were removed from the "predator" containers. All amphipods in containers 1 though 9 were then counted to see how many amphipods remained. Rocks were removed in order to find amphipods that were hiding underneath them and the percent loss was calculated. Chi-square tests were run on the total number of amphipods visible in the various tanks using Minitab. The tests were used to analyze each of the three springs and also each of the three treatments. A time schedule of the procedure is provided in Table 3.

Table 3. Time schedule followed during the experiment

Day	Procedure
1	Place fish into “predator scent” and “predator” containers
3	Remove fish, put in amphipods
6	Put fish back into “predator” containers
7	Count visible amphipods
10	Count visible amphipods
13	Count visible and total amphipods

RESULTS

During all three counts, the Petersburg Spring amphipods with the predator scent had the most visible *Gammarus minus* (9, 12, and 15 visible). The Blue Spring containers with the predator scent and the containers with the predator, as well as the Williamsburg tank with the predator all had none visible for all three counts. The rest of the springs ranged from 1 to 8 amphipods depending on the tank and the count (Table 4).

Table 4. Number of Gammarus minus *visible in each trial.*

		# Visible *Gammarus minus*					
Tank No.	**Description**	**Count 1**	**Count 2**	**Count 3**	**Total**	**Average**	**STDEV**
1	Petersburg - no predator	8	3	2	13	4.33	3.21
2	Petersburg - predator scent	9	12	15	36	12.00	3.00
3	Petersburg - predator	0	0	1	1	0.33	0.58
4	Blue - no predator	2	0	3	5	1.67	1.53
5	Blue - predator scent	0	0	0	0	0.00	0.00
6	Blue - predator	0	0	0	0	0.00	0.00
7	Williamsburg - no predator	0	1	0	1	0.33	0.58
8	Williamsburg - predator scent	0	3	3	6	2.00	1.73
9	Williamsburg - predator	0	0	0	0	0.00	0.00

Table 5. Number of Gammarus minus *lost during extent of experiment.*

Tank No.	**Description**	**Final Count**	**Percent Loss**
1	Petersburg - no predator	25	0
2	Petersburg - predator scent	24	4
3	**Petersburg - predator**	**16**	**36**
4	Blue - no predator	25	0
5	Blue - predator scent	24	4
6	**Blue - predator**	**20**	**20**
7	Williamsburg - no predator	25	0
8	Williamsburg - predator scent	24	4
9	**Williamsburg - predator**	**21**	**16**

The Petersburg amphipods also showed the greatest loss to predation (36%) and the Williamsburg amphipods showed the least loss (16%) (Table5). The results of the Chi-square tests run on the data from Table 4 can be seen in Table 6. The expected values used in the Chi-square tests were the average of the three values being compared. Because Minitab cannot accept non-whole numbers for expected values, some of the Chi-square and P-values differed depending on which expected value was assigned to which observed value. Therefore, average values are also shown for these tests in order to determine significance. The Chi-square analysis showed significant differences among the Petersburg treatment groups, but no significant differences among the Williamsburg treatments. There was also a significant difference among the non predator and predator scent treatment groups between the different springs.

Table 6: Chi-square analysis of each spring and each treatment. Average chi-square and average P-values are also shown

	Chi-Sq	P-value	Average Chi-Sq	Average P-value	Significant
Petersburg	20.580	<0.01	20.58	<0.01	yes
Blue	not enough data				
Williamsburg	3.333	0.189	4.555	0.113	no
	5.000	0.082			
	5.333	0.069			
No Predator	5.462	0.065	6.372	0.044	yes
	6.484	0.039			
	7.170	0.028			
Predator Scent	26.880	<0.01	26.880	<0.01	yes
Predator	not enough data				

DISCUSSION

Our data support the hypothesis that *Gammarus minus* exhibit varying degrees of microhabitat selection in order to escape predation based on their familiarity with predators. As seen in Table 4, the amphipods from the two springs having predators, Blue and Williamsburg, were in general less visible than those from Petersburg Spring. When there were no predators present, the behavior of the amphipods depended on their degree of familiarity with sculpins. The average number of amphipods visible from Petersburg Spring in the no predator tank was 4.33, while that of Williamsburg was 0.33. Blue Spring fell between these two extremes, having an average of 1.67 amphipods visible. This data corresponds to the relative familiarity with predators in their respective springs. Based on the results of the chi-squared analysis, these numbers are statistically significant (Table 6).

Although the behavior of the amphipods in the presence of a predator's scent was significantly different between the three springs, the observed behavior was not as expected based on familiarity. While Petersburg amphipods were still more visible than those of either Blue or Williamsburg amphipods, it was interesting to note that more amphipods from Williamsburg were visible than those from Blue. Future studies may be necessary to determine the cause of such unexpected results.

There were not enough data to test the significance of familiarity in the presence of predators because very few amphipods from any of the springs were visible. This could be evidence that these animals change their behavior based on pressures in their surrounding environments, such as predators. Petersburg amphipods display this phenomenon, as they reduce their visibility when presented with a predator. Compared to the number of *G. minus* visible when there is no predator or when only the scent is present, the number visible with a predator is significantly lower (Table 6). This is consistent with the results Wisenden et al. (1999), who tested the behavioral response to these cues that confer a survival benefit to *Gammarus* when exposed to a predator. They found that the anti-predator response to chemosensory cues confers an increased survival rate. By putting amphipods in an environment with predators with which they are not familiar, they were forced to behaviorally adapt to a new situation in order to survive. The results show how resourcefully the amphipods adjust to their surroundings. This "capacity for highly flexible and quickly adjustable anti-predator responses" can also be seen in aquatic insects, as discovered by Tikkanen et al. (1996).

Amphipods from Blue Spring and Williamsburg Spring showed no significant differences in behavior based on their surroundings. Regardless of the presence of a predator, few amphipods from these springs remained unhidden. This demonstrates that familiarity to predators is more important than the present environment. If an amphipod is accustomed to living in the presence of a predator, it will automatically hide in the substrate regardless of the occurrence of a predator. Other studies also demonstrate that behaviors which vary between populations are often fixed even when the organisms are removed from their natural habitat (Tikkanen et al. 1996)

Although almost no amphipods from any of the springs were visible in the presence of a predator, the percent of amphipods lost illustrated that familiarity still played an important role in the survival of the *Gammarus minus*. As demonstrated by Table 5, there was a 36% loss from the group of Petersburg amphipods occurring with a sculpin, a 20% loss in the comparable Blue container, and a 16% loss in the Williamsburg container. These data suggest that mortality rate was higher for those from Petersburg Spring, indicating that lack of experience with this particular predator has a considerable impact on fatality. This corresponds to data gathered by Tikkanen et al. (1996) that suggest that arthropods from sites with few predators do not exhibit protective behaviors to the same extent as those accustomed to areas with high predation.

For more accurate results, future experiments could involve larger numbers of amphipods. By increasing the sample size, the data would be more reliable and allow the correlations to be more pronounced. There were also many other variables that could have influenced the behavior of *Gammarus minus*. For instance, the size of the amphipods could have impacted the ability of the predator to locate them. As noted in Table 1, the amphipods from Williamsburg Spring were smaller and could therefore avoid predation more easily than the larger amphipods of Petersburg and Blue Springs. Environmental conditions also differed between the three springs (Table 1). Additional experimentation could test the effects of both size and environment on the avoidance behavior of the amphipods.

Related experiments could involve the effects that other species have on the behavior and physiological adaptations of *Gammarus minus*. Other species could include different predators or competitive species that coexist with the amphipods. Further research could determine if amphipods familiar with predators would adjust more rapidly to the presence of an unfamiliar predator than those without any past predator experience. Further insight into the results could be gained by researching why a higher number of amphipods overall are visible when a predator's scent is introduced to their environment. Although more research needs to be conducted, regardless of predator cues, if amphipods are accustomed to predators they will exert anti-predator behavior. However, if the amphipods are unfamiliar with sculpins, they will adjust and exert a higher rate of hiding behavior in the presence of a predator.

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