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# The Behavior of *Gammarus sp.* When Exposed to Predation Cues

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## ABSTRACT

Predator avoidance behaviors are a critical defense mechanism that can increase the chances of survival for potential prey species. We tested the avoidance behavior of the freshwater amphipod, *Gammarus sp.*, under two different chemical predation cues. The first was the presence of kairomone, which was derived from a species of fish, *Gambusia affinis*, that was fed a diet exclusively of amphipods. The second predation cue was potential alarm cue derived from macerated conspecific amphipods from the same population as the test species. Response variables included time spent moving after the introduction to the signal as well as the time spent in refuge. Movement of amphipods significantly decreased and time spent in refuge significantly increased when amphipods were exposed to alarm cue as compared to control organisms that received no exposure to predation cues. Exposure to the fish kairomone treatments significantly increased time spent in the refuge but did not significantly influence time spent moving during the trials. These results suggest that amphipods exhibit predator avoidance behavior primarily when there is evidence that an attack has occurred (evidenced by the death of conspecifics within the population), but will also demonstrate avoidance behaviors to a lesser degree when a predator is detected.

## INTRODUCTION

In aquatic systems, organisms often interact with their surrounding environment using chemical signals (Ferrari et al. 2010). These signals include cues from conspecifics as well as heterospecifics, and are especially important when the heterospecific happens also to be an active predator (Ferrari et al. 2010) (Chapman et al. 2015). All organisms that are actively preyed upon must possess at least some semblance of a defensive mechanism if they are to successfully survive, mate, and propagate another generation of their species, and the capacity for early detection of the predator can be critical (Lima & Dill 1990)(Dunn et al. 2008)(Chapman et al. 2015). The prey, after detecting the signal, will typically undergo a change in behavior and/or physiology that increases their chance of survival (Lima & Dill 1990).

Common predator avoidance behaviors for aquatic organisms include reduction of movement and or spatial avoidance of the detected predator (Wisenden 2000)(Chapman et al. 2015). *Gammarus sp.* is a species of amphipod often found in freshwater streams and ponds. With regard to organisms such as amphipods, specifically *Gammarus sp.*, the primary mechanisms can be reduced to avoidance behaviors. In other words, amphipods do not flee from a predator nor do they become aggressive and fight, but rather rely on natural camouflage to avoid predation. A problem, however, occurs when avoidance behavior interrupts other activities such as foraging and mating for an extended amount of time. An organism must weigh the risk of movement in the presence of a potential predator versus the reward of foraging and or mating (Lima & Dill 1990)(Chapman et al. 2015).

Waterfleas belonging to the genus *Daphnia*, another aquatic organism, have been known to migrate from the pelagic zone during the nighttime to the littoral zone during the day as part of their active predator avoidance behaviors (Meutter et al. 2004). Meutter (2004) asserted that this probably occurs due to intense predation pressure that occurs during the daytime in the pelagic zone on *Daphnia* (Meutter et al. 2004). Consequently, an opportunistic predator such as the larvae from zygopterans feeds on the *Daphnia* during the day within macrophytes where they co-occur (Meutter et al. 2004). When they measured the presence of *Daphnia* in the water column when the zygopterans or their “odor” was present they found that the presence of *Daphnia* was 10% lower than when the zygopterans or their odor was not present (Meutter et al. 2004). Similarly, an amphipod may exhibit avoidance behaviors in the presence of a predator, though in a slightly different manner. Amphipods have been known to avoid olfactory chemical cues of predators via reduced behavior (Baumgartner et al. 2003) (Wudkevich 1997), and that the avoidance behaviors of some members of *Gammarus* are inherited, or in other terms are a local adaptation to specific predator regimes (Abjornsson 2004). We tested the effect of two chemical signals on the behavior of *Gammarus sp.* to see if there was a tendency to reduce activity and or seek refuge in the presence of a potential predator. The first signal was from a heterospecific and was a kairomone cue, which means that the signal was from a predator, and in this experiment was taken from the a species of mosquito fish (*Gambusia affinis*) (Bronmark & Hansson 2000)(Chapman et al. 2015). We used the water in which the mosquito fish was residing to generate the kairomone. The second cue was a potential alarm cue derived from macerated conspecifics.

The potential alarm cue was especially interesting given that at any given time numerous signals are received and distributed in an aquatic environment (Solomon 1977)(Chapman et al. 2015). Thus the alarm cue portion of the experiment was designed to examine if the death of conspecifics in appreciable quantities signaled imminent danger to an amphipod. The amphipods collected for the experiment resided in extremely large populations, and thus a concentration comparable to that which was used in the experiment was feasible in a natural setting as well. Compared to the standard kairomone detection, these alarm cue trials were intended to examine how different cues might have similar or differing effects on predator avoidance behavior. This difference in behaviors is not mere speculation as a species of snails studied by Turner and Montgomery (2003) found that snails (*Physa acuta*) continued foraging in refuge if the predator was at a certain distance (Turner & Montgomery 2003). This shows that an organism may indeed remain active even if a predator is detected if a refuge is present. Thus, our hypothesis is that signals indicate different threat levels, and therefore that amphipods will respond to kairomone and alarm cue differently, as the signals indicate different threat levels.

## METHODS

### *Collection and Maintenance*

Amphipods were collected from a spring near Elliston, Virginia. This spring held a constant temperature of approximately 15 °C. Amphipods were collected and transported to campus in coolers filled with spring water. The housing unit consisted of a one hundred gallon tank of moderately hard water. Filters and aerators were also installed in order to maintain a proper oxygen level within the tank. Amphipods were fed a diet of watercress and or moss from the spring from which they were collected, and the water

was changed periodically. Animals were acclimated for 48 hours prior to running the trial.

### *Stimulus collection*

Concentrated alarm cue was created using three macerated amphipods per one mL of moderately hard water. Mosquito fish (*Gambusia affinis*) were collected from a pond near Erwin, Tennessee and used to collect predator kairomones. A total of 8 fish were individually placed into chambers with moderately hard water (100 mL per gram of body mass). Each fish was fed five amphipods per day for 48 hours. Following the 48-hour soaking period, the fish were removed and the stimulus from each individual chamber was mixed in order to avoid any variation in chemical cues.

### *Experimental Design*

A chamber was utilized as a container for the experiment and was filled with 150 mL of moderately hard water at around 15 °C. Black sand was used to provide a surface for the specimens and moss collected from the spring was used as refuge on one side of the chamber while the other was empty water column. A line drawn along the center of the chamber divided the two sections. Once the amphipod was transferred from the housing tank to the test chamber, a 30 second acclimation period was allowed. After the initial acclimation period, 2 ml of blank moderately hard water was added to the chamber with a micropipette. After allowing 60 seconds for distribution, the pre-stimulus period began with the observer timing both time spent in refuge and time spent moving. Once the five minutes were over, 2 ml of the stimulus (alarm cue, kairomone, or control) was added to the chambers, which provided a concentration of 4 individuals per 100 ml of moderately hard water. Another 60-second acclimation period was allowed, followed by

the post-stimulus observational period during which activity and time spend in refuge measurements were recorded. At the end of each trial, all materials were washed with tap water and distilled water prior to the next trial. Stimulus for treatments were prepared prior to trials and randomized during experiments. All stimuli (including control) were maintained at 15° C until trials began. Fresh alarm cue and kairomone water was prepared every day and was never frozen.

### *Statistical Analysis*

Statistical analysis of the data was conducted (Minitab 17 statistical software). A one-way ANOVA statistical test was utilized in the analysis of the data with  $\alpha=0.05$ . For post-hoc analysis a Tukey Test was utilized.

## RESULTS

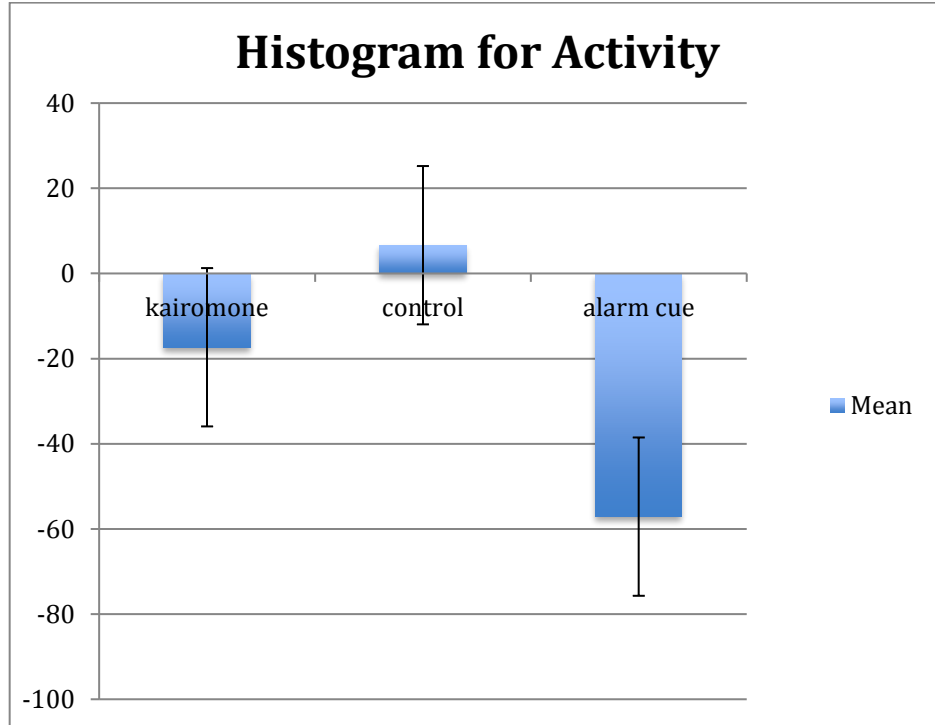


Figure 1: Activity of amphipods in experimental treatments. Results expressed as the time spent moving with directed locomotion for all. Directed locomotion was active movement either vertically or horizontally throughout the chamber. Each individual trial was measured by subtracting the post-stimulus results from the pre-stimulus results. Error bars represent 1 standard error.



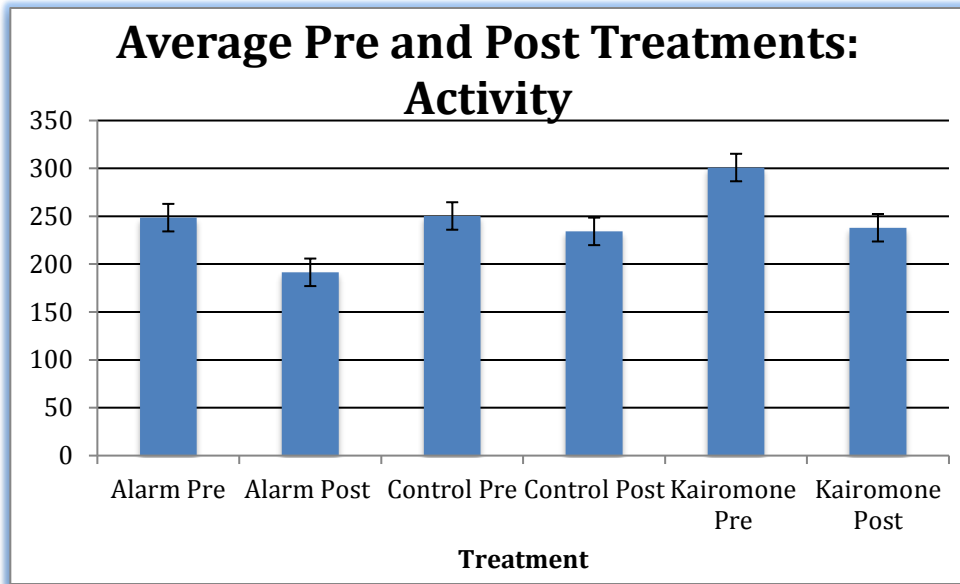


Figure 1: Activity of amphipods in experimental treatments. Results expressed as the time spent moving with directed locomotion for all. Directed locomotion was active movement either vertically or horizontally throughout the chamber. Each individual bar represents either the average pre or post-stimulus measurement for a given treatment. The time is expressed in seconds spent actively moving. Error bars represent 1 standard error.

Activity was measured for each trial, nine times for alarm cue and kairomone and nineteen for control. Alarm cue caused significant behavioral change in amphipods for activity, as they reduced their activity ( $p < 0.01$ , mean = -57.1, Figure 1). Kairomone did not cause a significant change in behavior ( $p = 0.079$ , mean = -17.3). Activity was measured as directed locomotion, or active movement either vertically or horizontally.

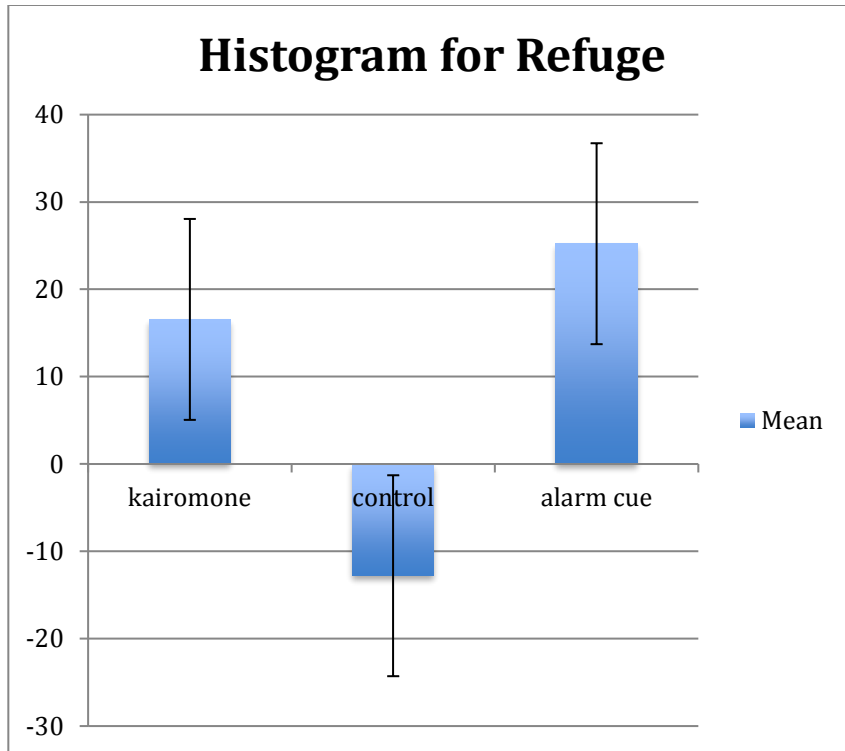


Figure 2: Time spent in refuge for amphipods in experimental treatments. Results expressed as time spent in refuge section of the test chamber. Each individual trial was measured by subtracting the pre-stimulus results from the post-stimulus results. Error bars represent 1 standard error.

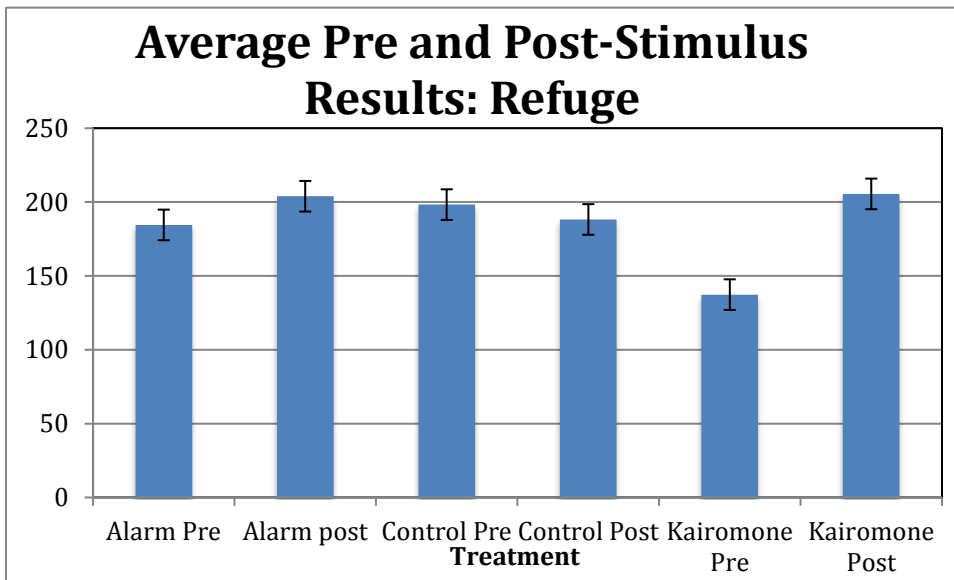


Figure 4: Time spent in refuge for amphipods in experimental treatments. Results expressed as time spent in refuge section of the test chamber. Each individual bar represents the average pre or post stimulus results for a given treatment. The time is expressed in seconds spent in refuge. Error bars represent 1 standard error.

Amphipods changed their behavior significantly in response to both predatory kairomone ( $p = 0.013$ , mean =16.56, Figure 2) and alarm cue ( $p =0.02$ , mean 25.22) by spending more time in refuge. The time, in seconds, indicates that the amphipods spent significantly more time on the refuge side of the chamber under the predation cue as compared to the control.

## DISCUSSION

We found that amphipods responded to both chemical cues. However, the results indicate that amphipods responded to different predation cues in different manners. The amphipods responded in two-fold fashion to the alarm cue signal by both reducing behavior and spending more time in refuge, while just reacting to the predatory kairomone in singular fashion. The alarm cue incited two significant behavioral trends: a reduction in activity as indicated by less time spent moving and an increase in time spent in refuge. For the kairomone treatment only an increase in time spent in refuge was seen. Simply seeing that the amphipods responded was not a surprising finding as other studies have noted a variety of aquatic organisms use chemical cues to interpret their surroundings, but the manner in which they responded was interesting as their response differed between chemical signals. (Mathis 2003; Mathis & Vincent 2000) (Pennuto & Kepler 2008) (Chapman et al. 2015).

In the experiment, amphipods demonstrated a different level of behavior based on the type of cue they were exposed to. Amphipods demonstrated a greater change in behavior for the alarm cue signal than for the kairomone, decreasing activity significantly while also increasing time spent in refuge. For the kairomone treatment, the organisms only increased time in the refuge.

These findings should not be considered anomalies as other studies have found such grading responses exist in predator avoidance behaviors in other organisms. One such example was described by Brown (2006) who tested the behavior of juvenile convict cichlids (*Archocentrus nigrofasciatus*) in response to alarm cue and with special regard to shoal size (Brown et al. 2006). They used varying concentrations of alarm cue (12.5% and 25.0% and a control), and saw that behavior differed between all three shoal sizes (Brown et al. 2006). Using varied concentrations of alarm cue (12.5% and 25.0%) and a distilled water control they saw that behavior differed between all three groups (Brown et al. 2006). Single cichlids exhibited a reduction in movement and foraging attempts under both the concentrations equally and thus demonstrated a hypersensitive or non-graded behavior pattern (Brown et al. 2006). Small shoals demonstrated similar behavior but only at the higher threshold, or in other words at the higher concentrations of alarm cue (Brown et al. 2006). Large shoals, however, exhibited a graded behavioral pattern across all concentrations, leading the group to find that shoal size affects the response of individual cichlids in the presence of alarm cue (Brown et al. 2006).

With regard to our experiment, this shows that amphipods and cichlids have the propensity to alter their behavior in a less than uniform way with regard to predation cues. In other words, amphipods can grade a signal as more dangerous than another just as cichlids grade signals differently when in shoals of different sizes. This means that behavioral changes occur in response to a set circumstance instead of unilaterally in the presence of danger. Amphipods alter their behavior in the presence of predators at large, but the evidence of an attack indicated by alarm cue proves more pressing of a danger

than merely evidence that a predator resides nearby as indicated by the presence of kairomone.

Another experiment by Barnes (2002) demonstrates this type of behavioral grading with wolf spiders (*Pardosa milvina*) (Barnes et al. 2002). Barnes (2002) found that while wolf spiders reduce movement in the presence of chemical cues from larger wolf spiders this behavior is also correlative with the age of the cue (Barnes et al. 2002). In other terms, wolf spiders reduce their movement significantly less in correlation with the age of the cue (Barnes et al. 2002). A fresh cue indicates a more pertinent risk to a wolf spider, and as the cue ages the risk to the spider also decreases (Barnes et al. 2002). Thus, wolf spiders grade the same chemical cue differently based off of the freshness of said cue.

Another study with wolf spiders (*Schizocosa ocreata*) by Lohrey, (2009) found that wolf spiders, like the amphipods in our experiment, vary their behavioral responses based on the type of signal (Lohrey et al. 2009). In their experiment, Lohrey (2009) tested the behavior of courting male wolf spiders in the presence of three different predator cues including visual cues, seismic cues, and acoustic cues (Lohrey et al. 2009). They found that wolf spiders respond to seismic (a simulated beak tapping) and acoustic (a bird call) cues by ceasing courtship behavior and movement at large (Lohrey et al. 2009). The response to the visual cue (a shadow of bird), however, was different resulting in an increase in movement (Lohrey et al. 2009). In addition to these tests, they also measured how long a return to normal courtship behavior took for both non-threatening and threatening (i.e. the aforementioned cues), finding that a return to normal behavior occurred more quickly when non-threatening stimuli were perceived by the spiders.

These results support the notion that invertebrates can respond to different predation cues in quite different manners, and show that unilateral behavior is not the case in certain organisms.

In addition to our study, another study conducted by Pennuto and Kepler (2008) tested amphipod predator avoidance behaviors. They tested whether an invasive amphipod species (*Echinogammarus ischnus*) and a native species of amphipod (*Gammarus fasciatus*) differed in terms of avoidance behaviors when exposed to a variety of predatory cues (five different species of fish with different foraging techniques) and different densities of fish (Pennuto & Kepler 2008). They found that though the organisms responded to a wide range of cues and also to increasing goby densities, ultimately their behaviors differed both from each other and with respect to specific predation cues from specific species of fish (Pennuto & Kepler 2008). This study shows that amphipods are capable of specified responses to certain cues, even cues that often indicate the presence of similar threats.

These studies reinforce the concept that amphipods alter their behavior based on the level of threat a predator cue represents. This leaves a great deal of room for further study given the broadness of the topic. For example, another line of research with regard to amphipods might be how multiple stressors such as chemical and thermal stresses affect behavior when coupled with predation cues. Any of these avenues of research would contribute to characterizing macro-invertebrate predator avoidance behavior.

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