Effects of Crayfish on Predator Avoidance Response in Freshwater Snails

Julia Berg, Lauren Hoff, Morgan Rondinelli, Kiernan Skelly

University of Michigan Biological Station
EEB 381: General Ecology
16 June 2016
Dr. Joel Heinen

Abstract

Cryptic substrate choice in Physidae snails was analyzed as a function of predator avoidance behavior. In the presence of Orconectes crayfish, snails demonstrated a preference for the most similar substrate in size, shape and color to their shells (measured by anecdotal observations). The preferred substrate was typically gravel, while rocks, sand and wood were less preferred. Impacts of perceived threat levels were tested in treatments involving varying degrees of crayfish presence. Snails did not demonstrate an increasing preference for the gravel substrate corresponding to level of predation. The one exception to the snails' general preference for gravel was documented in the chemical-cue trial, in which snails exhibited a slight preference for rocks. Without chemical cues from dead snails, in conjunction with chemical cues from crayfish, the snails were unlikely to perceive a threat. Results indicated that while Physidae snails did not respond to increasing levels of predation from Orconectes crayfish, they still responded to the threat of predation.

Introduction

Predation is a major selective pressure for prey animal species (Vermeij & Covich, 1978). In aquatic gastropods, antipredator behaviors are common (Alexander & Covich, 1991b) and take many forms (Feder, 1963), likely as a result of this selective pressure. As such, we sought to understand predator-prey interactions between freshwater snails and crayfish. Past studies have detailed significant impacts of predation by crayfish on the life history and behavior of freshwater snails (Crowl and Covich, 1990; Alexander & Covich, 1991b; Dickey & McCarthy, 2007). Given that the habitats of snails and crayfish overlap within many freshwater ecosystems (Lewis, 2001), their respective actions may largely be driven by their interactions within these shared habitats.

Physidae, known for their left-handed shell coil (Figure 1; Dillon, 2000), have been shown to exhibit predator avoidance mechanisms in the presence of *Orconectes* crayfish (Alexander & Covich, 1991a). These mechanisms include the ability to burrow into substrates (Snyder, 1967), float in the water and crawl above the waterline, both in nature and in captivity (Alexander & Covich, 1991b). Additionally, Alexander & Covich (1991b) observed increased predator avoidance by *Physidae* snails in comparison to the similar *Planorbella* snails, which have stronger shells that provide increased protection. This finding indicated that, due to their comparatively lower shell strength, *Physidae* have developed an adaptive response to crayfish predation. As a result of selective pressures from predation, cryptic behaviors can evolve as an avoidance strategy. According to Wickler (1968) and Heinen (1993, 1994), an organism demonstrates cryptic behavior by choosing surroundings that match its color, or by changing its color to match the surroundings.

In addition to the physical presence of a predator, snail behavior can be influenced by chemical cues associated with a risk of predation. Crowl & Covich (1990) found that *Physidae* are capable of detecting chemicals released when crayfish are actively foraging for snails. Similarly, Snyder (1967) reported that several *Physidae* exhibited predator avoidance mechanisms when exposed to water that contained these crayfish chemical cues. *Physidae*, as primarily a prey species, typically decrease activity or seek refuge when they detect chemical cues indicating the presence of crayfish (Dickey & McCarthy, 2007). Avoidance activity also depends on the length of exposure to the predators. As such, predatory evasive responses decreased after crayfish were removed from the tank, and snails which had climbed above the waterline returned to below the waterline (Alexander & Covich, 1991b).

The purpose of this study was to determine the preferred substrate of snails of the family *Physidae*, and to determine to what extent the presence of crayfish influenced substrate choice. Weber & Lodge (1990) observed strong correlations between substrate preference by *Physidae* and the presence of crayfish, as snails generally chose rocks when exposed to a predator. Additionally, Clampitt (1972) found that snails had a preference for hard substrates such as stones. Given these findings, we expected to observe a preference for rocks. We also anticipated that this preference would increase with the snails' perceived threat of predation, determined by both visual and chemical cues. In our trials, we introduced a predator at varying degrees (no crayfish present, crayfish present for the entire trial, crayfish present for half of the trial, and crayfish chemical cues present). This allowed us to quantify the effect of crayfish on the snails.

Our first hypothesis was that without the threat of predation, *Physidae* snails would have a weak preference for the rock substrate. Our second hypothesis was that *Physidae* snails that were directly threatened by the presence of a crayfish for the whole trial would have the strongest preference for the rock substrate. Third, we hypothesized that *Physidae* snails that were initially exposed to a predator but later had the threat removed would exhibit a strong preference for the rock substrate. We predicted that this preference would be weaker than the preference of snails exposed to crayfish for the entire duration of the trial. Fourth, *Physidae* snails, with only the apparent threat of predation from the presence of crayfish chemical cues, would have a moderate preference for the rock substrate.

Methods

We exposed snails to four levels of predator presence: no crayfish present, crayfish present for the full duration of the trials, crayfish present for half the duration of the trials and water in which the crayfish had been stored. In each trial, we placed snails in the center of a tank and gave them the option to locate themselves on one of four natural substrates: sand, rocks, gravel or waterlogged pieces of wood.

Site Description

We observed this predator-prey relationship between snails and crayfish in Douglas Lake, a freshwater ecosystem in Northern Michigan. Douglas Lake has diverse habitats that provide the opportunity to conduct research on a number of freshwater species. The unique location of the University of Michigan Biological Station (Heinen & Vande Kopple, 2003) further enhances these opportunities. Douglas Lake is home to at least three freshwater snail genera (Clampitt, 1972) and three species of crayfish (Keller & Hazlett, 2010). This particular study focused on the relationship between snails of the family *Physidae* and crayfish of the genus *Orconectes*.

Specimen and Substrate Collection

We chose to use *Physidae* snails because they had previously demonstrated predator avoidance behaviors, and because they were widely available in Douglas Lake. We collected *Physidae* snails from all four natural substrates (rock, gravel, sand and wood) along the shores of Douglas Lake, located in Cheboygan County, Michigan. Before trials, we stored snails in a small bucket filled with lake water, rocks and sand. We used new snails for every trial and released them back into Douglas Lake after use. Similarly, we collected crayfish from the shores of Douglas Lake and held them in a separate glass tank filled with lake water. We obtained all snails and crayfish during May and June of 2016.

We also collected samples of the four substrates on the shores of Douglas Lake, taking sand directly from the beach and gathering rocks and wood pieces in shallow water near the beach. For the purpose of this study, rocks were defined as being generally greater than sixty-four millimeters. We collected gravel using D-frame nets to filter sand out of the layer of sediment at the bottom of the lake. This left only the coarse-grained gravel, here defined as unsorted rocks above four millimeters in diameter (Wentworth, 1922). We then placed all substrate samples in boiling water for a minimum of fifteen minutes to kill any algae, eliminating food as a confounding variable in substrate choice during the experiment.

Tank Set-Up

We ran all trials in 37.85 liter glass tanks, with one tank designated for each treatment. Before placing the substrates into the tanks, we ran boiling water along the walls of each tank to kill any potential algae. Next, we placed all four tanks on top of black garbage bags to standardize what was visible under the four substrates. We hung black garbage bags between tanks in order to ensure that crayfish were not visible to snails in other tanks.

We then measured and divided each tank into four equal quadrants. Each quadrant was filled with one of the four substrates (Figure 2). To determine the placement of the substrates in the tanks, we numbered the quadrants of the first tank one through four, starting in the upper-left quadrant and moving counterclockwise. We then used a random number generator to assign each of the four samples a number one through four, placing them into their respective quadrants in the first tank. For the remaining three tanks, we rotated positions one spot counterclockwise. This randomization of substrate placement was done in order to eliminate potential bias toward a certain portion of the tanks. We then filled each tank with water up to a height of seven centimeters. For the control treatment and both treatments with live crayfish, we used water directly from Douglas Lake. For the crayfish water treatment, we used water from Douglas Lake which had been in the crayfish storage tank for a minimum of four hours.

Trial Process

We ran five one hour trials for each of the four treatments. For all four treatments, we placed ten snails at the center of the tank and started a one hour timer. In the full and half-duration crayfish treatments, we placed a crayfish in the center of the tank five minutes after the snails were placed in the tank. We left the crayfish in the full-duration crayfish treatment tank for the entirety of the remaining fifty-five minutes. In the half-duration crayfish treatment tank, we removed the crayfish after it had been in the tank for twenty-five minutes and carried out the remaining thirty minutes of the trail with no crayfish in the tank. At the end of the full hour, we located the snails in each tank and tallied them as belonging to one of the following placement categories: sand, rocks, gravel, wood, floating or wall. The sand, rocks, gravel and wood categories corresponded to a snail lying directly on one of designated substrates. The wall category corresponded to a snail not on a substrate, but stuck to the tank wall. The floating category corresponded to a snail not stuck to any surface, but floating in the water.

Statistical Tests

We ran statistical analyses in R (version 3.2.3). Initially we ran Chi-Square Goodness of Fit tests with all data points, including data points of snails in the categories wall or floating. Additionally, we ran Chi-Square tests that excluded data points of snails recorded as on the tank wall or floating, since they did not represent a snail choosing a naturally-available substrate. For the control treatment the null hypotheses were equal to an even distribution. For the other treatments, the null hypotheses were equal to the proportions of snails on each substrate given from the control trials (Table 1, Table 2; Ambrose & Ambrose, 2007). We used these null hypotheses because we assumed the control trials would provide us with a baseline of the snails' natural behaviors. For all tests, $\alpha=0.05$.

Results

Table 1. Statistical Results for Chi-Square Goodness of Fit Tests, Including Wall and Floating
For control treatment, H_0: $p_1=0.1667$, $p_2=0.1667$, $p_3=0.1667$, $p_4=0.1667$, $p_5=0.1667$, $p_6=0.1667$
For treatments two-four, H_0: $p_1=0.2308$, $p_2=0.0769$, $p_3=0.2885$, $p_4=0.1538$, $p_5=0.2308$, $p_6=0.0192$

Treatment	**p-value**
Control	0.005464
Crayfish Present for Entire Trial (55 mins)	0.2657
Crayfish Present for Half of Trial (25 mins)	0.02643
Water with Crayfish Chemical Cues	0.0003961

We first conducted statistical analyses on all data points, including those corresponding to snails found on the tank wall or floating. For the control treatment, we found that the proportions of snails found on each substrate differed significantly from the null hypothesis of equal distribution. Additionally, for the crayfish water and half-duration crayfish treatments, we found that the proportions of snails located on each substrate differed significantly from the null. For the full-duration crayfish treatment, the p-value was not low enough to reject the null hypothesis; we concluded that the proportions of snails found on each substrate did not differ significantly from the null (Table 1).

Table 2. Statistical Results for Chi-Square Goodness of Fit Tests, Excluding Wall and Floating
For control treatment, H_0: $p_1=0.25$, $p_2=0.25$, $p_3=0.25$, $p_4=0.25$
For treatments two-four, H_0: $p_1=0.3077$, $p_2=0.1026$, $p_3=0.3846$, $p_4=0.2051$

Treatment	**p-value**
Control	0.07028
Crayfish Present for Entire Trial (55 mins)	0.1479
Crayfish Present for Half of Trial (25 mins)	0.6996
Water with Crayfish Chemical Cues	0.01332

Our statistical results differed when we excluded the data points of snails found on the tank wall or floating. For the control treatment, we did not find that the proportions of snails located on each substrate differed significantly from the null hypothesis of equal distribution. Additionally, for the full and half-duration treatments we did not find that the proportions of snails found on each substrate differed significantly from the null. In the crayfish water treatment we did find that the proportions of snails found on each substrate differed significantly from the null (Table 2).

Given that our original hypotheses had not taken into account the possibility of crawlout behaviors onto the tank wall or floating, we chose to exclude these data points when discussing our statistical results. Our discussion analyzed data solely from Table 2, which only included snails found on the four natural substrates.

Discussion

Predator avoidance behaviors by freshwater snails likely increase their chances of survival, giving them a strong selective advantage. For *Physidae* snails, predator avoidance behaviors such as cryptic substrate selection are undertaken to reduce mortality caused by predators, such as the *Orconectes* crayfish. In our study, we examined two different behavior determinants: substrate choice and level of exposure of the snails to predatory crayfish. The results of this experiment did not indicate that *Physidae* snails responded to the increasing threat of predation, though they still exhibited a general preference for gravel in three of four treatments (Figure 3).

In the control treatment, we analyzed the snails' preferences for a particular substrate without any predatory influence. Though the Chi-Square test was not significant (p-value > 0.05, Table 2), we found that the snails preferred the gravel substrate in all five trials (Figure 4). Given past studies, we had hypothesized that snails would exhibit a weak preference for the larger rock substrate (Clampitt, 1972), however, this was not the case. This finding could be explained, in part, by evolutionary adaptations which drive predatory avoidance behavior. Although snails are unable to change the color of their shells over the course of their lifetime, they do exhibit cryptic behavior by hiding in substrates that match their natural colors (Cook, 1986). While we did not run any pigment analyses, such as a UV-Vis Spectroscopy, the snails were anecdotally observed to have a range of shell coloration that closely matched colors of the local gravel (Figure 5). Running such a test, which compares the wavelengths of pigment samples on the UV-visible spectrum (Veverica, 2015), could supplement these color comparisons. Also, the snails respective sizes may have contributed to their general preference for the gravel substrate (Figure 5). *Physidae* snails' shells generally range from between five and twelve millimeters in length (Alexander & Covich, 1991b), and, as such, were able to effectively burrow into the similarly-sized gravel. It is important to note that sand, another substrate conducive to snail-burrowing, was the second most popular substrate choice in this treatment (Figure 3). This finding indicated that a snail's ability to burrow, which Snyder (1967) previously observed may have played a more significant role in substrate choice than we had initially hypothesized.

For the full-duration crayfish treatment, we introduced a crayfish into the tank for the full extent of the trials. Here we sought to understand whether the presence of a live predator would influence snail substrate choice, and whether the amount of time that a snail spent under direct threat of predation would affect this choice. Ultimately, while the results of the Chi-Square test were not statistically significant ($p\text{-value} > 0.05$, Table 2), our results did indicate that the presence of the predator for the entire trial increased the gravel substrate preference when compared to the control (Figure 4). So, while our initial hypothesis that snails would prefer the rock substrate was unsupported, this finding did support the hypothesis that substrate preference would increase with the presence of a predator. Furthermore, these results were in line with the evolutionary concepts which informed our original hypotheses, detailing *Physidae* as being known to significantly alter their habitat use in the presence of predators (Turner, 1996). Additionally, studies have suggested that these behaviors are largely innate (Turner et al., 2006), indicating that they have evolved as a mechanism to increase fitness.

Results from the half-duration crayfish treatment, which tested how the removal of the crayfish halfway through the trial would influence snail behavior, were largely inconsistent with our other findings. Alexander and Covich (1991b) previously found that anti-predator crawlout behavior in *Physidae snails* decreased with time in trials where the predatory threat was introduced and later removed, but remained significantly higher than in predator-free controls. In this treatment, we found that snails' preferred substrate was still gravel, but that this preference was lower than in the control tank (Figure 4). In this case, neither the rock-substrate hypothesis, nor the increasing preference based on predation level hypothesis, were supported. The inconsistency of these results in comparison to previous results might be explained by the experimental design itself. Previous studies of reactions to threat removal typically had much longer trial times than ours. Similarly, these studies generally indicated that snails take two or more hours to retreat from their predator avoidance positions (Alexander, 1987; Alexander & Covich, 1991b). Since our study had trial times of only one hour, it was likely not long enough to observe any meaningful difference between the two live predation trials. Given the relatively slow-moving nature of the snails, one hour trials may have been too short for the snails to register and respond to the changing sensory information. This may have contributed to what appeared to be a more random distribution across substrates by the snails. Though sand was still the second most preferred substrate, the rock substrate and the wood substrate were both chosen in higher proportions than in the control.

For the crayfish water treatment, which tested the effects of chemical cues from crayfish-inhabited water on the snails' avoidance behaviors, the results deviated from our initial hypothesis. Contrary to our findings in the other treatments, the snails' preferred substrate was rock, as opposed to gravel. While our original rock-preference hypothesis was supported, the hypothesized increase in substrate preference based on predation was not. These results were largely unexpected, as we predicted that the preferred substrate would remain constant throughout each treatment. Even though the results of the Chi-Square test were statistically significant (p-value < 0.05, Table 2), they did demonstrate a slight deviation from our original hypotheses. Given the small sample size, this deviation may be attributed to the factors of chance present in any such study, but they may also be representative of a more general flaw in our study. While Snyder (1967) did note correlations between snail behavior and chemicals from predators, several other studies (Turner 1996; Crowl & Covich, 1990; Turner et. al, 1998; Alexander & Covich, 1991b) indicated that predator avoidance by the snails is contingent on the presence of chemical cues from both predators and dead snails. Crowl & Covich (1990) hypothesized that proteins from the blood of dead snails are modified by crayfish-produced enzymes, and that this enzymatic reaction would need to occur in order for living *Physidae* snails to react. To test this, Turner et. al (1998) used crushed snails in conjunction with predator presence to elicit a response from the *Physidae*. They found that avoidance behavior occurred most prominently in tanks with both treatments. Had our study introduced crushed snails into the treatment with crayfish water, we may have had more significant findings.

In all four treatments we observed an average rate of crawlout onto the tank wall of 20.9%, though this was a predator avoidance behavior not included in our original hypotheses. Crawlout has been observed in both laboratory and field settings and is a behavior in which the snails crawl above the water level as a means of minimizing the risk of predation (Alexander & Covich, 1991b). Still, this form of avoidance entails significant costs, as leaving the safety of the water both reduces foraging opportunities for the snails and puts them at greater risk of predation by birds and insects (Alexander & Covich, 1991b). The decrease in these components of fitness is often accompanied by a reduction in the primary risk of predation, and these tradeoffs are key factors in analyzing animal behavior (Skelly, 1992). While the scope of our study did not allow us to analyze how these variables interplayed, we did include this avoidance behavior – as well as the snails floating on the surface – in the first set of statistical results (Table 1). This allowed us to gain a more complete understanding of the range of avoidance behaviors.

In order to improve our study, and any related future studies, we would make several changes to our methods. To better execute what methods we did use, we would ensure that each snail was confirmed to be of the family *Physidae.* Additionally, we would insert plexiglass dividers between each of the four quadrants to prevent substrate mixing while still allowing the snails to move freely. To strengthen statistical power, we would increase sample sizes and run more trials. We would lengthen these trials to ensure that each snail had adequate time to both identify and locate itself on its preferred substrate. By also standardizing the time of day when we ran these trials, we would hope for more uniform results between trials. Additionally, setting trials at various times throughout the day would allow us to better explore the behavioral habits of the two species, and observing the treatments for the entirety of the trials would give us a better idea of the interactions between the two species.

Our results indicated that snails likely evolved to utilize multiple predator defense mechanisms, which could be a reason for the continued coexistence between *Physidae* and crayfish. Based upon our findings and past studies of the two species, we suggest that the direct threat of predation is a factor in substrate choice. Specifically, our results indicate a preference for gravel substrates by *Physidae* snails, which increased when a live crayfish was introduced. Our findings were less conclusive in quantifying the sensitivity of the snails to a predation threat presented by the crayfish, which may have been due to a variety of factors discussed here. Further studies can continue to investigate the evolutionary reasons behind the formation of these predator avoidance behaviors, and the interactions between these two species.

Acknowledgements

We would like to thank our professor, Dr. Joel Heinen, along with the teaching assistants, Kathryn Braddock and Emily Kroloff, for their mentorship on this project. Additionally, we thank the University of Michigan Biological Station for resources and allowing us to sample on the property. Lastly, we thank our fellow students of general ecology for the valuable discussions throughout the process of this project, particularly, Melanie Florkowski, Erin Gregoire, Cailin McLean and Matt Silbergleit for their helpful editing.

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Appendix

Figure 1. Sample of ten *Physidae* snails

Figure 2. Tank set-up before adding water or snails

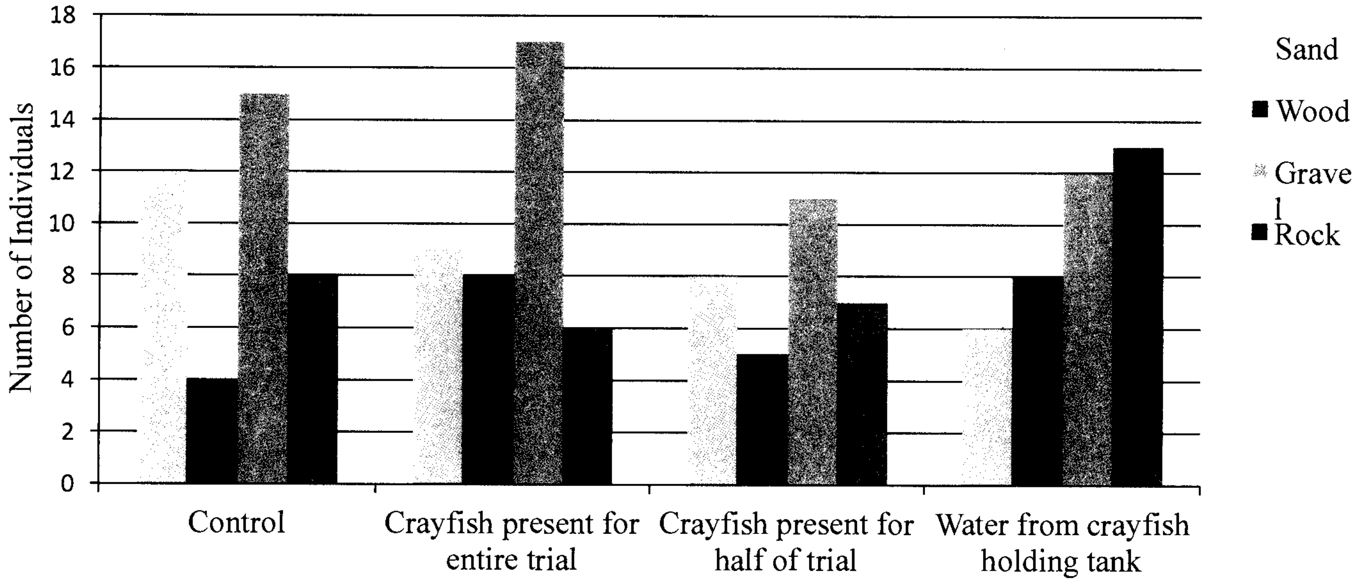

Figure 3. Distributions of snails across substrates for each treatment

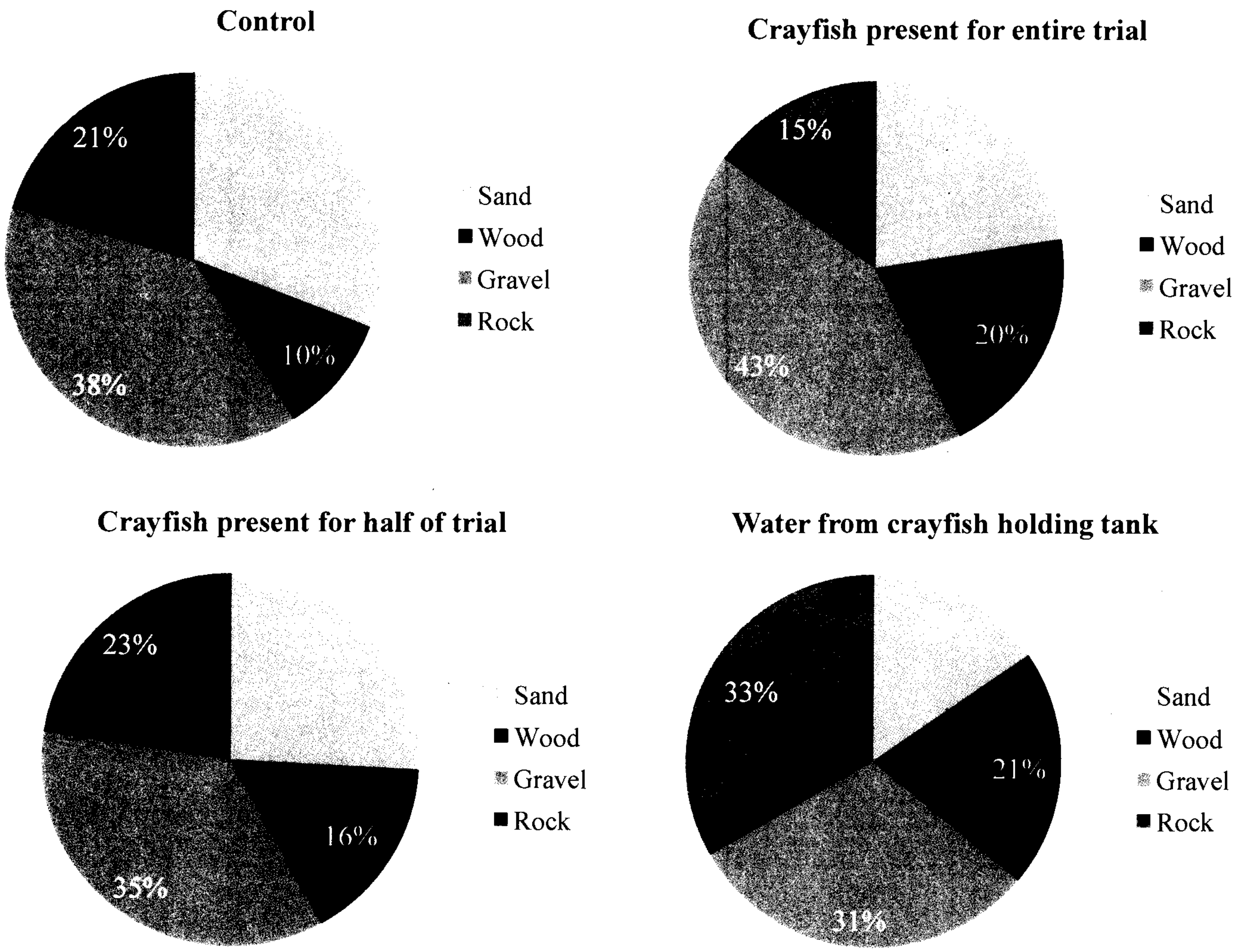

Figure 4. Distributions of snails within each treatment

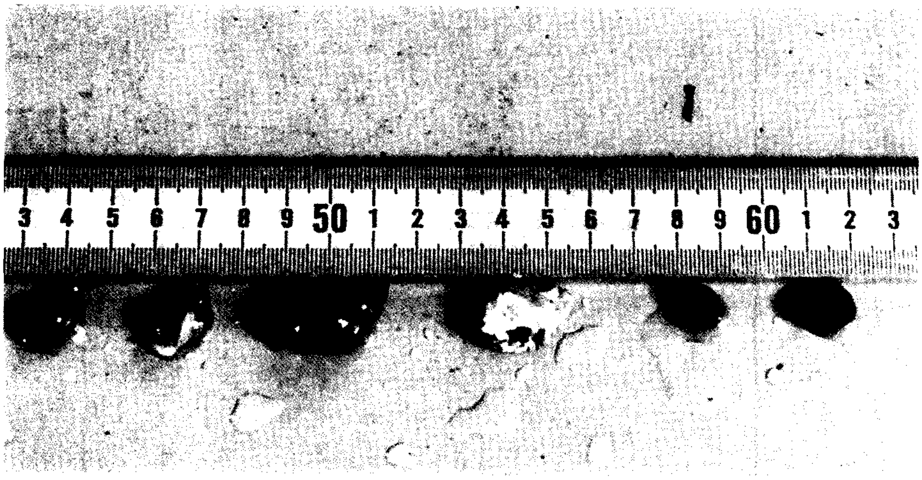

Figure 5. Three *Physidae* snails (on left) and three pieces of gravel (on right)