

**Predator-Avoidance Responses in Native and Exotic Freshwater Snail Species**

BIOS 35502: Practicum in Field Biology

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## **Abstract**

Insight into the likelihood of the success of a species in a particular environment can be gained, in part, from assessing the species' ability to avoid predation. Anti-predation responses to chemical cues from predators or injured prey are a common way to avoid predation. This study examines the predator-avoidance responses of *Campeloma decisum*, a snail species native to the UNDERC area in Vilas County, Wisconsin, and *Viviparus georgianus*, a species that has been introduced to the area. The behavioral responses of the two snail species to alarm cues from crushed snails, kairomones from crayfish, and a combination of the two chemical cues were recorded. The two species were found to exhibit different behavioral responses; *C. decisum* tended to engage in burial behavior while *V. georgianus* was found to exhibit a crawl-out response. In addition, *C. decisum* had a statistically significantly greater response to predator cues than *V. georgianus*. The results of this experiment provide evidence that the exotic *V. georgianus* has not yet acquired predator-avoidance responses and thus may not be threatening as an invasive species in the research area.

## **Introduction**

It is estimated that approximately 50,000 species are introduced to a nonnative environment in the United States annually, causing extreme damage to the environment as well as more than \$120 billion annually in economic losses (Crowl et al. 2008). Following habitat loss and landscape fragmentation, invasion by exotic species is considered to be the largest threat to global biodiversity. Observations of the interactions between native and invasive species can provide information about the roles an invasive species plays in a particular environment (Allendorf and Lundquist 2003). A key

component to survival in a new environment is the ability to avoid predators.

Investigating differences in predator-prey interactions and comparing the ability of native and exotic species to escape predation can provide insight into the possibility of different levels of success in a particular environment.

Previous research has been performed on the types of non-visual stimuli involved in behavioral responses due to predators (Culp and Crowl 1994). Such stimuli can be in the form of mechanical or chemical signals. Numerous experiments involving responses to predators have been performed on snails. Because snails lack both image-forming eyes as well as efficient vibration sensors to sense mechanical stimuli, any cues from a nearby predator must be in chemical form (Dodson et al. 1994). Dodson et al. (1994) defines chemicals that convey information between species involved in a predator-prey interaction as allelochemicals; when these chemicals benefit the receiving individual but may or may not benefit the sender they are called kairomones. While chemical cues from predators are known as kairomones, chemical cues from injured prey are known as alarm cues (Schoeppner and Relyea 2005). Often such chemical signals can affect prey in terms of its behavior, morphology, or life history characteristics (Dodson et al. 1994). For example, snails may crawl up vertical objects such as plants in order to escape bottom-dwelling crayfish predators (Lewis 2001). In an experimental setting, such a change in behavior is observed as crawling out of an aquarium, a response known as crawl-out.

Multiple studies have demonstrated that local adaptation to average predation intensity can occur in benthic or planktonic animals (Dodson et al. 1994). Freshwater gastropods face the risk of desiccation if they remain outside of water for long periods

and due to this risk these organisms have limited dispersal ability. According to Dalesman et al. (2007), species with low dispersal ability and a fairly sedentary lifestyle have a likelihood of developing a local adaptation to the predation threat they experience. For example, in some locations a certain snail species may have a crawl-out response when exposed to predator stimuli while in another location the same species will not crawl-out, but rather bury themselves in sediments or show no response at all. In addition, previous research on snails has found that certain species respond behaviorally to alarm cues from crushed conspecifics; some demonstrate an even greater response when exposed to both crushed conspecifics as well as a predator, while others require both signals to initiate predator avoidance responses (Dodson et al. 1994, Crowl and Covich 1990).

This experiment is designed to answer the questions: 1) what cues are required to provoke a predator-avoidance response in snails? and 2) how are the behaviors of native and exotic snail species similar or different in response to these cues? The banded mystery snail, *Viviparus georgianus*, is a species that has been introduced to the University of Notre Dame Environmental Research Center (UNDERC) area. The pointed campeloma snail, *Campeloma decisum*, belongs to the same family as the banded mystery snail and is native to the UNDERC area. Snails of the Viviparidae family to which these two species belong are characterized by having an operculum, indicating that these species are likely to use burial behaviors as a method of avoiding predators (T.A. Crowl, personal communication). I hypothesized that both the native and exotic snail species would show a predator-avoidance behavioral change when exposed to predator

and crushed conspecific cues but that the response of the native snail species would be greater than that of the introduced banded mystery snail.

## **Methods**

To examine the effects of predator cues on native and exotic snails, an experiment consisting of two snail species and six predator treatments was designed. I collected native pointed campeloma snails, *C. decisum*, from Brown Creek and Plum Creek at the University of Notre Dame Environmental Research Center and introduced banded mystery snails, *V. georgianus*, from Wildcat Lake, Vilas County, Wisconsin.

The experimental treatments consisted of a control (lake water), a crayfish cue, a crushed native snail cue, a crushed exotic snail cue, a combination of crayfish and crushed native snail cues, as well as a combination of crayfish and crushed exotic snail cues. Each cue was made using water from Tenderfoot Lake. In order to make the crushed snail cues, five snails of the corresponding species were crushed in a tank containing 5 liters of water. To create the crayfish cue, five *Orconectes propinquus* crayfish collected from Tenderfoot Lake were placed in a tank with 5 liters of water. The combination of crayfish and crushed snail cues consisted of 5 liters of water containing three crushed snails of the corresponding species and three *O. propinquus* crayfish restrained by a plastic Tupperware container with holes punched in all sides to allow water flow. The five predator cues, as well as 5 liters of lake water to act as a control, were aerated and allowed to sit for 24 hours.

Glass dishes 6.5 cm tall and 18 cm in diameter were used as experimental tanks. Each dish was filled with a 1.5 cm layer of small gravel and 800 mL of Tenderfoot Lake water. A total of ten dishes were assigned to receive each treatment, five dishes

containing *C. decisum* snails and five containing *V. georgianus* snails. Snails were randomly assigned so that each replicate, or dish, consisted of three snails of the same species. The snails were allowed a 25 minute acclimation period after which each tank received 200 mL of a predator treatment. I made observations of the positioning of the snails at the time the treatment was added and every five minutes thereafter for a two-hour duration, resulting in a total of 24 observations per dish. The behavioral responses of the snails, which consisted of buried, crawled-out, or normal behavior, were recorded as the percentage of snails in each position in each tank. A snail was considered to have a burial response if the majority of its foot or some of its shell was covered with gravel sediment. When a snail had the majority of its foot on the side of the glass dish, it was considered to have a crawl-out response. If a snail was sitting on or crawling across the gravel it was not considered to be showing a response.

The average of the 24 observations of each tank was calculated to obtain the average percentage of snails in each replicate present in each position throughout the duration of the observation period. This response behavior was arcsine, square root transformed to equalize variance and normalize the data. The average for each species-treatment combination was then calculated and used in the data analysis. A two-way ANOVA, with snail species and predator cues as the two main effects, was performed to determine if any significant differences in the predator-avoidance responses between species or among treatments existed. SYSTAT 12 was used for the data analysis. I used  $\alpha = 0.10$  to determine if results were statistically significant.

## **Results**

A two-way ANOVA was used to analyze how the predator avoidance responses (burial and crawl-out) of the two snail species were affected by predator cues. Statistically significant differences were found between the two species in terms of both burial ( $df = 1,48$ ,  $F = 488.635$ ,  $p < 0.0001$ ) and crawl-out ( $df = 1,48$ ,  $F = 157.813$ ,  $p < 0.0001$ ) responses (Figure 1). A statistically significant treatment effect was found for the burial response ( $df = 5,48$ ,  $F = 2.384$ ,  $p = 0.0520$ ). A Fisher's Least-Significant-Difference Test showed that the responses to the *V. georgianus* cue were statistically significantly different from the responses to the control, the crayfish cue, the combination of crayfish and *V. georgianus* cue, and the combination of crayfish and *C. decisum* cue (Figure 2). In addition, the interaction between species and treatment was found to have a statistically significant influence on the prevalence of the crawl-out response ( $df = 5,48$ ,  $F = 2.117$ ,  $p = 0.0794$ ).

Because the degrees of freedom in the two-way ANOVAs across all treatments were low, the treatments were analyzed in groups to increase statistical power. A two-way ANOVA analyzing the two species in terms of their responses to the control, crushed *C. decisum*, and crushed *V. georgianus* cues found statistically significant differences among treatments for the burial response ( $df = 2,24$ ,  $F = 3.549$ ,  $p = 0.0446$ ). A Fisher's Least-Significant-Difference Test showed that the burial response to the crushed *V. georgianus* cue was statistically significantly different from the response to the control ( $p = 0.0137$ ). The differences in crawl-out response to the three treatments were nearly statistically significant ( $df = 2,24$ ,  $F = 2.434$ ,  $p = 0.1090$ ). A Fisher's Least-Significant-Difference Test with  $\alpha = 0.15$  showed the crawl-out response to the crushed

*V. georgianus* cue to be different from that of the control ( $p = 0.0384$ ). In addition, a statistically significant interaction was found between the species and treatment in terms of the crawl-out response to these three treatments ( $df = 2,24$ ,  $F = 3.301$ ,  $p = 0.0542$ ). Two-way ANOVAs analyzing the reactions of the two species to the control and crayfish cue did not find a statistically significant difference in the responses according to the treatment or to the interaction between treatment and species.

Because of the significant difference in the form of predator-avoidance response between the two species, *C. decisum* burials and *V. georgianus* crawl-outs were labeled simply as responses. A two-way ANOVA found a statistically significant difference between the species in terms of the amount of response exhibited ( $df = 1,48$ ,  $F = 32.635$ ,  $p < 0.0001$ ) (Figure 3). The treatments were found to be nearly statistically significantly different in their ability to elicit responses from the snails ( $df = 5,48$ ,  $F = 1.920$ ,  $p = 0.1084$ ). The results of a Fisher's Least-Significant-Difference Test ( $\alpha = 0.15$ ) are shown in Table 1. When analyzed in a group with the control, crushed *C. decisum*, and crushed *V. georgianus* cues, a statistically significant interaction between species and treatment was found ( $df = 2,24$ ,  $F = 3.222$ ,  $p = 0.0576$ ).

Because of the presence of statistically significant interactions between species and treatments, one-way ANOVAs were used to analyze the effect of treatments on each species individually. These analyses found that while treatments did not have a statistically significant role on *V. georgianus* responses, they did have a statistically significant influence on *C. decisum* crawl-out ( $df = 5,24$ ,  $F = 3.305$ ,  $p = 0.0207$ ) behaviors (Figure 4). The results of a Fisher's Least-Significant-Difference Test are shown in Table 2. In addition, the effects of treatment on *C. decisum* burial was nearly statistically

significant ( $df = 5,24$ ,  $F = 2.029$ ,  $p = 0.1106$ ). The results of a Fisher's Least-Significant-Difference Test with  $\alpha = 0.15$  are shown in Table 3.

## **Discussion**

Chemical cues indicating the presence of predators are known to elicit responses in many animal species. A study by Schoeppner and Relyea (2005) involving larval anurans found that alarm cues did not elicit the same responses as consumed prey cues. Overall, the responses or defenses to the consumed prey cues were stronger than the responses to the alarm cues. In some species, the response to consumed prey cues is even greater than would be predicted if the responses to kairomones and alarm cues were simply added. One possible explanation is that the species elevate their response when they sense both cues at the same time. The heightened response may also be due to a signal released when the prey is consumed (Schoeppner and Relyea, 2005). Due to the combined effects of kairomones and alarm cues, I expected the snails in this experiment to exhibit the greatest anti-predator behavioral change in response to the combination of crayfish and crushed conspecific cues. However, the responses to the combination cues were not statistically significantly different from the control.

One possible explanation for these results involves the fact that in addition to behavioral responses, snails may also exhibit morphological responses, such as alterations of shell shape or thickness, to predator cues. For example, one study found that crayfish feeding on conspecifics provoked snails to grow narrow shells to reduce their risk of predation (Krist, 2002). According to Schoeppner and Relyea (2005), it is generally easier to remove a behavioral response than it is to undo a morphological change. Because of this, a species may not engage in a morphological response unless it

receives either consumed prey cues or a combination of both alarm cues and kairomones. When only part of this predatory information is received, a species may choose to respond in a behavioral manner so that its response can be easily reverted if it is found to be unnecessary (Schoeppner and Relyea, 2005). The fact that a behavioral anti-predation response was observed only when the treatment consisted of a crushed snail may be explained by this concept. As would be expected, the crushed snail cues by themselves elicited a behavioral response. When the crushed snail cues were combined with crayfish kairomones, a stronger response (possibly including morphological changes) might be expected. It may be the case that in this experiment the snails did not respond behaviorally but instead decided to initiate a morphological change, such as the strengthening of their shell, in response to the combination cues. However, according to this concept the crayfish cue by itself would be expected to cause a behavioral response as well. This was not observed, but it is possible that for the snails used crayfish kairomones are sufficient to elicit a morphological response. It is also possible that because the control water was collected from a lake with crayfish, the control may have contained crayfish cue. Because of this possibility, future experiments could use a well water control in order to ensure the absence of predatory cues. Another possible explanation for these results is that the crayfish used in this experiment may not have provided a kairomone, or that the amount was not sufficient to induce a response from the snails. If this was the case, it might be expected that the combination cues would still elicit responses due to the presence of the crushed snails; however, the alarm cue in these treatments may not have been strong enough given that only three snails were used in the combination cues while five were used in the crushed snail cues. Future studies which

take into account both behavioral and morphological responses to predator cues should be performed in order to explore the possible explanations for the results of this experiment. In addition, based on previous evidence that a crayfish and snail treatment in which active predation is allowed produces a predator chemical cue that some snails are able to detect (Crowl and Covich, 1990), such a treatment could be added to the experimental design in order to provide further information regarding responses due to differing predatory cues. Another possible improvement to this study would be to increase the number of individuals used to create the cues to ensure that the treatments contain chemical cues different from those of the control.

Researchers have suggested that closely related species may produce similar alarm cues, as will species that often coexist with one another. Species should respond to alarm cues similar to their own (Schoeppner and Relyea, 2005). This hypothesis offers an explanation as to why the crushed *V. georgianus* cue caused a statistically significant response in the *C. decisum* species. However, according to this hypothesis the crushed *C. decisum* cue would also be expected to elicit a response from the *C. decisum* snails. In general, the *C. decisum* snails were slightly smaller than the *V. georgianus* snails. This difference in size may have led to a lower concentration of chemical cues in the crushed *C. decisum* treatment, possibly explaining why *C. decisum* gave no response to its own alarm cues. Instead of using an equal number of crushed snails to make the treatments, this experiment should be repeated using another form of measurement, such as biomass, in attempt to control for the concentration of chemical cues present in each of the treatments.

The hypothesis that the response of the native *C. decisum* snail would be greater than that of the introduced *V. georgianus* snail was supported by the results of this experiment. The two species were found to exhibit different predator-avoidance behaviors in response to the cues; *C. decisum* tended to engage in burial behavior while *V. georgianus* was found to exhibit a crawl-out response. When the two different behaviors were compared and analyzed as general responses, *C. decisum* was found to have a statistically significantly greater response than *V. georgianus*.

According to Hanfling and Kollmann (2002), in order for an invasive species to be successful it is important that it is able to respond to new environmental conditions in a quick and efficient manner. The fact that the *V. georgianus* snails did not show statistically significant responses to the predator cues suggests that this species has not acquired a behavioral response to predatory cues in its new environment. This lack of anti-predator behavioral response could indicate that *V. georgianus* may not be threatening as an invasive species to the UNDERC area. However, identifying a species as invasive or not is a difficult task and other factors need to be taken into consideration before a definite conclusion is made regarding the possible role of *V. georgianus* as a successful invader. Such factors include, but are not limited to predator preference for *V. georgianus* in comparison to other species, the species' dispersal ability, and the vulnerability of the native community (Mack et al., 2000).

In order to verify the findings of this experiment, it should be performed again using more replicates and water from a source that lacks background cues. Because snails may develop a local adaptation to predation levels (Dalesman, 2007), the results of this experiment cannot be applied to snails outside of the collection populations. The

experiment needs to be repeated using snails collected from a variety of locations before any generalizations are made regarding the two species studied.

### **Acknowledgements**

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512.

**Table 1. Post-Hoc Analysis of the Effects of Treatment on *C. decisum* and *V. georgianus* Responses.** Treatments in the same row elicit statistically significantly different magnitudes of response when  $\alpha = 0.15$ .

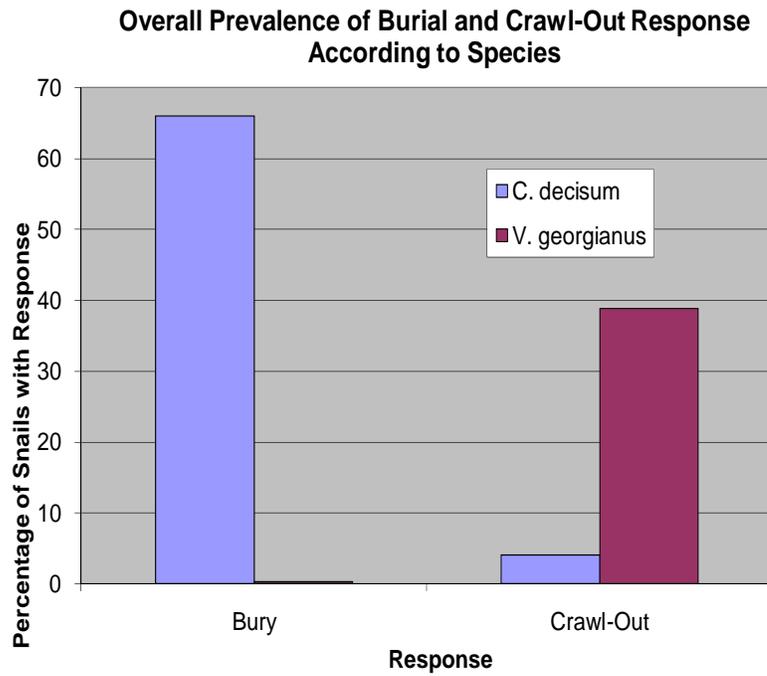
<b>Treatment I</b>	<b>Treatment II</b>	<b>p-value</b>
<i>V. georgianus</i>	Control	0.1070
<i>V. georgianus</i>	Crayfish	0.0575
<i>V. georgianus</i>	Crayfish + <i>V. georgianus</i>	0.0245
<i>V. georgianus</i>	Crayfish + <i>C. decisum</i>	0.0563
<i>C. decisum</i>	Crayfish	0.0952
<i>C. decisum</i>	Crayfish + <i>V. georgianus</i>	0.0430
<i>C. decisum</i>	Crayfish + <i>C. decisum</i>	0.0934

**Table 2. Post-Hoc Analysis of the Effects of Treatment on *C. decisum* Crawl-out Responses.** Treatments in the same row elicit statistically significantly different magnitudes of crawl-out response.

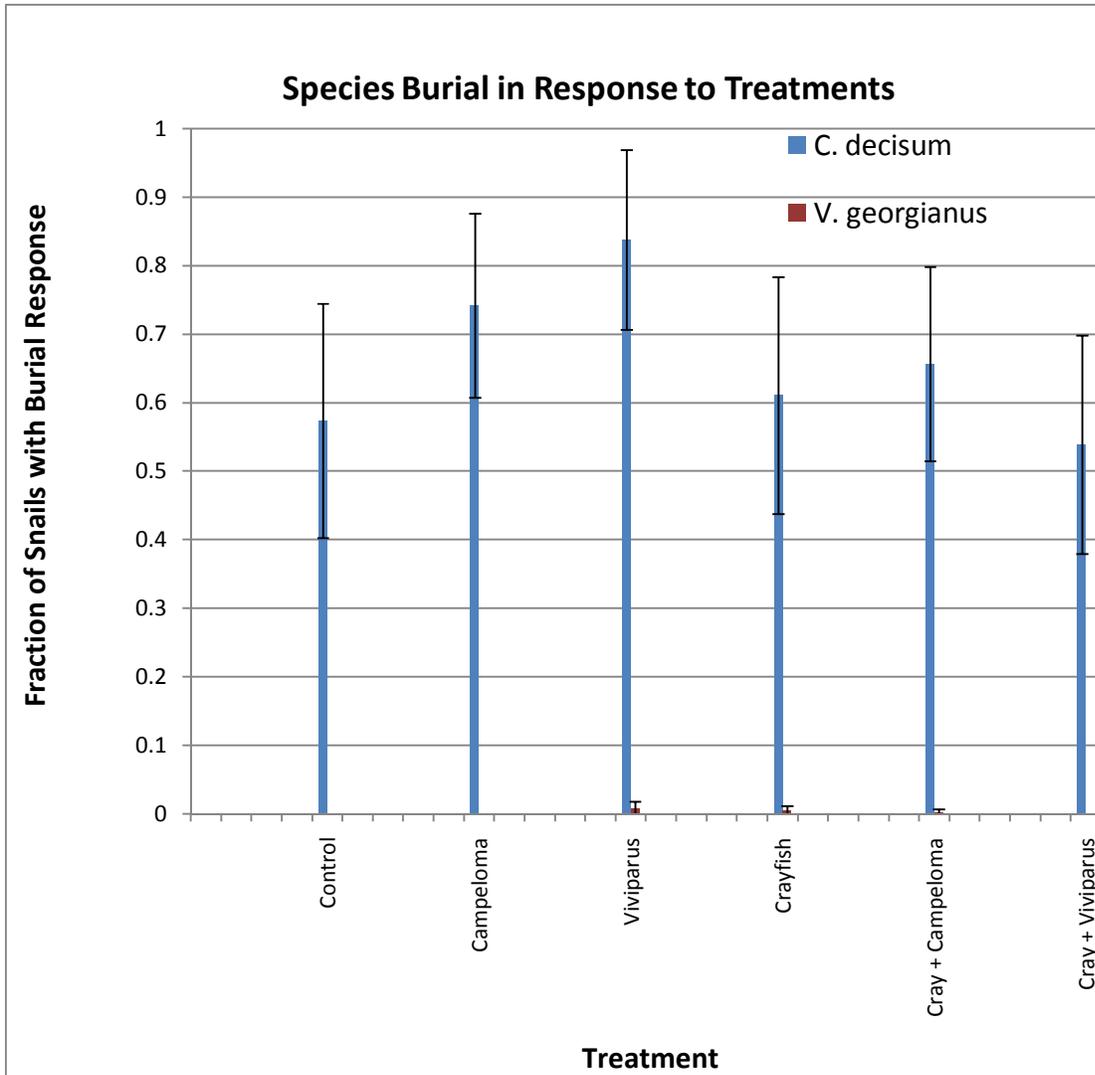
<b>Treatment I</b>	<b>Treatment II</b>	<b>p-value</b>
<i>V. georgianus</i>	Control	0.0252
<i>V. georgianus</i>	Crayfish	0.0139
<i>C. decisum</i>	Control	0.0148
<i>C. decisum</i>	Crayfish	0.0080
<i>C. decisum</i>	Crayfish + <i>V. georgianus</i>	0.0857
Crayfish + <i>C. decisum</i>	Control	0.0318
Crayfish + <i>C. decisum</i>	Crayfish	0.0177

**Table 3. Post-Hoc Analysis of the Effects of Treatment on *C. decisum* Burial Responses.** Treatments in the same row are considered to elicit statistically significantly different magnitudes of burial response when  $\alpha = 0.15$ .

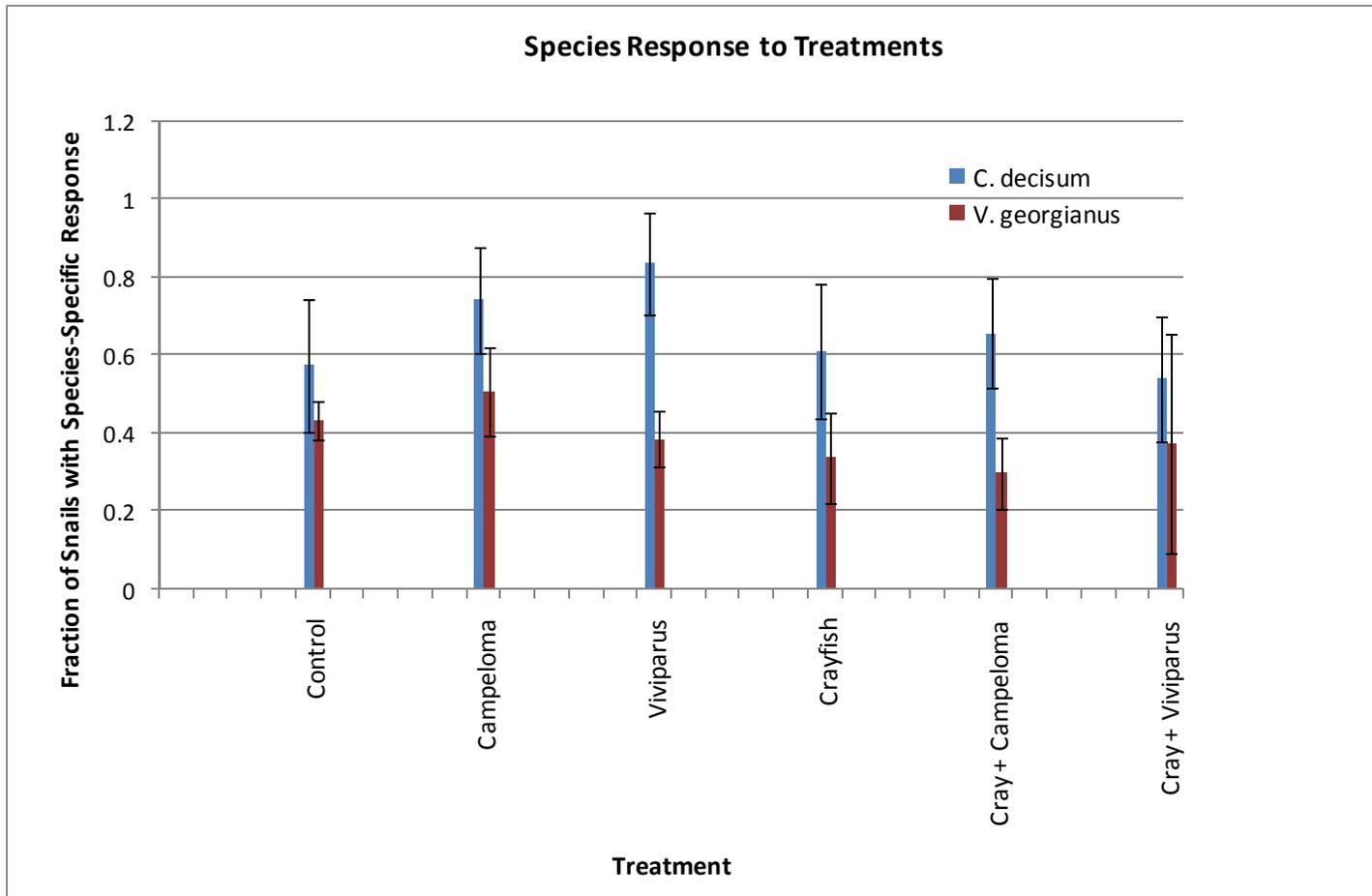
<b>Treatment I</b>	<b>Treatment II</b>	<b>p-value</b>
<i>V. georgianus</i>	Control	0.0211
<i>V. georgianus</i>	Crayfish	0.0421
<i>V. georgianus</i>	Crayfish + <i>V. georgianus</i>	0.0111
<i>V. georgianus</i>	Crayfish + <i>C. decisum</i>	0.0763
<i>C. decisum</i>	Crayfish + <i>V. georgianus</i>	0.1296



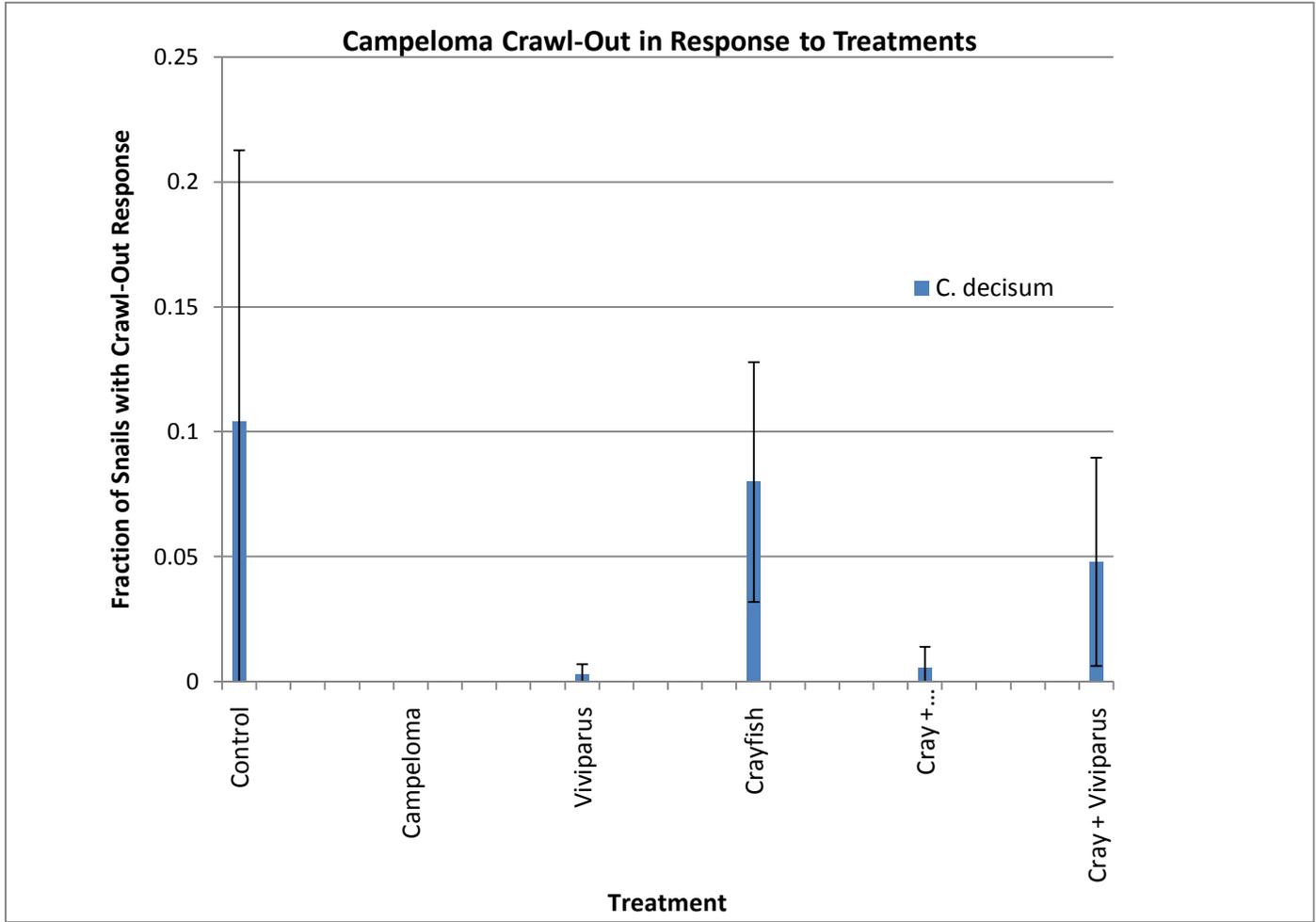
**Figure 1. Prevalence of total burial and crawl-out responses across all treatments in *C. decisum* and *V. georgianus* snails.** The two species demonstrated a statistically significant difference in responses; the greater percentage of *C. decisum* burial was found to be statistically significant ( $df = 1,48$ ,  $F = 488.635$ ,  $p < 0.0001$ ) as was the greater percentage of *V. georgianus* crawl-out behavior ( $df = 1,48$ ,  $F = 157.813$ ,  $p < 0.0001$ ).



**Figure 2. Fraction of snails from each species that demonstrated burial behavior in response to each of the treatments.** Treatments were found to have statistically significant differences in the amount burial response elicited ( $df = 5,48$ ,  $F = 2.384$ ,  $p = 0.0520$ ). The response to the *V. georgianus* cue was statistically significantly different from the response to the control ( $p = 0.0784$ ), the crayfish cue ( $p = 0.0345$ ), the combination of crayfish and *C. decisum* cue ( $p = 0.0479$ ), and the combination of crayfish and *V. georgianus* cue ( $p = 0.0400$ ).



**Figure 3. Fraction of snails from each species that demonstrated their species-specific response to each treatment.** The species-specific response for *C. decisum* is burial and for *V. georgianus* is crawl-out. *C. decisum* showed a statistically significantly greater amount of response to the treatments than *V. georgianus* (df = 1,48, F = 32.635, p < 0.0001).



**Figure 4.** Fraction of *C. decisum* exhibiting a crawl-out response to each treatment. The treatments were found to have a statistically significant influence on *C. decisum* crawl-out (df = 5,24, F = 3.305 p = 0.0207). The results of a Fisher's Least-Significant-Difference Test are shown in Table 2.