Predicting the effectiveness of chemical defenses based on the physical defenses of three gastropods in the presence of two crayfish predators

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ABSTRACT

Predation poses a serious threat to prey species' fitness with few species being immune to predation for all of their life cycle. Prey species invest energy into morphological and behavioral defenses in order to avoid predation. While these defenses offer the benefit of reduced predation, they also incur costs for the defended organism. Predators counteract these defenses with their own adaptations and by selectively foraging on prey items that offer enough nutritional benefit to account for the costs of consumption. We explored this interaction by observing the preferential browsing of two crayfish species— *Orconectes propinquus* and *Orconectes virilis*—on three gastropod species—*Bellayma chinensis*, *Lymnaea stagnalis*, and *Helisoma trivolvis*. Possible chemical defenses were isolated by removing the snail species' physical defenses. We predicted snails that invest more in physical defenses would possess fewer chemical defenses and that they would be browsed upon preferably. We found that this was not always the case and that *H. trivolvis* was more often preferred by the crayfish species than the other species. We believe that since crayfish do not strongly prefer the invasive *B. chinensis*, that this species will continue to spread with relatively little resistance, possibly endangering native gastropod populations. Further study is needed to adequately assess any chemical defenses and the nutritional value of these gastropods as well as the relative effectiveness of their defenses against predation. Analysis of possible mechanisms employed by crayfish to cope with these defenses as well as any long term fitness effects is also necessary.

INTRODUCTION

Predation poses a serious threat to prey species' fitness with few species being immune to predation for all of their life cycle (Broom et al. 2010). In order to cope with the pressures of predation, prey species have developed numerous behavioral and morphological ways to defend themselves. These adaptations include avoidance behavior (Sura and Mahon 2011), chemical defenses (Zang et al. 2012), physical defenses such as armor and spines (Brown 1998, Bollache et al. 2006), and differential coloration such as camouflage or warning coloration (Skelhorn and Rowe 2006). While defenses are important and useful for avoiding predation, an organism cannot possess every defense without incurring a significant energy deficit. Defenses are energetically costly and each added defense includes an associated energetic cost (Broom et al. 2010, Covich 2010). In fact, possessing one form of defense decreases the probability that an organism will have another defense (Broom et al. 2010).

Consuming prey protected by chemical defenses is costly to predators (Cruz-Rivera and Hay 2003). Predators can learn to avoid or remove chemical defenses behaviorally (Skelhorn and Rowe 2006), sequester toxins for their own defensive use (Dobler et al. 1998), metabolically modify toxins to make them less harmful (Sloggett and Davis 2010), or deal with prey toxins with morphological adaptations (Petschenka et al. 2012). Predators can lower the amount of energy needed to process toxic prey by avoiding prey items with high levels of chemical defenses and preferentially feeding on those that have low levels of toxins. Additionally, predators should select prey items that are nutritious enough to overcome the costs of processing chemical defenses. A previous study also used predator preference to form conclusions about the presence and relative effectiveness of chemical defenses and found that predators preferentially browsed on gastropods with lower levels of toxins (Neves et al. 2009).

All mollusks are essentially soft-bodied organisms despite the presence of shells in most groups (Benkendorff 2010). As such, they must invest heavily in defenses such as shells and chemical defenses. Gastropods are particularly interesting as some species are evolutionarily losing their shells and relying more on a variety of chemical defenses (Neves et al. 2009, Benkendorff 2010). These chemical defenses are very effective at deterring predators and it has been suggested that they have evolved in gastropods as a response to predation (Cimino and Ghiselin 1998, Neves et al. 2009).

This study will examine the chemical defenses of three freshwater gastropods—*Bellayma chinensis* (formerly *Cipangopaludina chinensis*), *Lymnaea stagnalis*, and *Helisoma trivolvis*—and how they affect the browsing preferences of two known crayfish predators—*Orconectes propinquus* and *Orconectes virilis*—with the assumption that lower browsing preference is associated with increased chemical defenses (as seen in Neves et al. 2009). We based our hypotheses on observable physical characteristics of the three gastropods with the general idea that prey only have a limited amount of energy to invest in defenses and those that invest in impressive physical defenses should have less energy to invest in chemical defenses (Broom et al. 2010). Because *B. chinensis* possess a large, thick shell and an operculum, we hypothesized it would not need extensive chemical defenses and should thus be preferred over the other two gastropod species by the crayfish predators. A previous study found that *L. stagnalis* and *H. trivolvis* have shells that are of similar strength (Nora and Mahon, *unpublished manuscript*). We hypothesized that because *L. stagnalis* is larger than *H. trivolvis*—investing energy into growing—and because the conical shape of the *L. stagnalis* shell is difficult for crayfish to handle (Brown 1998), that it should invest less in chemical defenses that deter crayfish predators. This means that *L. stagnalis* meat should be preferred over *H. trivolvis* meat. *Helisoma trivolvis* is also within the preferred size range of crayfish gastropod prey (Nystrom and Perez 1998) and possesses a shell that is preferentially browsed on by crayfish (Brown 1998), suggesting that it should invest the most in chemical defenses and thus be the least preferred by crayfish when physical defenses are removed. Finally, we hypothesized that the native crayfish, *O. virilis*, would have more distinct preferences for the native species than the naturalized *O. propinquus* because of its longer coevolutionary history with these species, possibly enabling it to counteract potential toxins.

METHODS

Study Organisms—We used three species of snails in our study. *Bellayma chinensis* (Viviparidae) is a freshwater snail that has invaded many of Wisconsin's inland waters, often as a result of aquaria disposal and anthropologic dispersal as hitchhikers on recreational boats (Solomon et al. 2010, Havel 2011). We collected *B. chinensis* from Brown Creek in Wisconsin on the University of Notre Dame Environmental Research Center (UNDERC) property. *Helisoma trivolvis* (Planorbidae) is a freshwater pulmonate snail that is found throughout North America (Norton et al. 2008). We collected *H. trivolvis* from Tenderfoot Lake on UNDERC property. *Lymnaea stagnalis* (Lymnaeidae) is a freshwater pulmonate snail that inhabits a variety of freshwater habitats including those present at UNDERC (Dalesman et al. 2007). *Lymnaea stagnalis* were collected from Plum Lake on UNDERC property.

Orconectes propinquus (Cambaridae) and *Orconectes virilis* (Cambaridae) were used as our crayfish predators. After being native to only southern and eastern Wisconsin, *O. propinquus* expanded its range and is now considered native to northern parts of Wisconsin as well (Olden et al. 2006). We collected *O. propinquus* from Tenderfoot Lake on UNDERC property. *Orconectes virilis* is native to a broad area in central Northern America including areas such as northern Wisconsin and the Upper Peninsula of Michigan (Pflieger 1996). We collected *O. virilis* from Plum Lake on UNDERC property.

Experimental design—Crayfish were randomly selected and presented ten alginate pellets containing meat from one snail species and ten pellets with meat from another snail species.

Crayfish were then left for ten hours overnight to forage on the alginate pellets. Snail species were paired to give three combinations. Each of these combinations was replicated 10 times for both crayfish species. Prior to the preference trials, randomly selected crayfish of the same species were placed into ten-gallon tanks filled with tap water and food was withheld for three days. Alginate pellets were standardized to contain 2% alginate powder and 2g of snail meat per 100mL of the water and powder mixture. Each pellet was made from 1mL of the alginate pellet mixture. In order to differentiate between pellets within each replicate, one species was randomly selected to be dyed green using commercial food coloring.

After the ten hour experimental trial, pellets were sorted and ranked individually based on the level of browsing they received. This rank was determined by which class the pellet fit: not browsed (0), lightly browsed (1), moderately browsed (2), heavily browsed (3), or completely consumed (4). A mean was taken from the ten pellets to give an overall ranking for each snail species in each trial. These rankings were then used to create a preference ratio based on the paired species of snail. If the first species listed was browsed on preferentially, the ratio was greater than one. If the ratio was less than one, the second species listed was browsed on preferentially. If there was no preference for either snail species, the ratio equaled one.

Statistics—We used the statistical program SYSTAT 13 (UNICODE) to analyze the collected data. Following the three day starvation period, some trials contained crayfish that had recently molted, were near to molting, or possessed physical irregularities—such as a missing claw—and these trials were excluded from our data set for statistical analyses. The presence of a color preference was analyzed using a one-way χ^2-test with the response variable of individual pellets being fed upon or not fed upon. A two-way ANOVA was used to determine if there was a significant difference in browsing ratio between the factors of predator species and snail combination. Since we were not interested in which preference ratios were different relative to each other, but rather if there was a significant preference for one snail species in each pairing, we set up a post-hoc hypothesis test with the null hypothesis that there was no significant preference for either species within a treatment. This was done by scaling the data to set a "no preference" line at zero and then testing whether or not the scaled preference ratios varied significantly from this line. Our null hypothesis was that if the ratio equals zero, there is no preference; however, if the ratio is significantly different from zero, there is a preference for one of the snail species. Shapiro-Wilk normality tests were used to test for normality and the data was log-transformed if it failed to meet the assumptions of normality.

RESULTS

Neither *O. propinquus* (Pearson's value=0.003, df=1, p-value=0.959) nor *O. virilis* (Pearson's value=0.295, df=1, p-value=0.587) preferentially browsed on dyed or non-dyed pellets. Because our preference ratios failed to meet the assumption of normality (Shapiro-Wilk p-value < 0.001), we used a log transformation to normalize our data (Shapiro-Wilk p-value = 0.228).

The preference ratios for the three snail combinations were not significantly affected by crayfish species alone ($F_{1,45} = 0.002$, $P = 0.964$). There was a significant effect of snail meat pairings on the preference ratios of pellets foraged on by crayfish predators ($F_{2,45} = 7.965$, $P = 0.001$). There was a significant interaction term for crayfish species and combination of snail meat meaning that the two crayfish species had different preference ratios for the snail meat pairings ($F_{2,45} = 6.106$, $P = 0.004$). Our post-hoc hypothesis test showed that *O. propinquus* displayed no significant preference for *L. stagnalis* or *B. chinensis* when they were offered together ($F_{1,45} = 0.172$, $P = 0.680$; Figure 1). This was also true for *B. chinensis* and *H. trivolvis* when they were offered together to *O. propinquus* ($F_{1,45} = 0.333$, $P = 0.567$; Figure 1). However, *O. propinquus* did significantly prefer *H. trivolvis* over *L. stagnalis* when they were offered together ($F_{1,45} = 17.733$, $P < 0.001$; Figure 1). *Orconectes virilis* showed a significant preference for *B. chinensis* alginate pellets to *L. stagnalis* pellets when these snail species were paired ($F_{1,45} = 4.044$, $P = 0.050$; Figure 1). *Orconectes virilis* also had a significant preference for *H. trivolvis* when offered with *L. stagnalis* ($F_{1,45} = 4.439$, $P = 0.041$) and when offered with *B. chinensis* ($F_{1,45} = 10.476$, $P = 0.002$; Figure 1).

DISCUSSION

Both *O. propinquus* and *O. virilis* showed some preference for *H. trivolvis* over the other species of snails present. *Orconectes propinquus* only showed this preference when offered *H. trivolvis* in the presence of *L. stagnalis* suggesting that the chemical defenses of *L. stagnalis* are more adapt to defending against this predator species or that *H. trivolvis* is more nutritious. This preference for *H. trivolvis* was not seen in *O. propinquus* when offered in addition to *B. chinensis*. Some possible explanations are that the chemical defenses of these gastropods are similarly effective at repelling *O. propinquus*, that they have similar nutritional values, or that the nutritional value of one species is enough to compensate for the energy required to deal with potential toxins, thus resulting in no net preference. We also did not observe a preference for *B. chinensis* over *L. stagnalis* by *O. propinquus*, suggesting again that there may be a complex relationship between nutritional value of a prey item and the energy required to cope with its chemical defenses. Another possible explanation is that *B. chinensis* may have fewer chemical defenses but that *O. propinquus* is more familiar to feeding on *L. stagnalis* and *H. trivolvis*, with which it naturally encounters on UNDERC property. Through these natural encounters, it is possible that *O. propinquus* has learned to recognize these snail meats and browsed on them preferentially in our trials based on a familiarity factor. A study by Skelhorn and Rowe (2006) found that predators can learn to selectively browse based on coloration and type of chemical defense present.

The preferences of *O. virilis* appear to be much more obvious. *Orconectes virilis* also preferred *H. trivolvis* over *L. stagnalis* suggesting again that the chemical defenses of *H. trivolvis* are less effective at deterring predation than those of *L. stagnalis* or that *H. trivolvis* is more nutritious. *Helisoma trivolvis* was also preferred over *B. chinensis*. Again, there may be a possible familiarity factor. This familiarity hypothesis is countered by observing the preference that *O. virilis* has for *B. chinensis* over *L. stagnalis*. Hypotheses attempting to explain these preferences will be explored later in this paper.

The results of this study contradict a similar study previously performed at UNDERC. This study found that when using alginate pellets, *B. chinensis* was more palatable than *Helisoma spp.* and *L. stagnalis* (Nickels and Mahon, *unpublished manuscript*). We improved on their study by standardizing the amount of alginate powder and snail meat present in the alginate pellets so that no species was preferred solely because its alginate pellets contained more food resource. We also took into account varying browsing magnitudes in order to achieve a higher resolution of the actual crayfish preferences. A final improvement was that unlike their study, we directly compared the preferences of our crayfish species by offering two species of snail meat at one time instead of one species and a set of control pellets. Because of these improvements, we believe that this study offers a much more accurate and resolute image of crayfish preference for these three snail species.

A previous study by Brown (1998) looked at shell strength and geometry and their effects on predation and found that snails with plano-spiral anatomy, such as *H. trivolvis*, are easier to handle and are therefore preferred by crayfish predators. This study also found that spiral shells, such as those of *L. stagnalis* and *B. chinensis*, were more difficult for crayfish to handle (Brown 1998). This could help explain the familiarity hypothesis because crayfish may associate certain chemical cues and defenses with lower handling times and preferentially browse on those species, even when the protective shells are absent. Conversely, molluscivore fish prefer larger, thin-shelled species (Lodge et al. 1998). A future study could explore the preferences of molluscivore fish based solely on the relative abundance of chemical defenses and nutritional value of these gastropods.

Another possible hypothesis is that *H. trivolvis* may have a smaller energy budget than *B. chinensis* or *L. stagnalis* or that it spends less of this budget on morphological defenses and more on avoidance behavior. This is supported by a hypothesis presented by Endler (1991). Endler (1991) suggested that it is better for prey species to interrupt the predation sequence as early as possible in order to avoid costly injury or catastrophic death. *Helisoma trivolvis* may fall under this model and invest mainly in defenses that allow it to avoid or escape predation early in a predation event. Previous studies have found that when *H. trivolvis* is presented with a predator, it—like other pulmonates—can escape predation by using its modified lung to climb and crawl out of the water (Alexander and Covich 1991, Covich et al. 1994, Nickels and Mahon, *unpublished manuscript*). The cost of this behavior is that *H. trivolvis* forages less while it is avoiding predation (Sura and Mahon 2011). This lowers that amount of food resources that *H. trivolvis* can uptake, possibly lowering its energy budget and leaving even less energy to invest in defenses for later in the predation sequence. Further study is necessary to analyze the energy budget of *H. trivolvis* as well as those of *B. chinensis* and *L. stagnalis* in order to fully describe their investments in a variety of defenses and behavioral changes.

The effects of *B. chinensis* on invaded ecosystems are not yet understood. Previous studies have suggested they may place high competitive pressures on native snails due to their large size and high population densities (Johnson et al. 2009, Solomon et al. 2010). *Bellayma chinensis* can also survive extreme conditions including desiccation for up to four weeks—an amazing feat for an aquatic species (Havel 2011). Combined with its already impressive physical defenses, the lack of a convincing crayfish preference for this species may suggest that *B. chinensis* is immune to the same kinds of predation pressures that other gastropods face in its invaded range. If native species are preferentially predated on while populations of *B. chinensis* continue to grow relatively unchecked by crayfish predation, eventually we could see a replacement of native gastropod species by *B. chinensis.*

Contrary to our original hypothesis, the species of snail with the greatest amount of physical defenses was not preferentially browsed on in the absence of these defenses. Further study is needed to more completely describe the complex interactions of gastropod defenses and the adaptations of their crayfish predators to deal with these defenses. Physical defenses were not an adequate predictor of relative chemical defenses, a result that has been observed in gastropods before (Neves et al. 2009). Because of this, additional analysis of the chemical defenses and nutritional value of *B. chinensis*, *L. stagnalis*, and *H. trivolvis* are needed. The effectiveness of different types of prey defenses could also explain our results and should be explored in future studies. We also ignored crayfish adaptations to possible chemical defenses and any long term effects of these toxins on crayfish. This study highlights the complexities of predator-prey interactions and the need to further investigate these interactions in order to obtain a better understanding of how organisms interact with native and invasive species.

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FIGURES

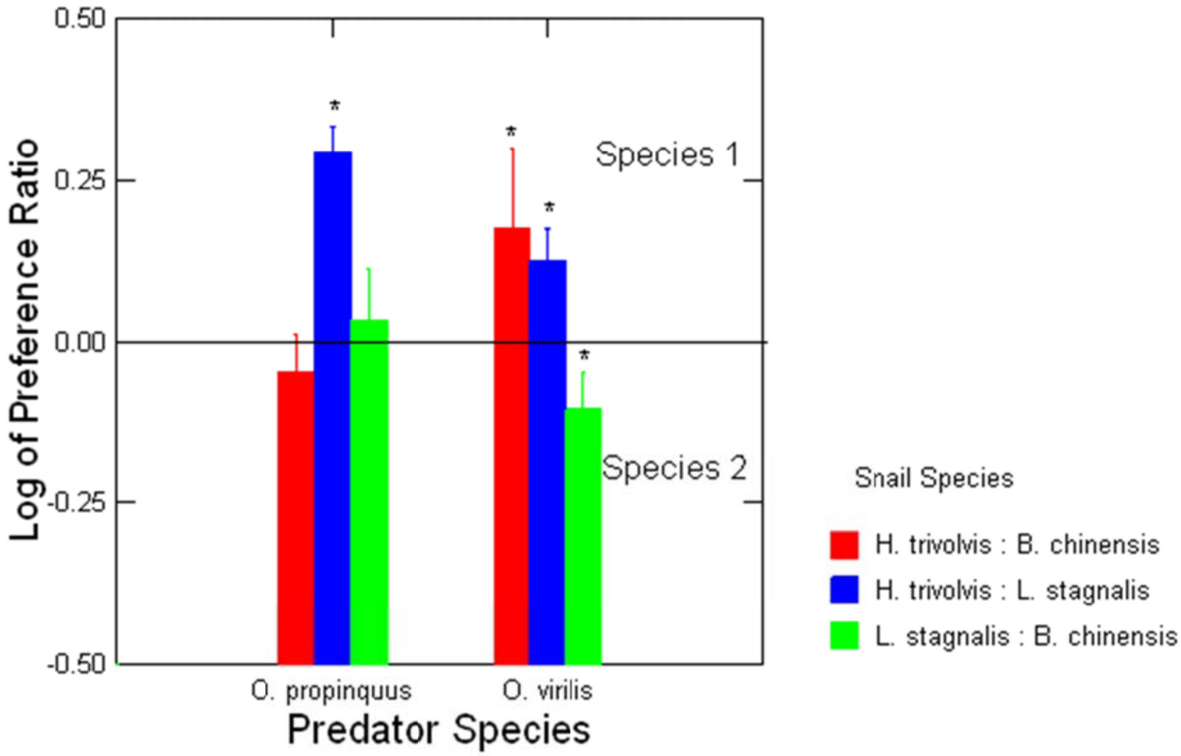

Figure 1. Preference Ratios of Crayfish for Snail Species. Ratios were determined by crayfish browsing on alginate pellets containing snail meat. The line at zero is the line of no preference. No preference is present if the scaled ratio does not vary significantly from this line. When the first snail species listed was preferred, the ratio was positive and the graphed ratio rose above the line of no preference. In cases where the second snail species was preferred, the scaled ratio was negative and the graphed average was below the line of no preference. Asterisks indicate which preferences were significant ($P < 0.05$).