Predator avoidance behaviours between native (*Helisoma trivolvis*) and invasive (*Cipangopaludina chinensis*) snail species when exposed to kairomones from crayfish (*Orconectes propinquus*) and pumpkinseed sunfish (*Lepomis gibbosus*) predators and alarm cues

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Abstract

Predator-prey relationships between invasive prey and native predators can show an interesting relationship of phenotypic plasticity of the trait, evolutionary history, morphological constraints and length of exposure. In this study, the difference between a native (*Helisoma trivolvis*) and invasive (*Cipangopaludina chinensis*) snail species being exposed to kairomones from two native predators - crayfish (*Orconectes propinquus*) and pumpkinseed sunfish (*Lepomis gibbosus*) - and alarm cues from conspecifics was looked at in terms of two predator avoidance behaviours of climbing and burrowing. This is one of the first studies focusing on how native and invasive species differ in their predator avoidance behaviour when exposed to both kairomones and alarm cues. A significant difference was found for the two avoidance behaviours between the snail species and chemical cues (kairomones and alarm cues). In the presence of only kairomones from predators, *Helisoma* did not alter their behaviour; however, in the presence of kairomones and alarm cues from crushed conspecifics, *Helisoma* exhibited increased burrowing behaviour. This burrowing behaviour goes against many previous studies looking at chemical cues using the same species, as climbing behaviour is more prevalent. The Chinese Mystery snails did not show distinct avoidance behaviours to either kairomones or kairomones and alarm cues from crushed conspecifics. The evolutionary history of a species, even between the same species, can therefore have a strong influence of which behaviours are carried out within an environment where species are exposed to multiple different cues.

Introduction

With invasive species invading new habitats, the ecological consequences could be detrimental as they can negatively affect the ecosystem by outcompeting other native species, depleting natural resources and overall decreasing the biodiversity of the habitat (Wilcove *et al.* 1998). The spread of invasive species is the second greatest threat to biodiversity, following habitat loss (Wilcove *et al.* 1998). The Chinese Mystery snail (*Cipangopaludina chinensis*) is an invasive snail species that crossed seas to North America in the Asian food trade and is now found in many freshwater ecosystems across North America. Over 500 lakes and rivers in the Wisconsin area have been invaded by the Chinese mystery snail.

The Chinese Mystery snail has invaded these waters in huge numbers due to their ability to reproduce and spread quickly, along with having a greater body size than native snail species
(Soloman et al. 2010). These natural morphological adaptations can allow the Chinese Mystery snail to have competitive advantages over the native snails and create unknown consequences to aquatic habitats.

For both native and invasive species, predator-prey interactions within a community can cause changes in the morphology, behaviour and life history of the prey and the predator (Turner and Montgomery 2003). Predator-prey dynamics can have a large effect on community structure and has allowed for a diverse range of adaptations in prey, allowing for defensive mechanisms to develop (Lakowitz et al. 2008). The behaviours to avoid and defend against predators can then be selected for, within the environmental conditions, to increase the fitness over those undefended behaviours (Lakowitz et al. 2008). With anti-predator behaviours being phenotypically plastic, an environmental variation in predators and predation risk causes different responses for different situations, allowing for a number of different behaviours seen in species to react to different predators (Hoverman et al. 2005). With a new invasive prey species in the community, the predator-prey relations in the community can change by allowing uncontrolled numbers of predators or native prey which can further create destructive effects.

When faced with the dangers of predators being in close proximity, some prey have adapted to respond to predators by detecting them through chemical cues (Turner 2008). Freshwater snails rely on chemical cues to respond to predators but also for foraging, mating and responding to environmental stressors (review in Lima 1998). Within aquatic ecosystems, two different types of chemical cues can be released and detected by snail species; kairomones from predators and alarm cues from crushed conspecifics (Pohnert et al. 2007). The snails are affected by these chemical cues by interpreting them as a threat and then responding with altered
behaviours. Chemical cues can indicate the presence, proximity and overall degree of danger of the predator (Dalesman et al. 2006).

The predator avoidance behaviours of the native snail species *Helisoma trivolvis*, has been shown in the presence of predators and alarm cues but the invasive species Chinese mystery snail (*Cipangopaludina chinensis*) has yet to be examined. *Helisoma trivolvis* reacts to chemical cues from crayfish (*Orconectes rusticus*) by increasing anti-predator behaviour to crawl out of the water (Hoverman et al. 2005).

Snails need to avoid their predators within their ecosystem in order to survive and therefore have developed behavioural trait shifts that allow them to protect themselves from predators by reacting to alarm cues along with the kairomones (Turner and Montgomery 2003). Dalesman et al. (2006) found that snails (*Lymnaea stagnalis*) responded to the presence of a fish predator’s (*Tinca tinca*) kairomones by moving towards the water surface and even climbing out of the water. When alarm cues from crushed conspecifics were also present along with the predator kairomones, *L. stagnalis* climbed out of the water more frequently (Dalesman et al. 2006).

With prey having to expend costly behavioural responses to different predators, it is important to reduce unnecessary behavioural responses and elicit a response to protect themselves (Dalesman et al. 2006). In a study by Turner et al. (1999) freshwater snails (*Physella gyrin*) respond to pumpkinseed sunfish kairomones by moving undercover, which confers protection from the fish. In contrast, the snails climb out of the water in response to kairomones from crayfish, which attack from the bottom (Turner et al. 1999).

This study addresses questions within predator-prey relations and the avoidance behavioural responses of two snail species, *H. trivolvis* (native) and Chinese mystery snail
(invasive), in relation to kairomones from two predators, crayfish and pumpkinseed sunfish, as well alarm cues from crushed conspecifics. The aim of the experiment was to determine (1) whether the snails exhibit different avoidance behaviours for predators with different hunting strategies (2) whether just predator kairomones elicit avoidance behaviours or if alarm cues are necessary for a response and (3) whether the two snail species exhibit similar avoidance responses to chemical cues. These behaviours should be seen this way due to the native \textit{H. trivolvis} having a much longer evolutionary time scale to co-evolve with the predators along with natural protective morphological characteristics possessed by the Chinese Mystery snail. Knowing the evolutionary history and aquatic habitat characteristics of both the native and invasive snail species can help predict how invasives will react in this new environment.

\textbf{Methods and Materials}

\textit{Experimental Species}

In this study we used two different freshwater snails found in lakes in Wisconsin and Michigan; a native species, \textit{Helisoma trivolvis}, and an invasive species, Chinese mystery snail (\textit{Cipangopaludina chinensis}). The \textit{H. trivolvis} were collected from Tenderfoot Lake and the Chinese mystery snails were collected from Brown Creek. The predators used for this experiment were the freshwater crayfish (\textit{Orconectes propinquus}) and pumpkinseed sunfish (\textit{Lepomis gibbosus}). The crayfish were collected from Tenderfoot Lake and the pumpkinseed fish were collected from Morris Lake and Tenderfoot Lake. All of these locations are found at the University of Notre Dame Environmental Research Center. Both snail and predator species were put into holding tanks with lake water from where they were collected to acclimate.

\textit{Experimental Design}
An experimental mesocosm was set up to represent a natural habitat for both the snail species. Using 38L tanks, approximately 5cm of sand was placed even on the bottom followed by 24L of tap water (19-16°C). Twelve wooden reeds (1/8 inch-1/2 inch) were placed in the sand of tanks where *H. trivolis* were tested and three one inch thick PVC pipes were stuck in the sand of tanks where Chinese Mystery snails were tested.

To test the effects of predator kairomones and alarm cues, an experimental set up of one control and five treatment trials, with each treatment trial replicated eight times along with measuring three behavioural responses. The six treatments were as followed:

1. Control – no predators or chemical cues (tap water)
2. Crayfish kairomones only
3. Pumpkinseed fish kairomones only
4. Alarm cues only
5. Alarm cues with crayfish kairomones
6. Alarm cues with pumpkinseed fish kairomones

Snails were taken out from holding tanks the night before and transferred to small tanks of tap water to allow them to acclimate for at least 12 hours to water with no chemical cues. Kairomone and alarm cues were generated an hour before the trial. For the kairomone treatments, either the crayfish or pumpkinseed fish was placed in a tank with 4L of tap water. For alarm cues, snails were placed in the tanks and then crushed at the end of the hour. For the combined alarm cue and kairomone treatments, the snails were placed in with the predator for one hour and then smashed at the end of one hour.
Each experimental mesocosm contained ten snails of the *H. trivolvis* or Chinese Mystery snail. Five hundred mL of well mixed water from the kairomone and alarm cue treatment tanks were added to each appropriate experimental mesocosm.

The behavioural response was recorded into three different categories based on the movement of the snails; burrowing, climbing or exposed (either in shell or out of shell on sand). After the chemical cues were added to the experimental mesocosm the behavioural response of snails was taken every 10 minutes up to 60 minutes. The burrowing response was classified as moving sand to burrow into the ground, climbing response was measured by climbing up glass wall of tank or “reeds”.

All six treatments were applied to a group of snails, each in random order. After the experimental trial is over, the snails were removed and put back into the holding tank and the experimental tank with snails getting 24 hours between treatments, before being used in another experiment. The tanks were washed out along with the rocks and dowels to remove any kairomones.

**Statistical Analysis**

One-way ANOVAs were used to determine whether presence of alarm cues vs. presence of kairomones showed significant effect compared to the control between the three behaviours over time. Three-way repeated measures ANOVA models (species x predators x chemical cues) were used to test the effects of snail species (*Helisoma trivolvis* vs. CMS), predators (crayfish vs. pumpkinseed) and chemical cues (kairomones vs. kairomones and alarm cues) on the three behaviours being measured (climbing, burrowing and no response) over time.

**Results**
A significant difference was found in burrowing (F=48.15, df=1, p=<0.0001) and climbing (F=57.73, df=1, p=<0.0001) and not significant when snails did not respond (F=1.01, df=1, p=0.2972) when alarm cues were either present or not present (control). When alarm cues were present the proportion of snails burrowing was 49.17% compared to when they were not present (21.70%). The proportion of snails climbing also differed when comparing alarm cues present (14.41%) and not present (37%). The proportion of snails that did not respond was close as when alarm cues were present 36.42% did not respond compared to when not present 41.32% did not respond. Over time, the presence or absence of alarm cues significantly affected climbing (F=5.75, df=5, p=<0.0001) but did not significantly affect burrowing (F=2.5, df=5, p=0.0364) and snails who did not respond (F=1.38, df=5, p=0.2396).

No significant differences were found on predators being present or absent (control) of the three behaviours along with presence or absence over the course of the experiment (Table 1).

There was a significant difference between the snail species for burrowing (F=82.94, df=1, p=<0.0001), climbing (F=10.62, df=1, p=0.0019) and showing no response (F=204.92, df=1, p=<0.0001, Fig. 1B). Helisoma trivolvis had a higher proportion burrowing (50.1%) while only 22.6% of Chinese Mystery snails burrowed. Again, a higher proportion of H. trivolvis were seen climbing (30.1%) while only 20.47 Chinese Mystery snails climbed. Helisoma trivolvis had a lower proportion with no response (19.84%) while Chinese Mystery snail had 56.98% with no response (Fig. 1B). Over time the two snail species also significantly differed in their burrowing behaviour (F=8.19, df=5, p=<0.0001) and showing no response (F=4.37, df=5, p=0.0021) while showing no significant difference between snail species in their climbing (F=2.124, df=5, p=0.0768, Fig. 3).
There was a significant difference in snails showing burrowing behaviour (F=8.07, df=1, 
p=0.0062) and showing no response (F=10.45, df=1, p=0.002) when exposed to two different 
predator kairomones, crayfish and pumpkinseed fish. No significant differences were found in 
snails climbing (F=0.005, df=1, p=0.9438) (Fig. 1A). Snail burrowing was seen in higher 
proportions when crayfish kairomones were present (40.63%) while addition of pumpkinseed 
fish kairomones had only 32.03% burrowing. Climbing response had similar proportions for 
addition of crayfish kairomones (25.16%) and pumpkinseed fish kairomones (25.37%, Fig. 1A). 
No response from snails was seen in higher proportions when pumpkinseed kairomones were 
present (42.60%) over crayfish kairomones being present (34.22%, Fig. 1A). Over time, the 
addition of predator kairomones did not significantly affect the proportion of snails burrowing 
(F=0.6186, df=5, p=0.6862), climbing (F=0.2697, df=5, p=0.9277) or showing no response 
(F=0.4998, df=5, p=0.2680).

A significant difference was seen in snails burrowing (F=76.79, df=1, p=<0.0001) and 
climbing (F=69.28, df=1, p=<0.0001) but not in snails showing no response (F=0.61, df=1, 
p=0.4369) when exposed to alarm cues and kairomones or just kairomones. The snails exposed 
to alarm cues and kairomones had a higher proportion of burrowing (49.58%) compared to just 
kairomones (23.07%). The opposite effect was seen in the climbing response when addition of 
kairomones (37.5%) and alarm cues (13.02%) was done. The proportions of snails with no 
response to chemical cues was very close as alarm cues had 37.4% not respond while 
kairomones had 39.43% not respond. Over time, the addition of chemical cues was significant in 
burrowing (F=4.88, df=5, p=0.001) and climbing (F=8.14, df=5, p=<0.0001) (Fig. 2).
A significant pairwise interaction of burrowing between species and chemical cues was found \((F=8.67, \text{df}=1, p=0.0047)\). All other interactions between the three factors were not significant for the three response variables (Table 1).

**Discussion**

*Snail Species*

Differences in anti-predator behaviour is seen between both native and invasive species due to factors such as differences in physical constraints and different amounts of time to gather information from predators to produce a defensive behaviour (Schoeppner and Relyea 2005). When comparing between the two species, *H. trivolvis* and Chinese Mystery Snail, a strong effect was seen when comparing between the three behaviours; burrowing, climbing and having no response (Fig. 1A). There was a trend seen in *H. trivolvis* with an overall increase in the behavioural responses of burrowing and climbing, which indicates that chemical cues are more likely to elicit response in the species. Trends were also found in both species in terms of increasing the number of responses over time with the addition of chemical cues and kairomones (Fig. 3). The slow response of the Chinese Mystery snail could be due to physiological or morphological differences in the snail compared to the *H. trivolvis*. The possibility of Chinese Mystery snail needing an increased response time to chemical cues, more likely alarm cues, could mean an increase in response not seen in this study as some chemical cue behavioural studies have one week of observations (Turner 1996).

With differences in behaviour seen between invasives and native species across all kingdoms and ecosystems, it is important to analyze to predict future consequences and removal procedures (Wilcove 1998). Invasive crayfish species have been found to forage slower and less
on *Cipangopaludina chinensis* compared to the native snail, putting the invasive species at a disadvantage (Olden 2009). The invasive crayfish could also consume much smaller snails compared to the native species of crayfish (Olden 2009). The results show a similar disadvantage to the native snail species, as a high proportion of Chinese Mystery snails did not respond to the cues at all. On the contrary, when comparing invasive and native turtles foraging behaviour on tadpoles, a higher number of tadpole species did not exhibit their anti-predator behaviour in response to kairomones from the invasive species over the native species (Polo-Cavia 2010). With the short evolutionary time scale the invasive prey and native predator species have interacted for could allow for the predator to not detect the invasive prey kairomones, like they do for some native species, which can put them at an advantage.

*Predators and Kairomones*

Predation can cause decreases in foraging, reproduction and growth in prey species, which is why anti-predator behaviour has been selected for (Gherardi 2011). Between the three responses of burrowing, climbing and not responding, predators did not have a large effect (Table 1). With the predator factor alone, a significant effect was seen from a higher proportion of snails burrowing in the presence of crayfish and not responding to pumpkinseed fish (Fig. 1A). With these results, snails did not react in with the distinct predator avoidance behaviours that were expected due to hunting strategies of the predators. These results do not necessarily mean that these predators do not feed on the snails, and do not affect the snail species behaviour, but kairomones alone do not always elicit an ant-predator behaviour unless combined with alarm cues. Turner (1996) found that freshwater snails do not seek refuge from pumpkinseed fish when only exposed to kairomones alone, but only when combined with crushed conspecifics or crushed conspecifics alone.
The lack of behavioural response to predators can infer possibilities for why a lack of response was seen in both the species, each having different reasons. Snail species in North America, including *H. trivolvis*, has been found to be preyed upon by both crayfish (Alexander and Covich, 1991; Lodge *et al.*, 1994; Brown, 1998) and pumpkinseed fish (Bronmark *et al.* 1992), which infers that the species has evolved a mechanism to reduce expending energy to respond to a predator when they are only present and not foraging on conspecifics (Phillips 1978). The lack of response to only presence of kairomones has also been found in snails reacting to starfish and only when starfish is feeding on conspecifics does a significant behavioural response take place (Phillips 1978). Similar studies with predator kairomones have found a decrease in feeding rates of *H. trivolvis* when exposed to predators, which infers that some behavioural aspects of the snails are affected by the presence of predators (Sura and Mahon, 2011).

The lack of response to predators seen in the Chinese Mystery snail behavioural response is most likely due to the fact that they have had only a very short time of co-evolving with the predator species. Therefore they have not had time to evolve behavioural traits to defend themselves against predators as quickly as compared to the native species who have the behaviour present. With the Chinese Mystery snail evolving with different predators in their native habitats, it can be proposed that the species does not have predators of similar taxa due to lack of response. When species who are phenotypically plastic are exposed to new environments and predators, there is a time lag for a new behavioural responses to be produced, as new behaviours are a product of variability in an environment (Padilla 1996).

*Alarm Cues*
When aquatic species experience alarm cues from crushed conspecifics, behavioural, physiological and morphological changes are seen. Presence of alarm cues has a very large effect on the behaviour the snails choose, which infers that this is the main cue for responding to predators over the kairomones present by themselves. In many studies, the snails follow a gradient of increasing anti-predator behavioural response from control to predator to alarm cue to alarm cue and predator together. In this study, the increased response was not seen until alarm cues were present with kairomones and was kept at same reactivity of burrowing when alarm cues were present alone. In a study by McCarthy and Fisher (2000), snails from two populations had a significant increase in anti-predator behaviour, in this case climbing to the surface, when exposed to alarm cues in conjunction with kairomones. Not only can the number of snails eliciting an anti-predator behaviour change with type of chemical cues present, but the behavioural response can change within the same species, showing phenotypic plasticity (Mach and Bourdeau 2011). When a snail species is exposed to alarm cues alone, they actively move away while when exposed to kairomones alone they retreat into their shells (Mach and Bourdeau 2011).

The interaction between snail species and chemical cues present shows an interesting relationship which a lot can be inferred from (Fig. 5). The relationship of alarm cues with *H. trivolvis* shows behaviour opposite to a lot of studies done, but the fact that they burrowed instead of climbing to protect themselves. *H. trivolvis* has been found to climb to near surface habitat when exposed to different predators, including crayfish (Hoverman *et al.* 2005), which was not seen in high proportions when the *H. trivolvis* were exposed to predator kairomones alone. The previous experiments did not look at the response to alarm cues, but other experiments in snail species have found that alarm cues with kairomones increase climbing.
behaviour (McCarthy and Fisher, 2000; Turner 2008). When looking at habitat use, studies have shown an increase in covered habitat use when exposed to alarm cues (Turner, 1996) which could be related to the burrowing results found.

With the large proportion of *H. trivolvis* burrowing instead of climbing from alarm cues and only responding to alarm cues, no matter which predator was present, leaves the question of why different behaviours can be seen within same species responding to same chemical cues? The behavioural responses of species must then be looked at on a population level, especially in aquatic habitats, as different lakes can differ in food, predators and water quality. Therefore the evolutionary history should be looked at in terms of the habitat the snail species has been brought up in at a local level and with our results compared to other studies, a form of “localized” evolution could have taken place (McCarthy and Fisher 2000). Since large aquatic predators, such as fish, are mostly visual predators, the burrowing response allows the snail to be hidden as they attack from above. Reasons for only reacting to the crushed conspecifics could be hypothesized in an energy cost/benefit analyses where it is important to respond to injured conspecifics, which indicates predator in area feeding, over when a predator is just present.

A question that is interesting to look at is why the Chinese Mystery does not show a significant behavioural response to alarm cues from conspecifics. In a large number of chemical cues on snail behavioural studies, alarm cues have an equal or greater effect when combined with kairomones as well on their own on a behavioural response to avoid predators. Evolutionary history of the Chinese Mystery snail could be drastically different than the native *H. trivolvis*, and morphological characteristics may have evolved over chemical cue recognition to avoid predators.
The interaction between predators with native and invasive prey is often overlooked by studies showing how they interact with each other, even though the predator recognition and avoidance plays a key role in managing these species. In this study, the Chinese Mystery snail has proved to show very little behavioural responses to both kairomones and alarm cues, inferring that the main defensive mechanism is based up morphological phenotypes which predators have place selective pressure on. Another finding of the study shows that the *H. trivolvis* species do not all respond similar to kairomones or alarm cues based upon their behaviour, indicating that a type of local evolution is taking place within aquatic habitats. With the behaviours shown between and within different native and invasive snail species, a great deal of evolutionary history can be analyzed from native phenotypes while also predicting how new, exotic species will interact and change with the new environment.
Figures and Tables

**Table 1.** Three-way repeated measures ANOVA testing the effects of predators, species, chemical cues and time on three snail behavioural responses; burrowing, climbing and no response. Each 10 minute interval up to one hour is the repeated factor.

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<td></td>
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<td>F</td>
<td>p</td>
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Figure 1. The effects of three different factors (A) predators; crayfish (CF) and pumpkinseed fish (PSF), (B) snail species; Chinese Mystery snail (CMS) and *Helisoma trivolvis* (HS) and (C) chemical cues; alarm cues and kairomones on three snail behavioural responses of burrowing, climbing and no response. Alarm cues in chemical cue response include kairomones. (*) indicates significance. (±SE, n=8)
Figure 2. The mean behavioural response of (a) burrowing (b) climbing and (c) no response with effects of alarm cues and kairomones over 60 minute time period. Graphs A and B are significant (n=8).
Figure 3. The mean behavioural response of (a) burrowing (b) climbing and (c) no response of Chinese Mystery snail (CMS) and *H. trivolvis* over 60 minute time period. Graphs A and C are significant (n=8).
Figure 4. Behavioural responses with the presence and absence of alarm cues (A) mean percentage of response, averaged over whole experiment and (B) mean percentage of response at every ten minutes up to one hour. The climbing with and without alarm cues shows a significant difference over time. (*) indicates significance (±SE, n=8).
**Figure 5.** Interaction between species and chemical cues on the three behavioural responses of (A) burrowing (B) climbing and (C) no response. (*) indicates significance. (±SE)
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