Effect of Conspecific Cue on Shelter Competition between Mixed Sex Intruders and Resident Female Crayfish *Orconectes propinquus*

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Abstract

One of the primary factors behind the expansion of invasive crayfish species across north temperate lakes is their ability to displace residents from shelters and claim them as their own. Shelter possession is a major component of crayfish fitness, as it provides vital protection from fish predation. I sought to explore the interaction and competition for shelter between resident and intruding crayfish by acclimating a female crayfish to a tank with a single shelter before introducing a conspecific intruder of either sex. I also introduced another variable – chemical cue saturated water from a tank containing high densities of crayfish (relative to standard lake densities), in order to see how a crayfish might respond when it perceives a large population around it. I predicted that males would be better intruders and thus more consistently displace the resident crayfish and that the addition of crayfish cues would increase the competition for shelter as a result of greater perceived risk of losing an undefended shelter. Contrarily, my results revealed that there was significantly less use of the shelter prior to intruder introduction when high-cue water was used and revealed no significant trends between shelter possession and the sex of the invading crayfish. These results suggest that crayfish in higher density lakes do not compete as aggressively for the shelters available and could be a result of reduced perception of predation.

Introduction

One of the main drivers of the spread of invasive species of crayfish is their ability to procure shelters in a new territory (Söderbäck 1994). Usually, these shelters are already occupied by a native species, so looking at the dynamics of resident displacement and shelter re-colonization by an invasive species can provide valuable insight into stopping the spread of unwanted invasive species of crayfish, such as well-known rusty crayfish (*Orconectes rusticus*) which is rapidly spreading across the Midwest as well as other areas of North America, including parts of the Northeast, Colorado, Wyoming, Oregon, and parts of Canada (Fuller). Before the rusty crayfish invasion, however, there was another invasive species of crayfish; *O. propinquus* was introduced to the region about -80 years ago, likely transported as bait for fishing, and has since spread across most of northern Wisconsin (Capelli and Munjal 1982). Its status as both an invasive that has displaced native species (*O. virilis*) and a “native” being displaced by a different invasive species (*O. rusticus*) makes it an ideal study organism in studying one of the primary drivers of crayfish invasions: shelter competition (Capelli 1982). Because shelter is an important resource that is also most often limiting (Bovbjerg 1970), shelter competition is one of the most important components of crayfish survival, especially when looking at displacement by invasive species. Species or individuals that are competitively superior (such as many invasive crayfish) are able to exclude other crayfish species or individuals from shelters. These excluded organisms face increased predation by fish and higher mortality (Nakata and Goshima 2003).

At the root of species interactions are individual competitions, so looking at specific interactions between crayfish can be used as an indication of a species' level of competitive superiority. Some determinants of this superiority have been well documented such as a size advantage (Bovbjerg 1953, Figler *et al.* 1999, Hill *et al.* 1993, Bergman and Moore 2003, Nakata and Goshima 2003) showing that larger crayfish are more likely to win interactions, a prior residence advantage (Figler *et al.* 1995, Peeke *et al.* 1995, 1998, Nakata and Goshima 2003) showing the advantage to the first crayfish to enter a shelter, and a sex advantage (Figler *et al.* 1995, 1999, Peeke *et al.* 1998) showing male dominance over females. Other influences, such as the effect of conspecific cues on behavior and ability to compete for shelters, have not been extensively studied and are therefore poorly understood.

Chemical cues released by predators have been shown to influence general behavior in aquatic invertebrates, including crustaceans (Rosen 2009), as well as more specifically influence shelter choice and competition (Boudreau 1993, Tierney *et al.* 2008). Conspecific chemical cues have been seen to affect other species of aquatic invertebrates including habitat choice in snails (Gerald and Lawrence 2005), reproduction rate and methods in daphnia (Lurling *et al.* 2003), and settlement choice in sea urchins (Nishizaki and Ackerman 2005). In crayfish, conspecific chemical signals have been shown to both strongly affect behavior and interactions between competitors directly and have some effect on neighboring crayfish without direct contact (Bergman and Moore 2005). To date, however, the effect of ambient cues produced by conspecific crayfish in higher density lakes on shelter competition has not been studied.

This study examined the effect of conspecific cue on mechanisms and result of shelter competition involving a female resident and male or female intruders. Furthermore, this study tested the relationship between the prior residence advantage and sex advantage in both normal conditions and in conditions of elevated crayfish chemical cue. I formed three hypotheses to be tested by this experiment: (1) that the addition of crayfish cue would increase the level of aggression shown, (2) that the addition of crayfish cue would increase the degree of competition for shelter, and (3) that male intruders would be more successful at obtaining shelter from female residents than would female intruders. To address these hypotheses, I conducted a series of shelter competition trials.

Materials and Methods

Crayfish were collected from Tenderfoot Lake (Gogebic County, MI) May through July 2010. All crayfish used were healthy and possessed intact appendages. Crayfish were housed in single sex, .22 m radius tanks filled with 15-30 L of water from Tenderfoot Lake. The tanks were aerated and changed out as necessary. They were fed Wardley® Shrimp Pellets every 3-4 days. All females used were non-gravid and all males were Form 2 (*i.e.* non-reproductive).

Trials were run in aquaria (25.4x50.8 cm) with an aquaria pebble substrate about 2 cm in depth. The aquaria were filled with either fresh lake water (control) or from lake water that was saturated with crayfish chemical cues (treatment). The cue water was prepared by placing 6-7 crayfish (mean size=25.614 cm, standard deviation=4.567 cm) in one of the holding tanks described containing fresh lake water. In designing this treatment, densities calculated by Capelli

(1975) were used as a starting point and were supplemented to increase the strength of the chemical cue. The water was conditioned in these tanks for four to five days before being used for trials.

In each aquarium was a single shelter formed by a tube 10cm long with a diameter of 5 cm made of polyvinylchloride (PVC) tubing with the interior roughened with a Dremel wood-cutting wheel (Blank and Figler 1996). The tube (interior and exterior) was darkened with dark green spray-paint (20018 camouflage green) and placed equidistant from both short sides of the aquaria along the right long side (Blank and Figler 1996). Roughly opposite the shelter on the other long side was an airstone to maintain sufficient levels of oxygen in the water.

For each trial, a female *O. propinquus* was placed in the tank and allowed to acclimate for at least 8 hours (referred to as the resident). Following acclimation, a conspecific female or Form 2 male (referred to as the intruder), size matched to within 10% of carapace length was placed in the tank and both crayfish's movements were observed for the first twenty minutes. General behaviors and the time at which they occurred were noted. Behaviors recorded include rearing, cornering, crossing, reverse walking, tail flipping, and use of chelae (Lundberg 2004, Drozdz *et al.* 2006, Mercier and May 2010). Additionally, duration of shelter occupancy was recorded for each crayfish. Following these observations, the crayfish were left in the tank with each other overnight (total trial duration following acclimation: 9-14 hours). The following morning, the crayfish's locations were noted.

The duration of occupancy was analyzed by a two-way ANOVA, comparing sex, and water type (control/high crayfish cue) to the amount of time each crayfish spent in the shelter over the course of the first twenty minutes of interaction. Two-way ANOVA's were also used to identify relationships between sex and water type to number of times rearing up on walls (used as an indication of activity) and number of aggressive contacts (characterized by use of chelae by one or more parties). Additionally, two-way ANOVA's were used to identify relationships between role (resident or intruder) and water type to number of times rearing up on walls and number of aggressive contacts. Finally, possible interactions between sex, role, water type, presence of aggression, and possession of shelter were interpreted through multiple Chi Square tests. All data analysis was conducted on SYSTAT 13 (2009).

Results

In general, this study suggests that there is decreased competition for shelter (as shown by total time sheltering) in high-cue water compared to the control and that the novelty of the tank is an influential component of crayfish behavior, specifically with regards to levels of activity. Resident crayfish were significantly less likely to be in the shelter after acclimation in the treatment than the control (χ^2_1=7.616, p=.006) (Figure 1). However, shelter possession after >10 hours of interaction was not significant (χ^2_1=.319, p=.572), even if one only considers resident possession (χ^2_1=.319, p=.572). Average time spent in shelter between resident and intruding crayfish was marginally non-significant (t_{104}=-1.703, p=.092), with residents having a slightly higher mean time in shelter than intruders. This is supported by a two-way ANOVA between crayfish role, presence of cue, and the number of crosses made by each crayfish (used as an indication of activity). Invaders made significantly more crosses than did residents ($F_{1.96}$= 6.977, p=.0096) (Figure 2) and there was a marginally non-significant difference in the number of crosses between water types. Crayfish made slightly less crosses in the control than the high-cue water ($F_{1.96}$= 3.101, p=.0814). A two-way ANOVA between role and cue with respect to the time spent in the shelter (by either resident or intruder) for each crayfish revealed a significant interaction between the two factors which complicates interpretation of the main effects, neither of which were significant ($p>.1$). Intruders crossed more frequently in high-cue water and residents crossed more in the control ($F_{1.102}$= 11.835, p=.0008) (Figure 3). Finally, when only looking at the intruders, a two-way ANOVA looking at sex and cue with respect to time spent in shelter revealed a marginally non-significant relationship between cue and time spent in shelter ($F_{1.49}$=3.295, p=.0756), with crayfish spending slightly more time in shelters in high-cue water than the control.

Discussion

Contrary to my predictions, I found that presence of cue is negatively correlated with crayfish's observable need for shelter. This was shown by a chi-squared analysis of the probability of finding a resident in a shelter following the acclimation period in either treatment or control water conditions. Interestingly, this trend does not extend to shelter possession in the morning when considering resident possession or overall possession, by either resident or intruding crayfish, of the shelter. This suggests a reduced need for shelter when exposed to only the cues of other crayfish, which is not continued in the presence of another crayfish. This could indicate that the crayfish interpret competition for shelter through direct interaction. Furthermore, the lack of desire for shelter in the presence of cue could be an indication that the crayfish are interpreting the cue as a sign of reduced predation. If crayfish numbers are high, there is a reduced risk of any one crayfish being consumed. This idea, known as the antipredator defense hypothesis, is especially well studied in birds (Lazarus 1972, Thiollay and Jullien 1998, Popp 1988), but as of yet has not been applied to crayfish. This could be tested in future experimentation by observing a crayfish in a tank with a shelter for a period of time and then adding conspecific cues and looking for a change in behavior.

Residents tended to spend slightly more time sheltering than the intruders. This could be justified by considering that the residents have already explored the tank during their acclimation time, while the intruder was introduced to a completely new habitat immediately before observation. This is consistent with the intruders making more crosses as well, as the crayfish would be expected to explore the entire tank. The significant interaction between cue and role when looking at time spent in shelter could be explained by this novelty effect on the intruder as well. Because the intruder is being introduced into water with cues of other crayfish, it would be expected for it to be more diligent in searching the tank to find them than it would if the water did not have the cues. Contrarily, the resident would make more crosses upon introduction of an intruder in the control because the new cues being released would be much more noticeable in otherwise clean water than they would in water already saturated with chemical cues.

Caution should be taken when interpreting these results, as there are some potentially influential factors that were not addressed in this study. Further experimentation is necessary to support or reject these findings. In the experimental tanks, there were several behaviors that could have confounding effects on the data produced. Included in these are crayfish making their own shelters (either under the pre-made shelter, or in the rock substrate – especially in corners), distractions produced by the aerator and the observer, and that crayfish were size-matched based on just carapace length. It has been shown that second to total length, chelae size is the determining factor in competitiveness (Tierney *et al.* 2008) and some trials had observable differences in chelae length between competitors. Fortunately, this should only have affected the mixed-sex trials, because chelae size is generally proportional to total length within the sexes (Nakata and Goshima 2003). These complications could be avoided by making the shelters more favorable to the crayfish being used by size matching crayfish with shelter size, as was done by

Nakata *et al.* (2001), using a less invasive means of aeration, more completely veiling the observer from the crayfish, and more detailed size matching which takes chelae size into effect in addition to total length.

Furthermore, there were some issues that were not considered when conditioning the water for the treatment. Some of the possible factors that were not considered when designing and obtaining high-cue water for trials include: the possibility of unforeseen influence on the specific cue composition in these tanks as a result of molting or fatality within the tank – the release of specific pheromones during molting or of alarm cues upon death could be present in the water of some of the trial tanks. Possible complications arising from how the crayfish were stored - the holding tanks used (in which the experimental crayfish were kept prior to trials) had much higher crayfish densities than the high-cue tanks, which could have influenced the magnitude or specifics of the reaction observed by the crayfish. And possible differences in concentration of cue throughout the water column in the tanks – because water was siphoned off to fill the trial aquaria there could have been variation in cue strength among trial tanks. These issues could be avoided in future experimentation by mixing the high-cue tanks before water extraction, using only water in which there has been no molting or fatalities, and either keeping the crayfish at more normal densities prior to trials, keeping the crayfish in isolation for a period of time prior to experimentation, or using only freshly caught crayfish.

Another aspect of the competition for shelter worthy of further exploration is the relationship of the different reproductive forms to a male intruder’s ability to displace the resident. Male crayfish alternate seasonally between reproductively active Form I and non-reproductive Form II. There are a number of morphological differences between the two forms; most relevant to this experiment is the presence of larger chelae in Form I crayfish compared to

Form II individuals (Hobbs and Lodge 2010). Tierney *et al.* (2008) found that there are also behavioral differences between reproductive forms of male crayfish, specifically regarding time spent sheltering. They found that Form II males spend significantly more time sheltering than Form I males (Tierney *et al.* 2008). With this prior knowledge, one would expect the competition to be greater for shelters with Form II males, as was used in this study. One possible confounding factor, however, is the increased aggression and receptivity to conspecific female cue in Form I organisms (Bovbjerg 1956, Tierney *et al.* 2008). Further experimentation could help detail the interaction of these influences and contribute to the understanding of shelter competition between residents and intruders as well.

The findings of this study can be applied to the natural system of crayfish shelter competition and the process by which invasive species are able to displace residents from their native territories. The reduced shelter use in the presence of high cue could be an indication of why some species are so quickly displaced by invasive species. In a high density of conspecifics, there would be less shelter use by the individuals of the species and therefore more open shelters for invasive species to claim. These invasive species, because they would not be exposed to conspecific cues, would experience a greater pressure for shelter than would the natives at high densities. My data also showed a strong preference for more activity in the intruder, likely due to the novelty of a new environment. The invasive species would also be spreading into new territory, which could suggest they would be more active than their resident counterparts. This would allow for faster movement and greater chances of finding shelter through exploration. With further research, these factors could be elucidated to better understand the mechanisms of the spread of invasive species.

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Figures

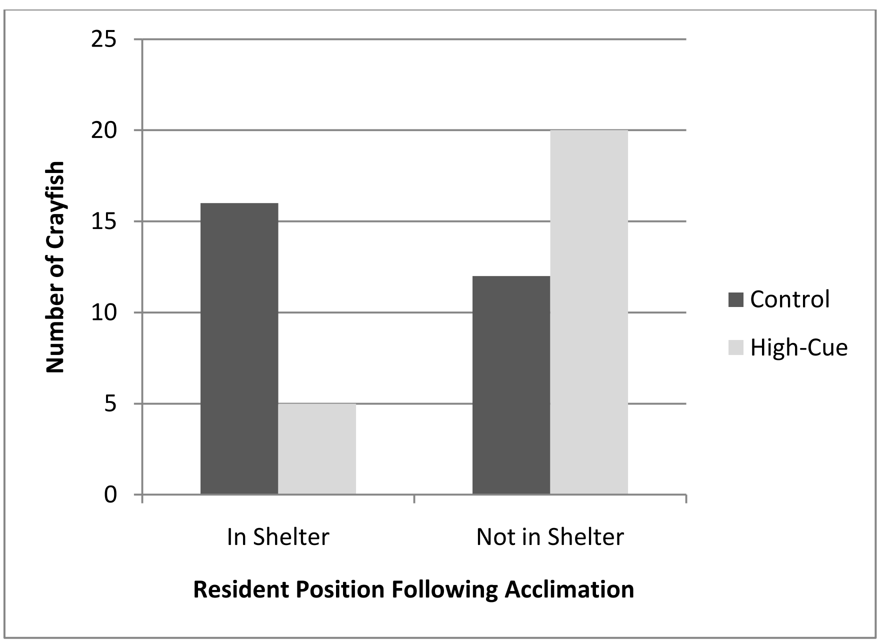

Figure 1.
Crayfish counts grouping by location of resident following acclimation period (>8 hours in tank alone) and by presence or absence of chemical cue in the water. Chi-square analysis revealed a significant relationship between cue and crayfish presence, with significantly less residents being inside the shelter when cue is present (χ^2_1=7.616, p=.006).

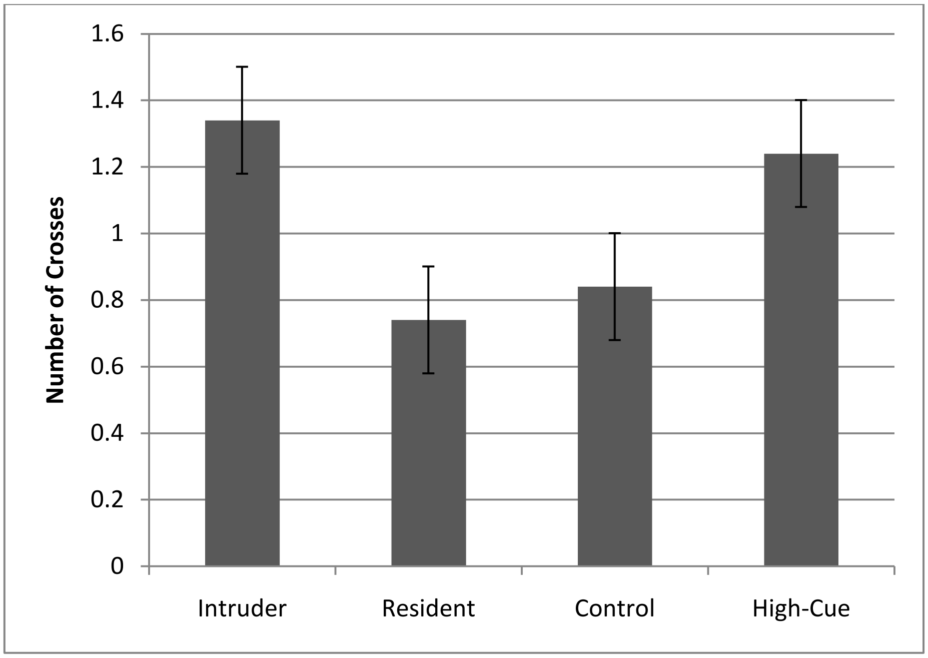

Figure 2.
An ANOVA revealed a significant difference between the number of crosses and the role of the crayfish in question. Intruders made significantly more crosses than residents ($F_{1.96}$= 6.977, p=.0096). This could be because Intruders are still adjusting to the new tank and are exploring more. The difference between water types was marginally non-significant ($F_{1.96}$= 3.101, p=.0814).

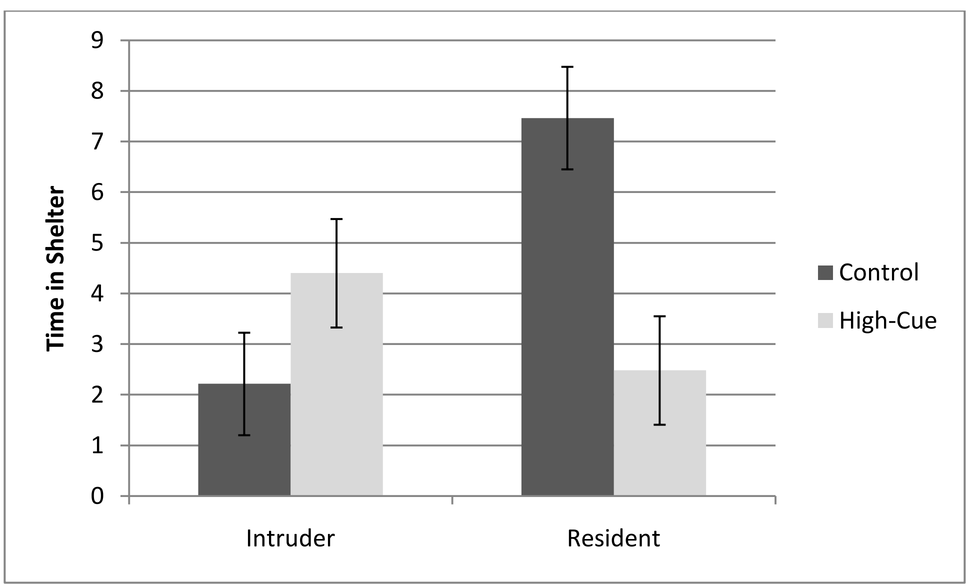

Figure 3.
Number of crosses performed by crayfish grouped by cue presence/absence and role of crayfish. There is a significant interaction between these two variables, with intruders sheltering more in high-cue and residents sheltering more in the control ($F_{1.102}$= 11.835, p=.00084). This can be explained through a combination of cue reception and tank novelty.