Abstract

Dominance hierarchy in regards to shelter preference was studied using the virile crayfish (Orconectes virilis). Dominance was determined in groups of six individuals, then placed in an enclosure with three treatments of shelter distribution—three to three, four to two, and five to one ratios were used. Data suggested that crayfish distribute themselves among shelters based upon the density of nearby shelters, rather than by the previously established dominance hierarchy. Within the five to one shelter distribution, crayfish generally assorted themselves within hierarchy with regards to shelter distance, with one exception. Individuals of epsilon dominance ranking selected shelters nearest to the alpha ranked crayfish. This behavior suggests that the volatile dominance position of lower-ranked crayfish may cause some individuals to seek safety and higher proportion of resources through submission and proximity to dominant crayfish.

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Introduction

Many organisms utilize aggressive or dominant behaviors in order to acquire and hold resources. In Northern Michigan, the virile crayfish, *Orconectes virilis*, demonstrates the outcomes of a strict social hierarchy. Crayfish individuals often compete in aggressive social contests, known as agonistic interactions, associated with winning resources and defense (King, 1973). These interactions shed light upon the methods which organisms use to obtain resources (Bergman & Moore, 2005). Intrinsic and extrinsic factors must be taken account when observing the crayfish behavior, as these elements may impact social aggression (Bovbjerg, 1956).

These agonistic interactions tend to be the most common way that individuals in a population establish their social hierarchy (Drews, 1993). Social dominance is distinguished by consistently out-competing other individuals in multiple social, aggressive interactions (Drews, 1993). The winner effect, an increase in probability of an individual’s chance to win based on its past history, also has an impact of whether or not a crayfish will be able to dominate in a fight (Hock & Huber, 2008; Bergman & Moore, 2005).

Physically larger crayfish are more likely to exhibit high aggression and corresponding dominance over their more passive counterparts. Studies have shown that the ability of a crayfish to dominate its opponent physically, along with past fighting experience, corresponds with agonistic displays (Guiasu and Dunham 1999). However, the attributes of a dominant male relative to his counterparts are but a portion of the factors influencing dominance behavior. Environmental concerns, such as the presence of shelter, food, and mates can directly contribute to dominant male conduct (Fero, et. al., 2006; Peeke et al., 2003).

Shelters hold high significance in social interaction among crayfish populations. As a territorial anchor, a crayfish’s chosen shelter demonstrates the value of its surrounding resources.
While a shelter may serve as a territorial marker, it also becomes a target for harassment and displays of dominance. Therefore, a dominant crayfish will spend less time within his chosen home than inferior individuals, instead attempting to demonstrate his authority by evicting others (Fero, et. al., 2006). In a conspicuous show of power, a socially superior crayfish will select a larger, more open shelter, while his subordinates will choose smaller, more easily defended locations (Martin & Moore, 2007). Little research has been done, however, on shelter preferences with other equal resources, despite the heavy significance crayfish place upon shelters.

The purpose of this experiment was to determine the shelter selection patterns of dominant male crayfishes in an enclosed environment. Close proximity to other individuals would give a dominant male many opportunities to assert his dominance. Therefore, we believe that if a large, dominant crayfish was placed within a tank of many individuals, he will attempt to gain a shelter and territory that allows him the maximum influence within the small enclosure. Secondary and tertiary individuals will divide territory in a similar manner, although being careful to avoid the domain of the prominent male.

**Methods**

**Location:** *Orconectes virilis* were collected from their natural habitat in Maple Bay located at Burt Lake in Cheboygan County, Michigan (45.486°N, 84.707°W) on 7/28/11 at 11:30 pm, 8/4/11 10:00 pm, and 8/10/12 at 12:00 am.

**Procedure:** Male individuals were dried and marked with a letter using a white out pen to distinguish between the individual organisms. When not used for testing, individuals were stored in plastic storage containers in the bottom of a large holding tank (Picture 3), keeping
them separate from one another. The length from the cervical groove to the end of the tail fan, the length of the carapace, was measured for each individual and recorded along with their letter.

The animals were initially assessed for their dominance by placing two crayfish in a 15.5x25.8x31.5 centimeter tank (10 gallons). These two animals were then allowed to battle each other until a display of dominance was shown or until 15 minutes were up. These displays of dominance were determined based on three actions: one individual chasing another, the dominant individual causing the second to tail-flip away from an encounter, or the subordinate submitting by bowing its head low to the ground (Moore 2007). Each organism fought against every other crayfish and the results were recorded. After every battle, the crayfish were placed back into their individual Tupperware containers for a fifteen minute resting period. If an individual won, two points were rewarded, if an individual lost, no points were given, and if there was no clear winner each crayfish received one point. The point system was utilized in order to determine overall dominance for each set of crayfish.

An artificial stream was built to study crayfish interactions. Water was pumped from the east branch of the Maple River into a rectangular pool constructed of cinder blocks, tarp, and a wooden board. Overall dimensions of enclosure were196x152x46centimeters, filled to a depth of 26 centimeters (Picture 1). The wooden board, used to maintain water depth, had holes with a diameter of 27 millimeters which allowed for a steady flow of water to escape the synthetic stream. Gravel was used as substrate. To prevent escape, enclosure was surrounded by a mesh screen. Shelters were made from PVC pipe attached to clear plastic, each with dimensions of 120x80 millimeters and were left open at both ends (Picture 2).

Trials took place among three different treatments. Shelters were split up into different densities around the makeshift stream, with five shelters on one side of the stream and one on the
opposite side. These shelters were placed as if they were along the edge of a circle in order to allow equal distances between each one. The diameter of this circle was one meter and the distances between each shelter was 10 centimeters (Picture 4). The same set up was done with treatments four and two shelters, as well as three and three (Pictures 5 & 6). Crayfishes were placed in the center of the test site and allowed to disperse themselves in the different shelters overnight. The next morning, between the time of 6:30AM and 8:30AM, the location of each individual and shelter was recorded from each side of the stream so that measurements were in the form of a Cartesian graph. This process was repeated for five different trials with a different set of six individuals each set.

**Statistical Analysis:** To investigate differences in shelter use and spacing as a function of size, we performed a two-way factorial MANOVA with the p-value set at 0.05. When significant was found, a Fisher-LSD post hoc test was done to determine where significant differences were located. In addition, a one-way ANOVA was conducted to determine if there were significant differences between carapace size and rank.
Results

The initial two-way factorial MANOVA test demonstrated no significance between the nearest neighbor distances, yet displayed significance for the distances between individuals and shelters (refer to Table 1).

Table 1: Results from the two-way factorial MANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>Error DF</th>
<th>P-Value</th>
<th>F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>134</td>
<td>0.014</td>
<td>3.532</td>
</tr>
<tr>
<td>Rank</td>
<td>10</td>
<td>134</td>
<td>0.605</td>
<td>0.826</td>
</tr>
<tr>
<td>Treatment*Rank</td>
<td>20</td>
<td>134</td>
<td>0.532</td>
<td>0.945</td>
</tr>
</tbody>
</table>

Table displays that treatment (shelter distribution) was the only significant factor in determining spread of crayfish for an assumed p-value of 0.05.

Our data showed a significant effect of rank on shelter distances (two-way MANOVA $F_{4,134,0.05} = 3.532$, $p < 0.05$). Fisher-LSD post hoc analysis of treatments showed that treatment 1 (5:1 shelter ratio) revealed a distinct trend in Alpha, Gamma, and Epsilon individuals (refer to Figure 1).

Data suggests an interesting trend in crayfish distribution. As expected, there was an observed increase in shelter distance in relation to decrease in rank, except for individuals of epsilon rank. These individuals had the second lowest average distance to shelter (Refer to Figure 2). Specific examination of crayfish distances to shelter within 5:1 shelter ratio set-up was then performed, once again using a two-way factorial MANOVA, demonstrating an overall p-value above threshold of $P=.05$. Average distance to shelter from lowest to highest rankings follows as such: Alpha, Epsilon, Beta, Gamma, Delta, Zeta.
Fisher-LSD post-hoc analysis of detailed 5:1 shelter ratio data show distinct crayfish preference for shelters based upon rank. Gamma and epsilon individuals sorted to the immediate vicinity of the Alpha, found in shelter 4.

Crayfish size data showed a trend that correlated with dominance hierarchy. Larger crayfish, on average, occupied more prominent positions within the population (refer to Figure 4). Data corresponds with findings from Moore (2007), suggesting that carapace size of crayfish is a key factor in agonistic interactions.

Treatments 2 and 3 (4:2 and 3:3 shelter ratios, respectively) showed no significance in trends for any dominance rank (refer to Figure 2). Detailed examination of nearest neighbor distances suggests no significant trend in assortment of individuals in relation to dominance (refer to Figure 1).

**Discussion**

Data demonstrates that shelter distribution, not proximity to other individuals, is essential to crayfish shelter selection. Study has demonstrated that shelters are a form of resource, akin to food or mates (Fero et al., 2006). Therefore, the trend seen in data is representative of the way in which crayfish of different hierarchy position assort resources amongst themselves.

Crayfish distribution of resources was most pronounced within treatment one, the 5:1 arrangement of shelters. The vast disparity in shelter concentration between clusters in this treatment brought out the most apparent trends in assortment. Data regarding this trial suggests that only crayfish ranked Alpha, Gamma, or Epsilon assorted themselves in a constant, statistically significant manner.
Figure 3 suggests that as dominance hierarchy position decreases, so do shelter distances. The Alpha chooses the shelter closest to all others, concentrating the amount of resources he receives. This corresponds to shelter 4 (Figure 4). Beta male takes a shelter with a slightly larger average distance to other shelters, and so the effect continues, until crayfish Zeta is the farthest removed from all available resources. Crayfish Epsilon, however, most frequently chooses shelter within a dense group, going against the general trend of data.

Crayfish Epsilon’s unique behavior, including the behavior of Gamma, may likely be attributed to his rank and the relative size of other individuals around him. Moore (2007) suggests that crayfish size is an essential factor in determining dominance and fight success rate in crayfish. Because our trial utilized fighting as a recognizable form of dominance, the relative size of individuals contributes much to the overall dominance hierarchy determined before placing crayfish within the enclosure. Figure 5 indicates that crayfish form 4 groupings of sizes, placing the statistically significant crayfishes Gamma and Epsilon in highly volatile positions—each is liable to shift up or down. Their average size during our trials was very similar to other individuals, making them part of large size classes whose dominance rankings shift frequently. Study has shown that similar sized individuals often change dominance over time (Graham & Herberholz 2009).

Detailed analysis of treatment one shows that crayfishes Gamma, Alpha, and Epsilon preferentially select shelters 3, 4, and 5, respectively. The size class of Alpha places him in a position of dominance that is unlikely to be challenged by these neighbors. Thus, we theorize that Gamma and Epsilon individuals are demonstrating a behavior of submission to alpha, in exchange for safety from their highly volatile dominance hierarchy.
By placing themselves near to Alpha, Gamma and Epsilon individuals retain a close distance to nearby resources (Figure 3). Crayfish Alpha, who is unlikely to be confronted, provides protection from challengers to Gamma and Epsilon’s dominance positions. This behavioral strategy is somewhat conservative, in that Gamma and Epsilon individuals relinquish other possible resources in exchange for safety.

This behavior, provided that shelters are resources equivalent to food, mates, etc., could be applied to a broad range of interactions. If this close association with dominant crayfish occurs broadly over populations of similarly sized individuals, it could be responsible for the formation of pods or groups in native habitat of *O. virilis*. Individuals in volatile positions could cement their rank by submission and close association to a dominant crayfish, and receive priority food or mate choice over individuals likely to challenge them. Long-term trends of this behavior could give such individuals an advantage in fitness.

Further study needs to be done regarding different groupings of shelters and other possible strategies taken by crayfish in volatile positions. Would the same interaction between crayfish occur when resources are abundant? If the added rank safety of submission outweighs the risk of frequent agonistic interactions, crayfish should display the same behavior in the wild. Observation of natural movement and shelter preference tendencies is necessary. Also, examinations of other possible strategies could prove useful. Perhaps a counterpart to the conservative strategy exists, in which crayfish attempt to utilize the freedom of isolated inhabitation. This radical, high-risk, high-reward behavior could generate highly fit crayfish in a shorter period of time than the conservative behavior, provided that ample resources are available outside of the closely associated population.
Appendix

Picture 1: The complete set up with the mesh covering in place.

Picture 2: An example of one of six shelters made.
Picture 3: The holding tank where the crayfish were placed when not being tested.

Picture 4: The shelters set up in the first treatment; as a five to one ratio.
Picture 5: The shelters set up for treatment two; a ratio of four to two.

Picture 6: Treatment three; shelters in a three to three ratio.
Figure 1: Nearest neighbor distances display no significant distance between crayfish individuals.

Figure 2: Nearest shelter distances demonstrate significant differences between treatment one (5:1 shelter ratio).
Figure 3: Average distance from the shelters and crayfish ranking. This graph illustrates a general increase in distance as position decreases, except for the epsilon (5th) crayfish which had an average distance similar to the alpha crayfish (1st).

Figure 4: Average distances of crayfish individuals to each shelter. The alpha male typically was found in the fourth shelter, next to the epsilon in the fifth.
Figure 5: Average sizes of crayfish carapace length and dominance hierarchy. The alpha male was consistently larger and different than the rest of the individuals, while the epsilon organism was similar in size to the gamma, delta, and zeta organisms.


