The influence of reproductive state on the agonistic interactions between male and female crayfish

(Orconectes rusticus)

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Summary
Research analyzing social dynamics has shown that systems can be structured by individuals of different age, size, sex and reproductive state within a population. Extensive research on male–male interactions in crayfish has illustrated the mechanisms that underlie the agonism and social relationships that develop between conspecifics. Despite the wealth of knowledge on male–male interactions, there is a lack of knowledge on the role that reproductive state plays in modulating aggression between the sexes in crayfish. The purpose of this study was to investigate aggression between crayfish of different sexes and reproductive forms. Form I and II male crayfish were paired against glair and non-glair female crayfish in agonistic bouts. Fight outcomes, intensities and mating events were calculated from the paired interactions. Form I male crayfish won significantly more bouts with females than form II males. In addition, reproductive females won more bouts than non-reproductive males. Thus, reproductive state of the individual has a significant influence over the outcome and level of aggression within agonistic encounters. This finding may indicate that the individuals are approaching interactions with motivational states (reproductive needs versus resource acquisition).

Keywords: agonism, crayfish, sex, mating.

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Introduction

Aggression is an important behavioural mechanism involved in structuring social dynamics among many animal populations (Francis, 1988). Animals act aggressively toward one another when engaged in competitive interactions in the presence of resources such as shelter, food, and mates (Wilson, 1975). Aggressive interactions for resources between individuals of the same species are often termed agonistic interactions in order to differentiate these interactions from predator–prey interactions (Scott & Fredericson, 1951). Agonistic encounters consist of physical and non-physical interactions that vary in intensity and outcome between individuals.

Several different characteristics of organisms can influence the intensity and outcome of agonistic interactions. Factors such as age (elephants, *Loxodonta africana*: Archie et al., 2006), size (ants, *Leptothorax gredleri*: Heinze & Oberstadt, 1999; brown trout, *Salmo trutta*: Johnsson et al., 1999; elephants: Archie et al., 2006), sex (dippers, *Cinclus cinclus*: Bryant & Newton, 1996; capuchins, *Cebus capucinus*: Perry, 1998), social status (crayfish, *Orconectes rusticus*: Bergman et al., 2003) and reproductive state (crayfish: Stein, 1976; lemurs, *Lemur catta*: Cavigelli & Pereira, 2000) can alter the probability that a certain organism will win an encounter and gain access to valuable resources. As a result of these agonistic interactions, many animals develop social hierarchies or dominance relationships which may alter future access to important resources such as mates, food, or shelters (Cloutier et al., 1995; Daws et al., 2002; Bergman et al., 2003; Archie et al., 2006).

The intensity of interactions can increase significantly when the resource in contention is accessed by a reproductively-active individual (Cavigelli & Pereira, 2000). Reproductive receptivity can be episodic in nature, as in mammals, which can lead to differences in aggressive behaviour during reproductive and non-reproductive periods (Creel et al., 1996). Physiological changes during the mating season, i.e., hormonal shifts, are considered the underlying mechanism which, in turn, leads to higher levels of aggression (Turner & Iverson, 1973; Cavigelli & Pereira, 2000). A number of animals have seasonal changes in their reproductive state which can cause fluctuations in both the number and intensity of agonistic bouts (crayfish, *Orconectes propinquis*: Stein, 1976; wild dogs, *Lycaon pictus*: Creel et al., 1996; lemurs: Cavigelli & Pereira, 2000). Crustaceans are one group of animals that exhibit seasonal changes in reproductive states (Stein, 1976).
In Cambaridae, male and female crayfish have different reproductive forms, which are seasonally dependent (Crocker & Barr, 1968; Stein, 1976; Muck et al., 2002; Stebbing et al., 2003). These reproductive changes are cyclical in that both males and females molt between reproductive and non-reproductive form more than once. Given the cyclical nature of the reproductive form in both sexes and subtle differences in this timing, there exists an overlap between the reproductive morphotypes in nature (Holdich, 2002).

Adult male crayfish molt between a reproductive form (form I) and a non-reproductive form (form II). Male reproductive (form I) crayfish have larger chelae and have been shown to be behaviourally more aggressive than non-reproductive (form II) males (Crocker & Barr, 1968; Stein, 1976; Garvey & Stein, 1993; Guiasu & Dunham, 1997, 1998). Males with larger chelae have also been shown to increase their likelihood of winning agonistic encounters (Stein, 1976; Garvey & Stein, 1993). Adult female crayfish have been shown to switch between a reproductive (glair) and a non-reproductive (non-glair) form (Wetzel, 2002). Females have also been shown to increase both the intensity of interactions and the likelihood to win encounters when carrying eggs and offspring (Figler et al., 1995, 2001, 2005). Given the overlap between these reproductive forms and differences in levels of aggression, it is possible that changes in aggression and/or dominance associated with reproductive form could have a significant impact on resource acquisition and holding and, in particular, mate recognition, acquisition and copulation.

To date, research focusing on male–female interactions has illustrated how form I males respond to glair females (Ameyaw-Akumfi & Hazlett, 1975; Hazlett, 1985; Dunham & Oh, 1992). In general, male crayfish may become submissive in the presence of female odours (Hazlett, 1985; Dunham & Oh, 1992) and this appears to be a prelude to mating behaviour (Belanger & Moore, 2006). There is a gap in research on the agonistic interactions between male crayfish and female crayfish in different reproductive states (Stein, 1976). Differences in aggression and agonism may stem from differences in resource needs. Crayfish in the reproductive form (either male or female) may be using aggression in connection with mate choice or mate acquisition, whereas non-reproductive crayfish may be using aggression to acquire other resources, within the context of social hierarchies. In order to understand the social dynamics that exist within a mixed-reproductively active population, the role of aggression and agonism among all crayfish in a population needs to be studied. The research in this paper outlines the
analysis of aggression and agonism between crayfish of differing sexes and differing reproductive state. We predict that agonistic interactions between size matched crayfish should depend upon the reproductive state of the individuals involved and that individuals in a reproductive state will be more aggressive than those in a non-reproductive state.

Materials and methods

Animals

Crayfish (Orconectes rusticus) were collected from two field sites and housed in two different laboratories. The first group contained male and female crayfish collected from Maple Bay in Burt Lake, MI, USA (lat. 45°28’N, long. 84°40’W) from June–August 2005. All crayfish were physically and socially isolated in flow-through tanks supplied with fresh lake water from Douglas Lake. These crayfish were housed at the University of Michigan Biological Station (UMBS) in semi-natural conditions. All crayfish were housed under natural light and temperature fluctuations (approx. 14:10 light/dark cycle and 20–22°C lake water). Crayfish were fed a diet that consisted of detrital material introduced from the circulating lake water. The second group contained male and female crayfish collected from the Portage River, Wood County, OH, USA (lat. 41°37’N, long. 83°65’W) from August–October 2005. All crayfish were physically and socially isolated in flow-through tanks in an environmental chamber at Bowling Green State University (BGSU) with regulated temperature (21°C) and light/dark cycle (14:10). Crayfish diet consisted of one commercial rabbit pellet three times per week.

For both populations, crayfish were physically and socially isolated for a minimum of one week. This was done in order to eliminate all effects from previous social interactions (lobster: Karavanich & Atema, 1998; crayfish: Zulandt Schneider et al., 2001). Crayfish were fought against individuals from the same site of origin (UMBS or BGSU) to negate any differences due to place of origin that might have been present. The two collection sites are approximately 480 km apart. Burt Lake (UMBS site) is almost directly north from the Portage River (BGSU site). Description of the distribution of these two groups within the treatments and statistical analysis of the behaviour exhibited by these two groups is given below.
Reproductive morphology

*Orconectus rusticus* has both reproductive and non-reproductive forms. Male and female reproductive forms were identified based on external morphology. Reproductive males (form I) were identified by their defined ischial hooks, used in grasping the female during copulation, and sharp pointed white copulatory stylets, used in spermatophore delivery. Form I males are present during late summer to spring months (Crocker & Barr, 1968; Hobbs, 1974; Berrill & Arsenault, 1982). Non reproductive (form II) males appear morphologically different than form I males by having reduced ischial hooks and shorter cornified yellow stylets. Form II males were found during the summer months when mating does not typically occur, although there are significant periods of overlap between the two forms (Berrill & Arsenault, 1984).

Female crayfish that are reproductively (G) active contain glair glands that become visible on the underside of their abdomen. Glair is thought to be used for egg attachment to the female’s abdomen during excretion. The glair binds the eggs to the female’s swimmerets for protection (Holdich, 2002). Females have been shown to become sexually active during September to October (Berrill & Arsenault, 1984). Non-reproductive (N) females that do not have visible glair glands are present typically during May to September (Holdich, 2002).

Experimental design

Four different experimental comparisons were designed to investigate differences in agonistic behaviour between male–female interactions with respect to their reproductive forms. This setup follows that used by Simon & Moore (2007). Each of the following setups will be labelled as experiments A, B, C and D ($N =$ number of trials used per behavioural analysis):

- **A**: II + N, non-reproductive (form II) male paired with non-reproductive (Non-glair) female; $N = 10$
- **B**: I + N, reproductive (form I) male paired with non-reproductive (Non-glair) female; $N = 9$
- **C**: II + G, non-reproductive (form II) male paired with reproductive (Glair) female; $N = 12$
- **D**: I + G, reproductive (form I) male paired with reproductive (Glair) female; $N = 17$. 
Figure 1. Average carapace length (a), chelae length (b) and weight (c) of male (open bars) and females (black bars) in the different reproductive pairings with reproductive (G) and non-reproductive females (N) against reproductive (I) and non-reproductive (II) male crayfish. Asterisks indicate significant differences using a two-way ANOVA ($p < 0.05$).

Animals used in each trial were size matched to within a 10% difference in carapace length and, when possible, chelae length. All interactions that involved non-reproductive males (II + G and II + N) consisted of males and females with a similar chelae size that on average varied by $9 \pm 1.3\%$. Interactions with reproductive males exhibited a much larger chelae size as compared to females $21 \pm 1.4\%$ (Figure 1). Crayfish were run only once during this experiment. Dyad trials where crayfish did not engage in interactions with conspecifics were not included in analyses ($N = 25$). Therefore, a total of 96 crayfish were used in 48 dyads.

A separate statistical analysis was run comparing agonistic behaviour exhibited by males and females from both origins (UMBS and BGSU). There were no statistically significant differences between agonistic behaviours related to origin of collection. (ANOVA male: $N = 39, F = 2.151, p =$
Male–female agonism

0.1125; female: $N = 39$, $F = 1.068$, $p = 0.3760$). The I + N group contained only crayfish from UMBS, these animals ($N = 9$) were excluded from this analysis changing the overall $N$ from 48 to 39. Thus, crayfish from BGSU and UMBS were grouped for final statistical analysis regardless of origin (Simon & Moore, 2007).

**Fight arena and set-up**

A glass aquarium ($23 \times 24 \times 23$ cm) was used to run fight trials. A stationary camera (Sony DCR-TRV900) was positioned underneath the aquarium to provide a close analysis of all aggressive and mating behaviours. The tank was divided in half with a black removable divider to allow the crayfish to acclimate to the environment before encountering the conspecific. Animals acclimated for 20 min prior to the start of a trial. The naïve crayfish were isolated with the divider to prevent mechanical, visual, and chemical interaction before the start of each trial. Recordings of each trial began once the divider was removed.

The aquarium was emptied, rinsed, and refilled with fresh water before each trial to eliminate residual chemical odors from previous fights. Crayfish were marked with correction fluid on the bottom of their chelae to aid in the identification of males and females of each trial for further analysis. Correction fluid (Liquid Paper®) has not been shown to alter behavioural interactions in previous experiments (Bergman et al., 2003).

**Behavioural analysis**

Social behaviour was analyzed and quantified using an ethogram (Table 1) to determine the outcome of each agonistic bout every second until the loser

<table>
<thead>
<tr>
<th>Intensity level</th>
<th>Behaviour</th>
</tr>
</thead>
<tbody>
<tr>
<td>−2</td>
<td>Tail-flip away from opponent or a fast retreat</td>
</tr>
<tr>
<td>−1</td>
<td>Retreat — slowly back away from opponent</td>
</tr>
<tr>
<td>0</td>
<td>No response or threat display</td>
</tr>
<tr>
<td>1</td>
<td>Approach without a threat display</td>
</tr>
<tr>
<td>2</td>
<td>Approach with threat display — antennal whip, meral spread, or raised posture</td>
</tr>
<tr>
<td>3</td>
<td>Initial claw use — boxing, pushing, or touching with closed claws</td>
</tr>
<tr>
<td>4</td>
<td>Active claw use — grab opponent with claws or hold other crayfish with claw</td>
</tr>
</tbody>
</table>
crayfish retreated, defined by tail flipping or walking away from the opponent. A blind analysis was performed by an unbiased researcher using the ethogram to distinguish outcomes of interactions between agonism and mating behaviour. A two-way factorial ANOVA was used to analyze size differences in chelae, carapace and weight of combatants. The $2 \times 4$ design included factors for the sex of the combatant and the reproductive status (I + G, I + N, II + G and II + N). Since the two-way factorial ANOVA had an overall significant difference, a post-hoc analysis using a Tukey-HSD test was used. The intensity, duration, and winner of agonistic bouts between each experiment were analyzed to quantify changes in aggressive behaviour. A one-way MANOVA with a Tukey-HSD post hoc test was used to examine significant differences of time to intensities 2 and 3, and the total duration of agonistic bouts. Mating was defined to occur when the male flipped a female over and grasped and held both chelae over the head of the female. Mating events, winners of encounters, and larger chelae winners were analyzed using Chi-square for overall differences and Tukey Multiple Comparisons post hoc test for specific differences between experimental groups with $q_{\infty 0.05 4} = 3.633$ (Zar, 1999). For analysis, the I + G treatment group was split into those trials in which mating was observed, I + G (M) ($N = 8$), and those when it was not observed, I + G (N) ($N = 9$). Significant results are represented by ($q$) being greater than $q_{\infty 0.05 4} < 3.633$ from the multiple comparisons test, which is equivalent to $p < 0.05$.

Results

Analysis of size differences

The overall ANOVA analysis showed significant differences in the three dependent size measures as a result of reproductive condition ($F_{9,199} = 8.87, p < 0.001$), sex of the combatant ($F_{3,82} = 46.98, p < 0.001$), and an interaction between reproductive condition and sex ($F_{9,199} = 5.86, p < 0.001$). Given the significant differences from the overall general model, a planned comparisons post-hoc analysis (Tukey-HSD) was performed to examine significant differences in any of the size measures between paired combatants (Figure 1). The only significant differences that appeared with the planned comparisons were found with reproductive males paired with
Male–female agonism

either reproductive or non-reproductive females. In both conditions, reproductive males had significantly larger chelae than females in either condition ($p < 0.02$: reproductive male vs. reproductive female and $p < 0.03$: reproductive male vs. non-reproductive female).

Overall win/loss

The reproductive status of the individual crayfish altered its ability to win agonistic encounters ($\chi^2_{0.05,3} = 8.77$, $p < 0.05$, Figure 2). Reproductive crayfish paired with non-reproductive combatants exhibited differential fight success ($q_{\infty 0.05,4} > 3.633$, Figure 2). Reproductive male crayfish won agonistic encounters against females of either reproductive state (Figure 2) and reproductive females won significantly more fights against non-reproductive males than they did against reproductive males (Figure 2). Reproductive females that engaged non-reproductive males were equally likely to win a bout (Figure 2). These results suggest differential fight success based on the reproductive form of the mixed-sex conspecifics.

Figure 2. The proportion of wins of reproductive (G) and non-reproductive females (N) against reproductive (I) and non-reproductive (II) male crayfish. Different letters (a), (b) and (c) indicate a significant difference between treatments using a Tukey multiple proportions test ($N = 48; p < 0.05$).
The proportion of wins by the crayfish with the larger chelae in the dyad. in the pairing of reproductive (G) and non-reproductive females (N) against reproductive (I) and non-reproductive (II) male crayfish. Different letters (a) and (b) indicate a significant difference between treatments using a Tukey multiple proportions test ($N = 48; p < 0.05$).

Win/loss in relation to the larger chelae size

The crayfish with the larger chelae won a higher proportion of fights irrespective of the reproductive status of the crayfish involved in the encounter ($\chi^2_{0.05,3} = 8.9, p < 0.05$, Figure 3). This overall significant difference is driven from the results from larger chelae winners in the I + G grouping (15 out of the 16 fights were won by the larger chelae crayfish) and the I + N grouping (8 out of the 9 fights were won by the larger chelae crayfish). These two groups (I + G and I + N) were not significantly different from each other ($q_{0.05,4} > 1.32$), but were significantly different from the II + G and II + N groups ($q_{0.05,4} > 6.19$ (I + G vs. II + G), 6.99 (I + G vs. II + N), 4.14 (I + N vs. II + G) and 4.96 (I + N vs. II + N)). Crayfish with the larger chelae did not win significantly more fights in the II + G and II + N groups.

Dyads with mating events

Mating events occurred when both crayfish were in reproductive form ($q_{0.05,4} > 3.633$). No mating events were recorded between any of the
other three Experiments (A, B or C) that all consisted of only one or no reproductive animals. This data suggests that mating interactions only occur when both individuals are in reproductive form.

**Duration of encounters and time to intensities**

The progression of interactions, as indicated by the times to intensity levels 2 and 3, and the overall duration, differed significantly among pairing types (overall MANOVA, $F_{12,109} = 3.018$, $p < 0.001$, Figure 4). The time to intensity level 2 illustrates a significant difference between experiment D (M) and experiments A, B and C ($p < 0.05$, Figure 4a). The time to reach

![Figure 4](image-url)

**Figure 4.** (a) The average duration ± SE (in s) to reach intensity level 2 during agonistic encounters. I and II represent reproductive and non-reproductive males, and G and N represent reproductive and non-reproductive females. I + G-M represent those pairings, in which both crayfish were reproductive, where a mating event took place. Different letters (a) and (b) indicate a significant difference between treatments using a one-way MANOVA followed by a Tukey-HSD post-hoc test ($II + N, N = 10$; $I + N, 9$; $I + G, N = 9$; $II + G, N = 11$; $I + G-M, N = 8$; $p < 0.05$). (b) The average duration ± SE (in s) that it took to reach intensity level 3 during agonistic encounters ($II + N, N = 10$; $I + N, N = 9$; $I + G, N = 9$; $II + G, N = 11$; $I + G-M, N = 8$; $p < 0.05$). (c) The average duration ± SE (in s) of agonistic encounters ($II + N, N = 10$; $I + N, N = 9$; $I + G, N = 9$; $II + G, N = 11$; $I + G-M, N = 8$; $p < 0.05$).
intensity level 3 of experiment D (M) was significantly longer than all the other experiments \((p < 0.05, \text{ Figure 4b})\). Reproductive males and females took longer to escalate encounters to the third intensity level than did non-reproductive males and females. Experiment D (M) featuring both reproductive crayfish resulted in the longest lasting encounters \((p < 0.05, \text{ Figure 4c})\). The average duration of bouts in experiment D was significantly longer than experiment C.

**Discussion**

Our results show that sex and reproductive state influenced the level of aggression and the outcome of agonistic encounters. Form I males were shown to be more likely to win encounters than all female conspecifics regardless of reproductive state, but females increased their likelihood of winning an encounter when they were in reproductive form and males were in non-reproductive form. Mating events were only observed when both crayfish were in reproductive form indicating that the potential outcome of social interactions also changes with reproductive state. Encounters between reproductive crayfish that eventually mated were slower to escalate when compared to fights between non-reproductive pairs or mixed-reproductive pairs. Furthermore, encounters between reproductive male and female crayfish in which mating occurred were longer in duration than all other pairings. Surprisingly, agonistic encounters between reproductive pairs, which did not end in mating, showed no significant differences in rates of escalation or duration. These results suggest reproductive state alters the outcome of agonistic interactions and that certain fights (those that end in mating) have altered temporal dynamics (duration and rate of escalation).

Within natural populations, crayfish engage in social interactions with individuals consisting of differential age, sex, size and reproductive state (Fero et al., 2007). These social interactions consist of purely agonistic behaviour over resources such as shelters, food, or social behaviour that could lead to mating. In crayfish, age and size are correlated, which suggests that older crayfish are larger than younger individuals (Belchier et al., 1998; Holdich, 2002). Both size and age have been shown to increase the likelihood of a crayfish winning an encounter (Bergman et al., 2003; Bergman & Moore, 2003). Sex and reproductive state (female crayfish with eggs) have also been
shown to be factors that influence social dynamics. Maternal crayfish have been shown to exhibit both an increased fight success and increased intensity of fights over shelters than non-maternal crayfish (Figler et al., 1995, 2001, 2005). Form I males typically have a larger chelae to body ratio and exhibit higher levels of aggression as compared to form II males. The outcome of interactions between males of different reproductive forms shows that form I males are dominant over form II males (Garvey & Stein, 1993; Guiasu & Dunham, 1998). The results in this study have shown that the reproductive state of female crayfish can also increase fight success over non-reproductive males. Thus, it appears that generally reproductive crayfish have an increased level of aggression and fight success over non-reproductive individuals. We propose three possible mechanisms to explain these results. First, the outcome of interactions can be influenced by the differences in chelae size between conspecifics. Second, the difference in fight dynamics and outcomes may be due to changes in the neurohormonal levels that influence aggression. Third, it is possible that the underlying motivation for engaging in agonistic encounters changes from resource (social status, shelters or food) acquisition to a fight based on mate acquisition. It is important to note that these mechanisms are not mutually exclusive and could be working synergistically.

In crustaceans chelae size has been studied extensively as a factor that influences the outcome of social interactions (Rutherford et al., 1995; Sneddon et al., 2000). In I + G and I + N fights the crayfish with the larger chelae won significantly more fights (Figure 3). In these fights the average chelae size difference was 21%. However, in interactions II + G and II + N the average chelae size difference between conspecifics was less than 10%, and larger chelae was no longer predictive of fight outcome (Figure 3). The role of chelae size in determining the outcome of social interactions between males and females appears to change with reproductive status.

Social status has been shown to alter fights, where dominant male crayfish and females bearing eggs are more likely to win encounters with conspecifics (Bergman et al., 2003). These examples illustrate how an individual’s motivation (food, shelter, eggs and status) can influence fight outcomes. A change in an individual’s motivation can be a result of physiological changes or differences within an animal. Aggression can be altered by neurohormonal differences, which have been found between crayfish that vary in reproductive state and social status. The regulations of the neuromodulator serotonin and
other agonists have been shown to alter aggressive behaviour in male crayfish (Huber & Delago, 1998; Tierney & Mangiamele, 2001). Reproductive females exhibit neurohormonal differences that could be altering their aggressive behaviour (Fingerman, 1995; Sagi et al., 1997).

Social interactions may also be changing, during an interaction, from agonistic behaviour to mate recognition. Crayfish can communicate sex and reproductive status during social interactions (Ameyaw-Akumfi & Hazlett, 1975; Zulandt-Schneider et al., 2001). If crayfish communicate differently based on dominance status and/or reproductive status, then the agonistic interaction could change from an interaction based on aggression to an interaction for mate selection. In our study, males and females exhibited mate recognition during social interactions only when both males and females were in the reproductive state. Mating attempts were never recorded in any other pairings of individuals. Therefore, it is possible that chemical signaling exhibited during interactions with reproductive pairs is physically or chemically altered as compared to non-reproductive pairings (Stebbing et al., 2003; Belanger & Moore, 2006). Villanelli & Gherardi (1998) showed that form I males respond differently to reproductive and non-reproductive female odours. Males and females of both reproductive and non-reproductive status engage in agonistic bouts over resources such as shelters (Holdich, 2002), but it is possible that agonistic bouts over resources are different than bouts over mating opportunities. Crayfish mate recognition is difficult to distinguish from agonism, because mating is normally recognized when males aggressively subdue a female mate by flipping and pinning down her chelae (Villanelli & Gherardi, 1998).

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