

# Role of Sensory Signals in Maintaining Dominance Hierarchies in Crayfish

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**Abstract:** Animals often change their behavior in response to changing environmental conditions; this is evident in crayfish, who establish social hierarchies in their communities. These hierarchies are formed through a series of agonistic bouts, and provide stability in communal environments through controlled access to resources, and preferential mating. However, these hierarchies are also easily disrupted, resulting in a reversal of the roles of dominants and subordinates. These disruptions can be caused by a variety of factors, including molting, access to food, and individual aggression. We tested the hypothesis that sensory signals, such as vision and olfaction, can alter levels of aggression in juvenile *Procambarus clarkii* and *Orconectes rusticus*. Two size-matched crayfish were isolated for one week before an initial agonistic bout. During the next week, they were re-isolated with dividers that allowed for visual and/or olfactory contact, before a final agonistic bout. Changes in aggression levels between the two fights were calculated through a dominance index. Crayfish that were separated by a divider that allowed for olfaction but not visual contact displayed a significant increase in aggression during their second agonistic bout; crayfish that were separated by solid, opaque dividers, and dividers that allowed for visual and olfactory contact displayed a modest increase in aggression during their second fight. The results of these experiments suggest that sensory signals in the environment play a role in modulating crayfish aggression and hierarchy stability. The opaque, olfaction divider thus provides a mechanism for naturally heightened aggression levels, opening the possibility for targeted drug treatments.

## Introduction

Crayfish are highly aggressive crustaceans that tend to form social hierarchies when living in communities. These hierarchies are formed through aggressive dyadic interactions between two naïve animals, who don't have an existing relationship. However, if two acquainted animals are isolated for a long enough period of time, they will forget the hierarchy between them, and the only way to re-establish this hierarchy is through another set of aggressive interactions. It is presumed for there to be little changes in levels of aggression, or in the outcome of a second agonistic encounter, unless molting or a significant environmental change has occurred.

However, reversals and variable aggression do occur between crayfish that have an existing hierarchy. The purpose of this study is to elucidate specific environmental sensory cues that might lend to variable levels in aggression. Crayfish interact with each through physical contact with their chelae, often used in agonistic encounters to assert dominance, and through urine secretion, a method of communicating social status. It is believed that these two mechanisms allow for the maintenance of the established social hierarchy. Thus, this study attempts to study the effect of the disruption of these mechanisms on the aggression of crayfish in their re-establishment of a hierarchy.

It was hypothesized that since any form of communication between the two animals would help reinforce the existing hierarchy, an opaque divider preventing all forms of contact would yield the greatest increase in aggression. To determine the role of sensory mechanisms on the aggression in crayfish, dividers that allowed for visual and/or olfactory contact were used. It was additionally hypothesized that a divider allowing for both visual and olfactory contact would yield the least increase in aggression. The dividers that allowed for olfaction and visual contact might allow the hierarchy to persist, lowering the aggression in latter fights due to an acceptable existing social structure.

The dividers that allowed for olfaction contained holes, permitting the antennules of crayfish to pass through, but nothing wider. This ensured that no harmful physical contact could occur between the separated animals. Antennules serve as a means of communication between crayfish, allowing them to sense the presence of others. This, coupled with secreted pheromones, including urine, and other chemical signals, would allow for prolonged contact between two

crayfish. Although vision in invertebrates is comparatively weak, it has been shown to play a role in female mate choice of dominants, and could serve to preserve a hierarchy.

Thus, to gain a comprehensive understanding of the role of different sensory cues in influencing crayfish aggression and hierarchy preservation, different dividers between fights must be used that allow for communication via specific sensory signals. The relative importance of these signals in maintaining dominance hierarchies in crayfish can then be determined based on their effect on crayfish aggression and social status.

## **Background**

Relative levels of aggression in crayfish are controlled by a variety of environmental, chemical, social, and neural cues. They act in different of ways, from altering social interactions, to changing postures and locomotion in crayfish. However, the most significant factor in predicting the outcome of agonistic encounters in invertebrates is past experience, controlled through winner and loser effects (Kasumovic et al. 2010). Winner and loser effects dictate the outcome of future interactions, through a record of past interactions. Animals who have displayed success in maintaining dominance and have shown high levels of aggression in prior encounters are likely to continue doing so in future encounters; similarly, animals who have displayed submission and reclusiveness in past encounters are not likely to change in future encounters.

These winner and loser effects are not permanent however, and have been shown to be altered with elevated levels of certain neurotransmitters. Specifically, it has been shown that octopamine injection in winning large crayfish has decreased their capacity to win in pairings against smaller opponents (Momohara et al. 2013). Additionally, serotonin injection leads smaller losing crayfish to win more often against larger opponents. This indicates that the success of crayfish in agonistic encounters, and the role of winner and loser effects, is influenced by neurotransmission.

Once a crayfish social hierarchy has formed, it is unlikely to change unless there are severe disruptions in the community. The preservation of this hierarchy is helped by a social setting, where chemical signals and pheromones are constantly exchanged, maintaining roles of dominance and subordination. Crayfish urine release is significantly higher during agonistic

encounters than during their resting states (Simon and Moore 2007). During times of chelae contact, both urine release and anterior current generation were shown to be elevated. This indicates that the secretion of urine plays a significant role in communicating social dominance. It has been shown that the chemical signals in the urine that is dispersed by animal-generated currents in the American lobster, have a direct correlation to communicating social status. Thus, the stability and low levels of aggression in an existing hierarchy might be attributed to this continued pheromone release.

Due to the weakening of the effect of pheromones with distance, it has also been postulated that visual stimuli allow for communication of social status in crayfish. The dominant postures in crustaceans, caused by elevated levels of serotonin, have long been used as a visual signal for mates (Atema and Steinbach 2007). These postures are also seen as a potential method of signaling dominance to nearby crayfish. However, some believe that maintenance of crayfish social hierarchies require visual, olfactory, and tactile communication, through a system of multimodal signals (Partan and Marler 2005). A response can only be elicited if information derived from multiple outputs is received. This is particularly true in choosing mates, as a recent study showed that *Procambarus clarkii* females rely on both chemical and visual stimuli to assess a conspecific (Aquiloni et al. 2009). To confirm this hypothesis, this study seeks to determine what forms of sensory communication are necessary in preserving a hierarchy and controlling aggression.

The study of the formation of a dominance hierarchy among juvenile crayfish has allowed their actions to be characterized by four different behaviors: attack, approach, retreat, and escape. Often, at the start of agonistic encounters, there will be a higher proportion of attacks and escapes, signifying the struggle to form a hierarchical structure; however, after time, approaches and retreats will be used to maintain the hierarchy (Issa et al. 1999). A dominance index serves as a strong tool to determine relative levels of dominance and aggression in crayfish. It is particularly helpful in measuring changes in aggression over different time scales, as was used in a recent study that measured social dominance over time in juvenile crayfish (Herberholz 2007). Thus, a dominance index will be used to determine what role visual, olfactory, and tactile signals play in changing aggression in juvenile crayfish following a baseline fight.

## **Materials and Methods**

### **Construction of Isolation and Experimental Tanks**

Tanks were made out of white acrylic boxes of dimensions (14.5 cm x 7.5 cm x 10.5 cm). Dividers were then constructed matching the height of the tank, and wide enough to be inserted and held in place inside the tank. These dividers were classified either as opaque or transparent. A portion of both the opaque and transparent dividers had holes of diameter 1 mm drilled across them, allowing for olfaction and slight physical contact. Isolation tanks contained an opaque divider, preventing all forms of contact. Experimental tanks included the four different types of dividers.

### **Capture and Isolation of Crayfish**

Juvenile *Procambarus clarkii* and *Orconectes rusticus* were obtained from a commercial supplier (Atchafalaya Biological Supply Co., Raceland, LA, USA) and isolated in tanks containing an opaque divider for one week before an initial fight. One-hundred-and-twenty size-matched crayfish (size: mean 3.28 cm  $\pm$  SD 0.27 cm, measured from rostrum to telson) were assigned to four experimental groups. The maximal size difference allowed in size-matching pairings was  $\pm 0.1$  cm. The average size difference across the four groups was 0.04 cm  $\pm$  SD 0.03 cm. In each pairing, crayfish were identified as having either one or two dots on its carapace, which were drawn on at the start of isolation.

Isolation tanks were filled with water up to the 7 cm mark on each end of the divider. A thin layer of wire mesh was placed over the divider to prevent crayfish from tail flipping across the divider. A hole of diameter 2 cm was cut on each side of the mesh to allow for a dissolved oxygen bubbler to be placed in the water on each end of the tank. An aquarium lid was placed over the wire mesh, and isolation tanks were stored in a closed room under a 12:12 light dark cycle at 20 °C. Tanks were checked periodically to ensure the survival of the crayfish, and to ensure that the marked dots remained.

### **Baseline Fight and Experimental Isolation**

After one week of isolation, pairs of crayfish were removed from their isolation tanks and measured from rostrum to telson. This was done to ensure that no molting had occurred during

isolation, as that would lead to an unfair size advantage for one animal. If no molting had occurred, crayfish would be transferred to a test arena (14.5 cm x 7.5 cm x 10.5 cm), filled up to the 7 cm mark with water; the area was constructed out of an opaque acrylic box to ensure that no other visual stimuli could affect the agonistic bout. The arena was separated by a retractable opaque divider. Crayfish were given three minutes to acclimate to their new surroundings before the divider was removed. All interactions were recorded for thirty minutes following the removal of the divider by a video camera (Canon Vixia HF R300) mounted above the arena.

After thirty minutes, both crayfish were transferred from the fight arena to an experimental tank. Experimental tanks had either an opaque or transparent divider. Half of each type of divider had holes, which allowed for olfactory contact between animals. Upon re-isolation under experimental conditions, an adult crayfish food pellet was dropped on each side of the tank. Dissolved oxygen bubblers were inserted through a thin layer of wire mesh, and crayfish remained under the experimental condition for one week.

Fight arenas were washed with soap and dried prior to other fights.

## **Second Experimental Fight and Disposal**

After one week in an experimental condition, each pair of crayfish was removed from the experimental tank, and measured from rostrum to telson. If no molting or significant size changes were observed, they were once again moved to a fight arena. This second fight followed a similar procedure as the first fight. Crayfish were given three minutes to acclimate to the fight arena, while separated by an opaque divider. Following that, the divider was removed, and their interactions were recorded for thirty minutes.

After thirty minutes, both crayfish were removed from the arena, and transferred to a junk tank, where they would only be allowed to interact with other crayfish who had completed experimentation. The fight arena was once again washed with soap before any future fights. All isolation and experimental tanks were also washed thoroughly with soap, and left to dry before any future isolations.

Fight recordings were stored in either mini-DV format (Sony DVC60PRL Mini DV Tape) or as an MPEG-4 file on an SD card (Sony 64GB Class 10 UHS-1 SDXC Memory Card).

All SD card recordings were transferred to an external hard drive for storage and future video analysis.

### **Negative Control Setup**

A condition without the presence of any divider following an initial fight was run. This was to account for any possible changes in aggression due to transfer to a new environment (fight arena). A similar procedure to the experimental condition was observed for these trials; however, after the baseline fight, both crayfish were returned to a tank without any divider. For the first thirty minutes of re-isolation, an opaque divider was placed in the tank, and food pellets were delivered to each side. This was to ensure that both crayfish had the opportunity to feed after the initial fight. The divider was then removed, and isolation continued as per normal for the remainder of the week.

### **Video Analysis**

Agnostic behaviors observed during fights were analyzed using single-frame analysis on a TV monitor (Panasonic 2010-Y). Crayfish were identified during analysis through the number of dots on their carapace. Each fight was scored using a rubric to characterize agonistic behaviors, and provide sufficient information for the calculation of a dominance index. This dominance index was used as a benchmark for aggression, and a comparison between changes in aggression between the first and second fight was used to determine the effect of sensory signals on crayfish hierarchies.

Aggressive behaviors could be characterized as either attacks or approaches; attacks hold twice the weightage of approaches in the calculation of the dominance index. Attacks are characterized as “launching toward an opponent with outstretched claws with the possibility of physical contact.” Approaches are characterized as “an advance toward an opponent with lowered claws.” Submissive behaviors are characterized as either retreats or escapes; escapes hold twice the weightage of retreats in the calculation of the dominance index. Escapes entail “the use of tail-flips or a speedy fleeing mechanisms to escape an opponent.” Retreats are characterized by “moving away, usually through walking, from an opponent.” A dominance index (DI) is calculated as follows:  $(\text{total number of aggressive acts}) / (\text{total number of aggressive and submissive acts})$ . This yields a number ranging from 0 to 1, and helps

characterize the social status of an animal. The total aggression or submission between fights is calculated through dividing the sum of all aggressive/submissive encounters in the second fight by the sum of aggressive/submissive encounters in the first fight. This yields a ratio that indicates how levels of aggression changed between fights.

## Statistical Analyses

Data is presented, when applicable, as a mean value, with standard error bars from the mean. The changes between the counts of specific aggressive or submissive behaviors between trials for each condition follows a non-parametric statistic, and thus a normal distribution cannot be assumed. Thus, a Wilcoxon signed-rank test was used to measure changes in behavioral counts. Changes in experimental aggression and submission between paired crayfish in all four trials will be analyzed using a Kruskal-Wallis H test. Changes in dominance indices of animals following treatment will be analyzed using a Kruskal-Wallis H test.

## Results

In Figure 3, the number of crayfish surviving two weeks under the open divider condition is shown to be 50% of the number initially isolated. Due to the low survival rate of crayfish under this condition, data regarding agonistic behavioral performance cannot be provided. However, the overall level of aggression in surviving crayfish after the open divider condition was comparatively low, as the crayfish had maintained their hierarchy over the past week with no disruptions.

Figure 1 indicates sixteen trials conducted under the opaque condition, thirteen trials under the vision condition, ten trials under the olfaction condition, and fourteen trials under the visual-olfactory (VO) condition. The ratio of reversals between the first and second trials remained statistically constant across all conditions except the olfaction condition, where 50% of paired crayfish experienced a reversal.

Figure 1 shows the changes in behavioral counts for *Procambarus clarkii* that were paired with an opaque divider following one week in isolation and a baseline fight. A Wilcoxon signed-rank test found that crayfish experienced a significant increase in behavioral escape counts during the second agonistic encounter ( $W=15<25$ ,  $p=0.01<0.05$ ). Behavioral counts for attacks and escapes also experienced modest increases.



The changes in behavioral counts for *Procambarus clarkii* paired with a transparent divider, only allowing for visual contact, are shown in Figure 2. All behaviors showed a modest decrease in count following the baseline fight.

Changes in behavioral counts for *Procambarus clarkii* paired under a divider allowing for only olfaction are shown in Figure 3. Mean behavioral counts for both attacks and escapes experienced significant increases between baseline and experimental fights. A Wilcoxon signed-rank test found a significant increase in attack counts ( $W=2<8$ ,  $p=0.009<0.05$ ) and a significant increase in escape counts ( $W=4<8$ ,  $p=0.02<0.05$ ). Mean counts for approaches and retreats experienced a slight decline from the baseline to the experimental fight.

Figure 4 shows the changes in behavioral counts for *Procambarus clarkii* that were paired with a divider allowing for both olfaction and vision (VO) following a baseline fight. Although mean counts for attacks and escapes experienced increases, neither was significant ( $W=25>21$ ,  $p=0.08>0.05$ ) and ( $W=18.5>17$ ,  $p=0.06>0.05$ ) respectively. Mean counts for approaches and retreats remained relatively constant across baseline and experimental fights.

Changes in total aggression and submission between fights for *Procambarus clarkii* paired under all four different dividers is shown in Figure 5. A Kruskal-Wallis test conducted for aggression following treatment found a significant difference across the four categories ( $p=0.03<0.05$ ). However, a Kruskal-Wallis test conducted for submission following treatment found no significant difference across trials ( $p=0.06>0.05$ ). A Wilcoxon signed-rank test conducted for changes between baseline and experimental aggression in the olfaction condition found a significant increase ( $W=3<8$ ,  $p=0.01<0.05$ ). A Wilcoxon signed-rank test conducted for changes between baseline and experimental submission in the olfaction condition also found a significant increase ( $W=5.5<8$ ,  $p=0.025<0.05$ ).

Table 3 shows the dominance indices of *Procambarus clarkii* during baseline isolation fights and during a second agonistic bout following treatment. A Kruskal-Wallis test conducted on the dominance indices of animals following treatment found no significant differences, indicating that no one divider led to an increased probability of reversal ( $H=4.1<7.8$ ,  $p=0.2>0.05$ ). However, crayfish separated by a divider that allowed for olfaction did experience increases in the number of reversals, as compared to the other three conditions.

A comparative species study was conducted using pairs of juvenile *Orconectes rusticus* that were separated by a VO divider following a baseline fight. Figure 6 shows the changes in mean behavioral counts between fights following introduction of the VO divider. A Wilcoxon signed-rank test found a significant increase in attack counts ( $W=4<5$ ) although an accurate p-statistic could not be provided due to only 10 trials taking place. Counts for approaches and retreats also showed modest increases following treatment, while counts for escapes experienced a small decline.

Figure 7 shows the ratios of changes in aggression in *Orconectes rusticus* following VO treatment and the baseline ratio of aggression of *Procambarus clarkii* compared to the baseline aggression of *Orconectes rusticus*, which was assigned a value of 1. *Procambarus* juveniles displayed heightened aggression and submission compared to either *Orconectes* juveniles under VO treatment, or from a baseline fight.

The results of these experiments indicate that disruption of contact after hierarchy formation provides a potential mechanism for increasing aggression in crayfish and disrupting existing hierarchies.

## **Discussion and Analysis**

Hierarchy formation and maintenance in crayfish proves to be of delicate structure. Disruption of the signals necessary in maintaining hierarchies was shown to lead to altered levels of aggression and submission in subsequent agonistic bouts. The role of olfaction in maintaining hierarchies in crayfish has been shown to be of particular importance following the results of this experiment. While visual contact tended to preserve existing hierarchies and reduce levels of aggression in subsequent fights, olfactory but not visual contact tended to increase aggression in subsequent fights. This could be due to knowledge of an intruder, but no means of visually identifying and maintaining a hierarchy with that intruder. Since animals paired under the VO and transparent condition displayed no significant increases in behavioral counts, it can be presumed that visual contact is critical in maintaining crayfish hierarchies.

Dividers that allowed for olfaction but not visual contact allowed crayfish to communicate only through specific channels. Secreted chemicals and urine, along with slight physical contact through antennae were the only forms of communication allowed. It is

interesting that this contact alone led to heightened aggression and submission, because urine secretions are thought to communicate social status. Perhaps knowledge of this status without the means to physically establish it might promote the increased aggression and reversals observed during the experimental fight.

The measured differences in aggression also extended to the between-species study. The relatively lower aggression seen in juvenile *Orconectes* crayfish could be attributed to their larger size. Increased growth at an early developmental stage caused *Orconectes* crayfish to be slightly larger than their *Procambarus* counterparts during testing. However, the differences in aggression might also be attributed to different levels and concentrations of neurotransmitters. Future studies should conduct *in vivo* cellular physiology for *Orconectes* and *Procambarus* crayfish following experimental fights to determine concentrations of serotonin, octopamine and dopamine. These neurotransmitters have been implicated in previous work to be associated with the outcome of agonistic bouts. Thus, measured changes in these neurotransmitter levels between animals or following divider placement would further strengthen the argument for the increased aggression caused by the olfactory divider. Additionally, correlation between neurotransmitter levels and the changing aggression caused by sensory dividers might lead to potential drug treatments to control aggression levels.

Crayfish are strong model organisms for the study of aggression due to their size and our comprehension of their neural circuitry. Their naturally aggressive behaviors allows for quantification of their aggression and submission through the calculation of dominance indices (DI). Thus, through manipulating their aggression either through environmental and sensory stimuli or through drug treatments, we can better understand the neurotransmitters and circuitry involved with promotion of aggressive behaviors. Human mental disorders such as Bipolar Disorder (BPL) and schizophrenia are strongly correlated with alterations in aggression and mood. Similar serotonin (5-HT) and dopamine receptors in both vertebrates and invertebrates allows for translational aggression studies. Thus, through a sensory mechanism of modulating aggression, future studies with drug treatments to lower or control aggression can progress.

Several limitations were present during experimentation that merit future consideration. First, the scoring of fights was conducted manually, which leaves room for human error. The development of programs that could digitally store and analyze fights could provide a basis for

measuring behaviors in the future. The timing during the day of fights also leaves room for error. The circadian rhythms of crayfish could influence crayfish locomotion and thus fight performance. Seasonal timing also prevents a limitation in experimentation. Fights took place over the autumn and winter, when crayfish in the wild tend to display different behavior due to limited rainfall. This could potentially influence crayfish aggression levels and agonistic behavior. Finally, crayfish size presents a possible source of error. Crayfish were isolated between 2.8 cm to 4.0 cm (rostrum to telson) in *Procambarus clarkii* and 3.2 cm to 4.5 cm (rostrum to telson) in *Orconectes rusticus*. Variations in sizes across fights could lead to altered levels of aggression. However, size-matching of crayfish helps limit the potential influence of this limitation.

Future studies should attempt to engineer an experimental protocol capable of isolating crayfish in a condition with no divider for one week, to fully establish a negative control group. The increased levels of crayfish aggression under the olfaction divider condition should continue to be investigated either through cellular studies or further behavioral studies. Possible drug treatments should be considered that would modulate levels of crayfish aggression from the newly formed “hyper aggressive” state. Additionally, future studies should look at the dynamics of crayfish hierarchies, and specifically isolate what secreted chemicals or what specific sensory signals influence the stability of social hierarchies.

The primarily goal of this study was to determine the role that sensory signals played in the dominance hierarchies formed by crayfish following a baseline fight. Sustained visual contact was shown to maintain hierarchy stability and stabilize aggression levels, while olfactory contact was shown to disrupt hierarchy stability while increasing aggression levels. This clearly indicates that the stability of social hierarchies in crayfish is highly dependent on sustained contact, and disruptions in these forms of contact can have drastic consequences for populations and individual crayfish. This study also showed that olfactory contact following a baseline fight could yield a state in which two crayfish would have unusually high levels of aggression toward one another. This provides an opportunity to study the mechanics behind social encounters in crayfish, while looking at possible neurological markers for these states of high aggression.

## References

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## Appendix

**Table 1.** The number of reversals represented as a ratio to the total number of trials for each experimental condition in *Procambarus clarkii*.

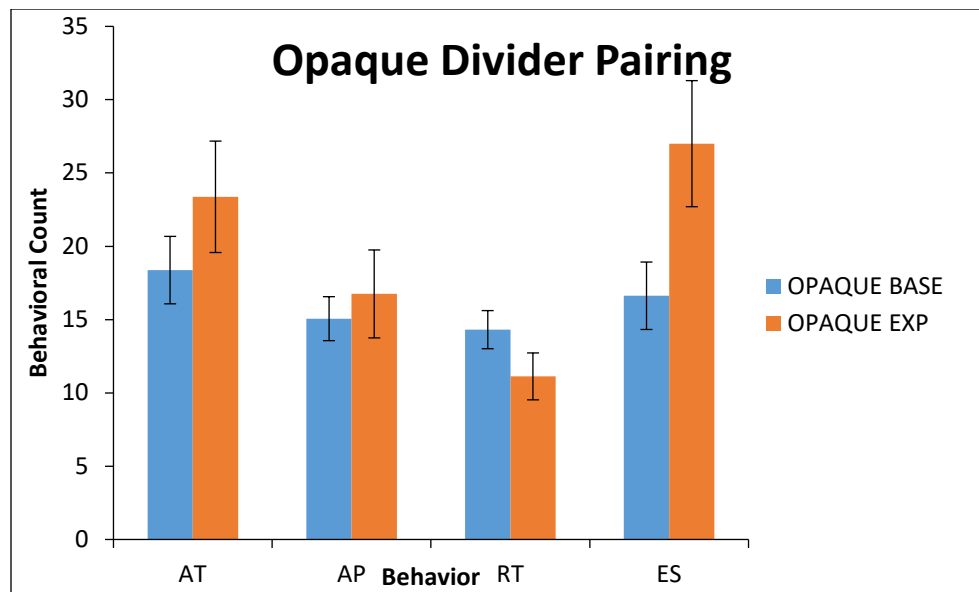
Condition	Opaque	Vision	Olfaction	VO
Number of Trials	16	13	10	14
Number of Reversals	4	2	5	3
Ratio of Reversals	0.25	0.15	0.5	0.21

**Table 2.** Survival rates for *Procambarus clarkii* stored in an open divider experimental condition.

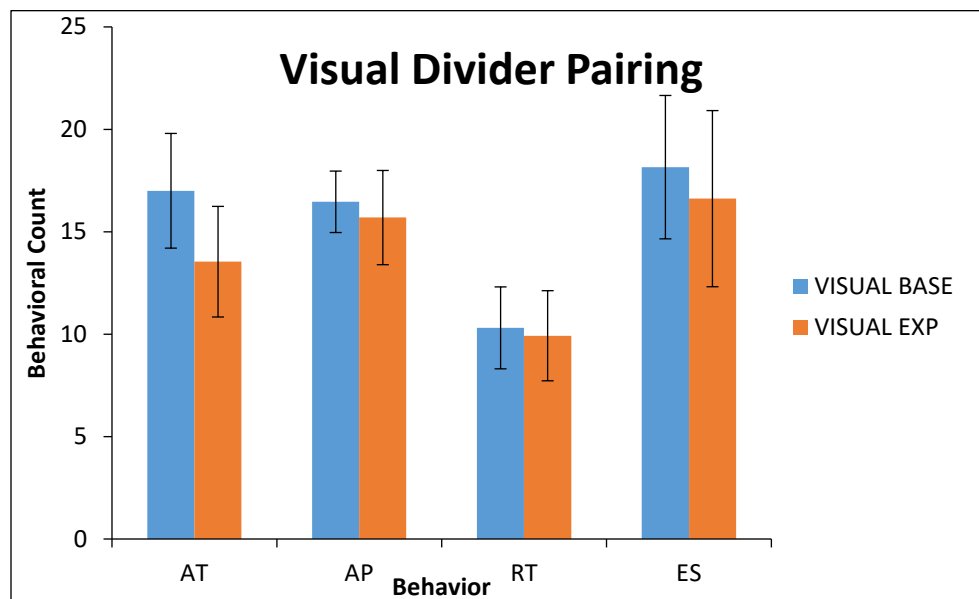
Pairs at Start	1 <sup>st</sup> Week Survival	2 <sup>nd</sup> Week Survival	Fraction Survived
12	10	6	0.5

**Table 3.** The adjusted dominance indices for animals under each experimental condition. Lower index values for former dominants and higher index values for former subordinates indicates the occurrence of reversals during the second agonistic bout.

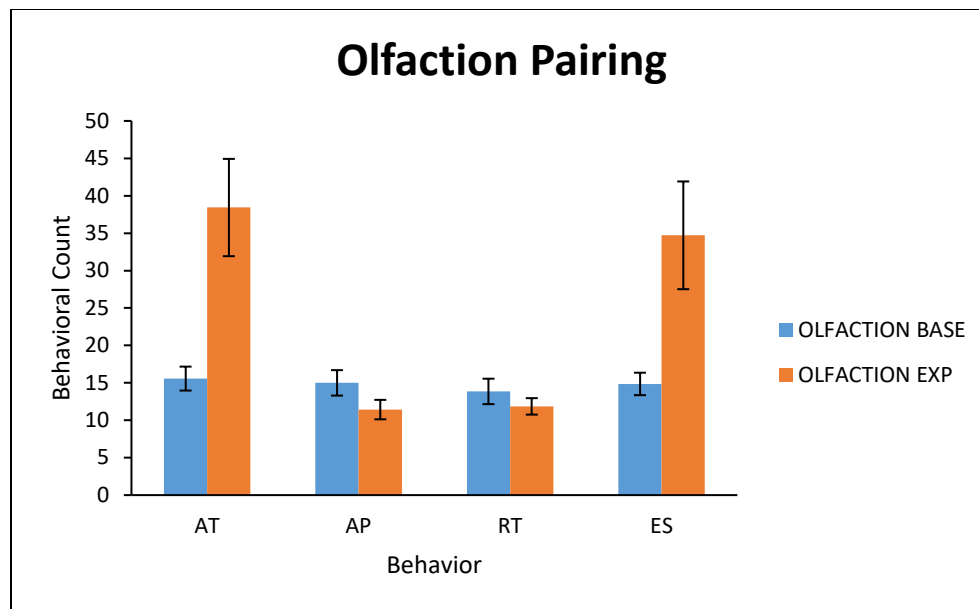
	Dominant	Former Dominant	Subordinate	Former Subordinate
Opaque	0.951236	0.740975	0.127281	0.309339
Vision	0.922811	0.1590	0.8834	0.2470
Olfaction	0.957575	0.5607	0.092183	0.525403
VO	0.974144	0.865296	0.111475	0.233209



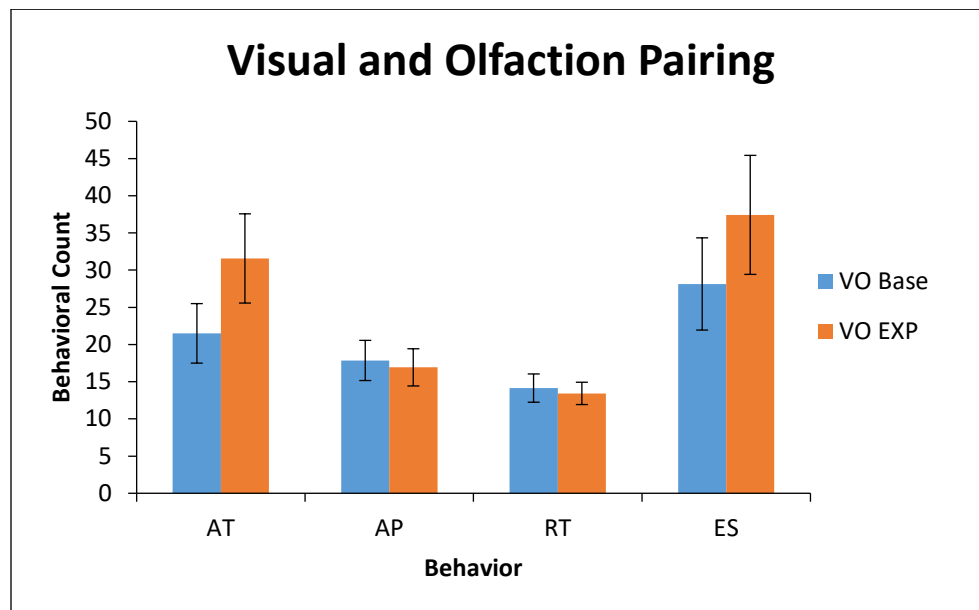
**Figure 1.** Quantifying the mean behavioral counts taken during agonistic bouts for *Procambarus clarkii*. These counts were taken during an initial baseline fight, and a second fight, following a second week in isolation with an opaque divider. Data is reported with standard error bars.



**Figure 2.** Quantifying the mean behavioral counts taken during agonistic bouts for *Procambarus clarkii*. These counts were taken during an initial baseline fight, and a second fight, following a week separated by a transparent visual divider. Data is reported with standard error bars.

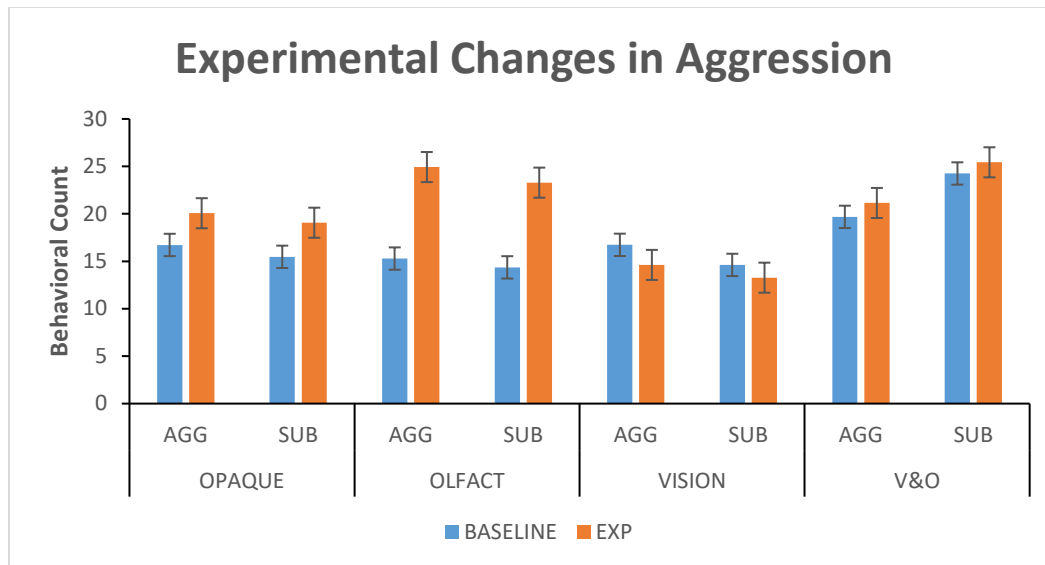


**Figure 3.** Quantifying the mean behavioral counts taken during agonistic bouts for *Procambarus clarkii*. These counts were taken during an initial baseline fight, and a second fight, following a week separated by a divider allowing only for olfactory contact.

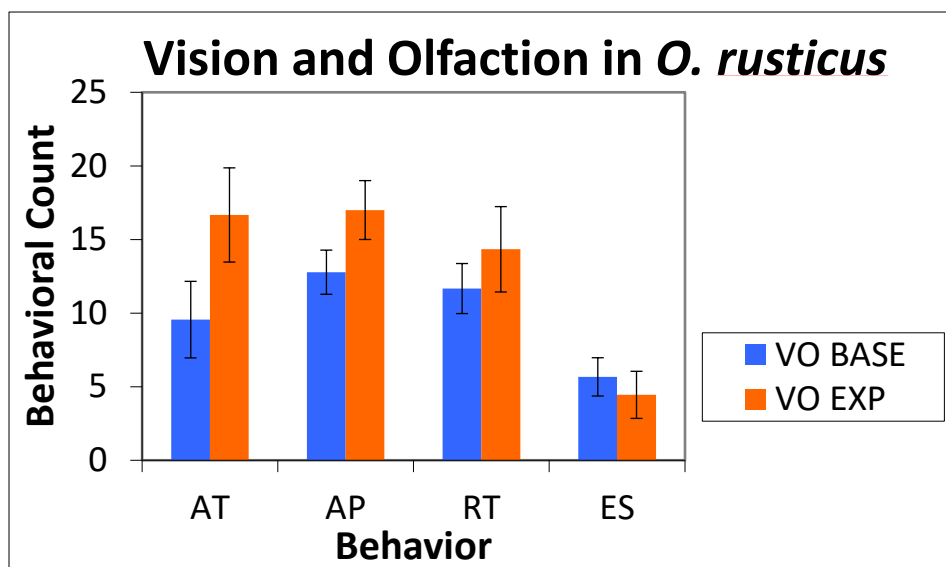


**Figure 4.** Quantifying the mean behavioral counts taken during agonistic bouts for *Procambarus clarkii*. These counts were taken during an initial baseline fight, and a second fight, following a week separated by a divider allowing for both olfactory and visual contact.

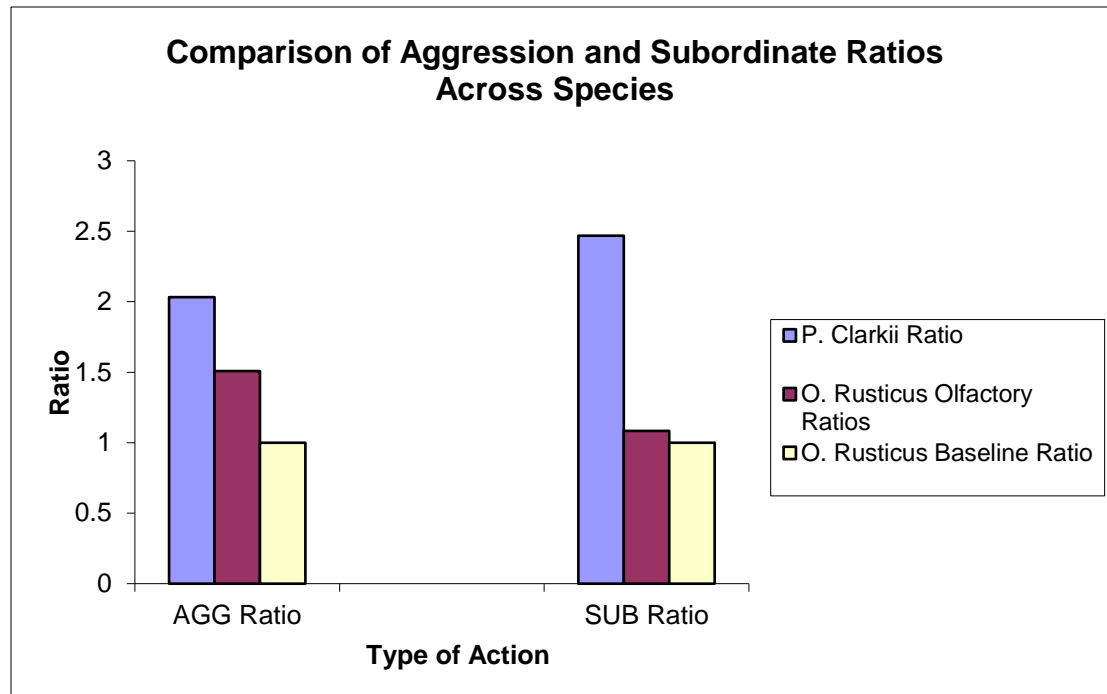




**Figure 5:** Changes in total aggression, taken as the weighted average of aggressive and submissive behaviors, between baseline and experimental fights. Experimental conditions shown include an opaque divider, an olfaction divider, a vision divider, and a VO divider. Data is presented with standard error bars.



**Figure 6:** Quantifying the mean behavioral counts taken during agonistic bouts for *Orconectes rusticus*. These counts were taken during an initial baseline fight, and a second fight, following a week separated by a divider allowing for both olfactory and visual contact.



**Figure 7:** Comparison of the ratios of aggression and submission across *P. Clarkii* baseline, *O. rusticus* baseline, and *O. rusticus* olfaction. In determining these ratios, the mean aggression and submission for *O. rusticus* baseline was given a value of one. The ratios of aggression and submission of other groups were determined as a ratio compared to the values in the *O. rusticus* baseline trial.