

ARTICLE

Crayfish Aggression and the Androgenic Gland in a Behavior Lab for Non-Majors**Kristina S. Mead***Biology Department, Denison University, Granville, Ohio 43023.*

Male *Procambarus clarkii* were matched by size and largest claw length and observed interacting in pair matches before and after removal of the androgenic gland or a sham operation. Although results were not significant, trends suggested that males showed less aggression after the removal of the androgenic gland. Average bout duration did not decrease, but mean intensity of interaction

decreased. This exercise was part of a student lab for non-majors. Students were positive about the lab, indicating that they learned about quantifying behavior, about hormonal involvement in aggression, and that the lab made them want to do more science.

Key words: crayfish, androgenic gland, aggression

For a new class for non-majors called "Sex, Gender, and the Brain," I wanted to create a lab experience that would expose students to the quantification of behavior and enable students to make the link between hormones and aggression, hopefully in a charismatic animal that would be easy to work with and (because of time constraints), not under purview of IACUC. My original inspiration was an article by Barki et al. (2003) that described how androgenic glands from male crayfish could be implanted into immature females, causing regression of female secondary sex characteristics, and the development of some male secondary characteristics, including some social behavior. This seemed too ambitious for my class, but I felt that we might try simply removing the androgenic glands from males, to see if male-male aggression was decreased. We therefore designed the simple lab that follows, to test the hypothesis that removing the androgenic gland from male crayfish would result in decreased aggression in size-matched crayfish. Secondly, we wanted to see if a possible decrease in aggression level stemmed from a decrease in the duration of aggressive interactions, or from a decrease in the overall intensity of aggressive interaction.

The androgenic gland is a small gland found associated with the terminal portion of the vas deferens in male isopods, amphipods, and decapods. The androgenic gland is thought to be the primary source of male hormone in malacostracan crustaceans (Charniaux-Cotton, 1962), which include crabs, lobsters, shrimp, mantis shrimp, krill, amphipods, and isopods. The androgenic gland has been found to affect sexual differentiation in males and in females. Primary sexual characteristics affected include gonadal sex reversal in freshwater shrimp (Nagamine et al., 1980a,b), inhibition of vitellogenesis (yolk deposition) and maintenance of spermatogenesis in several crayfish species (Taketomi and Nishikawa, 1996; Fowler and Leonard, 1999; Khalaila et al., 1999). The gland also affects secondary sexual characteristics, including development of pleopods in prawn (Touir, 1977), claw morphology and other features in freshwater shrimp (Sagi and Cohen, 1990; Sagi et al., 1990), development of first abdominal appendages, development of a red patch on the claw, aggressive behavior, and inhibition of oosetae (feathery structures that hold eggs in a brooding female) in

various crayfish species (Taketomi and Nishikawa, 1996; Khalaila et al., 1999; Karplus et al., 2000). These effects and others are succinctly reviewed in Sagi and Khalaila (2001).

MATERIALS AND METHODS

Sixty-two crayfish (*Procambarus clarkii*) with rostrum-telson lengths of 70-95 mm and maximum claw lengths of 17-36 mm were obtained from Carolina Biological supply. Crayfish were housed individually in opaque plastic containers with shelters made of PVC pipe half-sections for two weeks before experiments began. Crayfish were fed dry cat food and the water was changed twice weekly.

Crayfish were examined to see if they were male, female, or intersex. In males, the first pair of swimmerets is long and prong-shaped and serves as a sperm-transfer organ. Female swimmerets are not modified. Females have a seminal receptacle located between the fourth and fifth pairs of walking legs, and visible oviduct openings at the base of the third pair of walking legs. Of the 62 specimens, 49 were female and 13 were male. Since body size and claw size are intrinsic indicators used by crayfish (Bergman and Moore, 2003), we set up matches between male crayfish with similar telson-rostrum lengths and maximum claw sizes matched to within 10%. The two crayfish slated for a match were placed into an opaque-walled test arena (rat cage) 25 x 50 x 20 cm filled with clean water up to about 10 cm depth. Each of the two contestants was simultaneously placed in the tank, under a smaller shelter container. Matches started after 5 minute acclimation, when the small shelter containers were removed, and observations continued for up to 10 additional minutes. Matches were scored every 15 seconds, using the following table of behaviors from Bergman and Moore (2003) (see Table 1). The summed aggression over the match was calculated by adding up all the aggression scores for the duration of the match. Match duration was defined as the amount of time during which at least one of the two crayfish exhibited behavior that did not score a 0 according to Table 1. Match intensity was calculated by finding the mean aggression score over all the 15-second periods of the match. JMP6 software (SAS Institute 2006) was used to calculate t-tests and ANOVAs.

Activity:	Points
Fast retreat	-2
Turns away, slowly backs from opponent	-1
No observed response to opponent	0
Approaches the other crayfish without threat display	1
Approaches the other crayfish with threat display (meral spread or antennal whip)	2
Boxing, pushing, or touching with closed claws	3
Active claw use with open claw	4
Unrestrained fighting	5

Table 1. Crayfish behavior scoring

After the matches, males either had their androgenic gland removed or had a sham surgery. After being anaesthetized in a bath of ice and water, male crayfish were gently pinned to a dissecting tray and surrounded by more ice and water. The rest of the procedure was done under a dissecting scope. The fifth pair of walking legs were each removed proximal to the opening of the vas deferens. Using forceps, the terminal portion of the vas deferens was located and removed. The vas deferens looked like a thick cream-colored cotton thread. The androgenic gland, which is associated with the vas deferens, came out along with the vas deferens (Figure 1). Sham surgeries were done as above, but forceps were used to root around in the base of the fifth walking leg, without removing the vas deferens and androgenic gland.

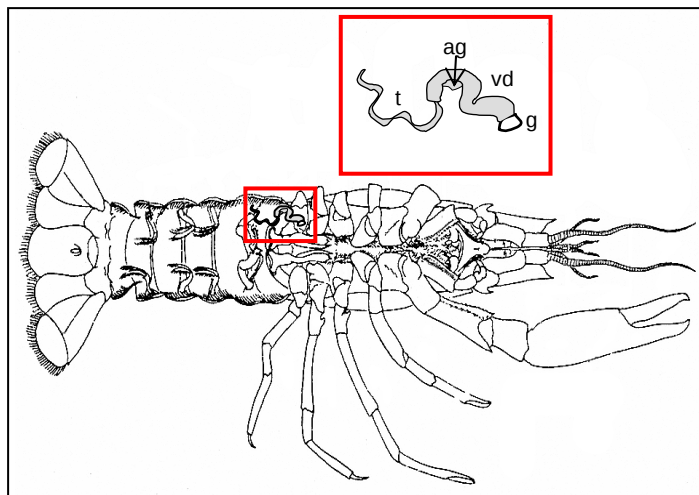


Figure 1. Male crayfish showing location of male reproductive tract accessible via the base of the fifth walking leg. Ag = androgenic gland, g = gonopore, t = testes, and vd = vas deferens.

Three weeks after surgery, crayfish were rematched and their summed aggression over the match, duration of interaction, and average intensity of interaction were calculated as before.

Setting up and observing the matches and then performing the surgeries is doable in a typical three-hour undergraduate lab. In round one, our 12 male crayfish had a total of 23 matches. With six groups of students running matches, this took about 1.5 hours. Each surgery took about 10 minutes to anaesthetize the animal, followed by

5-30 minutes for the surgery (still on ice), depending on the skill of the student.

RESULTS

All animals survived the fights and the surgery, although one male escaped and one male molted. Typically interactions started slowly, with little or no activity for the first couple of minutes, followed by approaches with and without meral spreads. This was sometimes followed by increasing escalation to the point of hitting or grappling with claws. Unrestrained fighting was relatively rare. Often, the crayfish disengaged after a couple of minutes and retreated to their own corners. While the results were not statistically significant, there was a decrease in mean total aggression from 17.3 ± 5.2 to 8.1 ± 3.8 after the removal of the androgenic gland (t-test, $p=0.13$, Figure 2). The sham group showed no significant change in aggression (t-test, $p=0.51$).

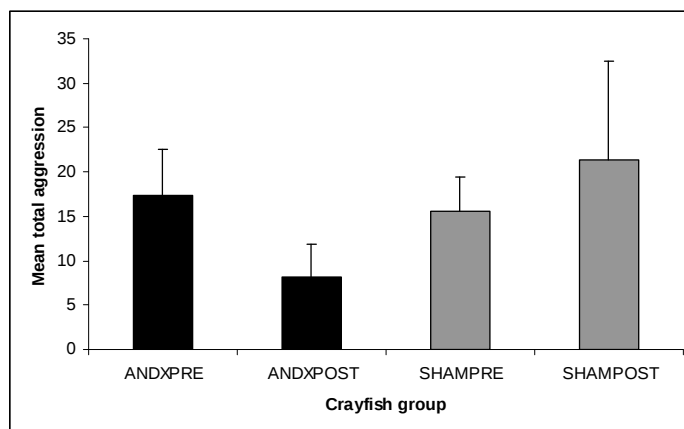


Figure 2. Crayfish mean total aggression before and after androgenic gland removal (black columns) and sham surgery (gray columns). Error bars indicate standard errors of the mean. N = 6, 6, 6, and 4 for the pre-andrectomy, post-andrectomy, pre-sham, and post-sham groups.

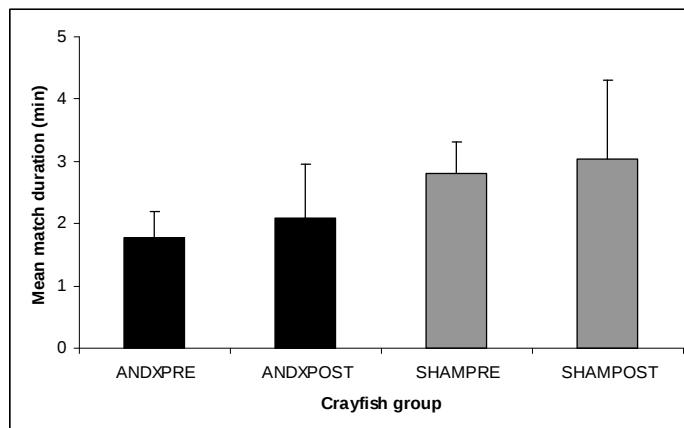


Figure 3. Crayfish mean match duration before and after androgenic gland removal (black columns) and sham surgery (gray columns). Error bars indicate standard errors of the mean. N = 6, 6, 6, and 4 for the pre-andrectomy, post-andrectomy, pre-sham, and post-sham groups.

Crayfish matches were relatively short in length, ranging from 15 seconds to 9 minutes. There did not appear to be any effect of andrectomy (t-test $p=0.76$) or sham (t-test $p=0.83$) on match duration, which was typically less than 3 minutes (Figure 3).

Mean intensity of interaction is shown in Figure 4. Again, while not statistically significant, there is a trend toward a decrease in the mean behavioral intensity from 1.04 ± 0.5 to 0.39 ± 0.27 after removal of the androgenic gland (t-test $p=0.21$), but not after the sham (t-test $p=0.79$).

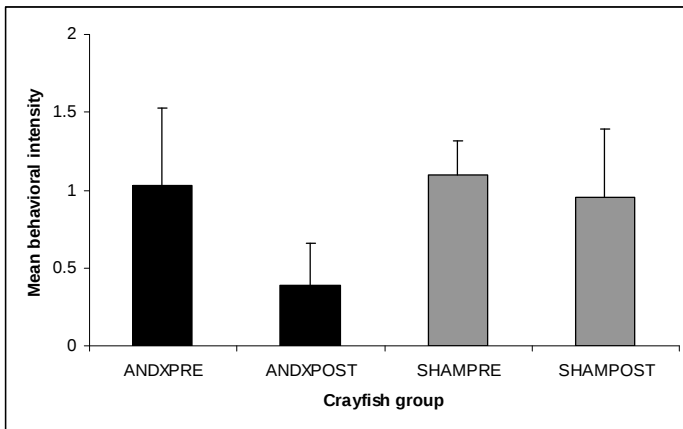


Figure 4. Crayfish behavioral intensity before and after androgenic gland removal (black columns) and sham surgery (gray columns). Error bars indicate standard errors of the mean. N = 6, 6, 6, and 4 for the pre-andrectomy, post-andrectomy, pre-sham, and post-sham groups

DISCUSSION

Students responded well to this lab. In an anonymous survey, students were asked to answer the following questions with numbers of 1-5 (low-high). Their responses were as follows in Table 2.

Question	Score (Mean ± SE)
Did this lab help me understand that you can quantify some aspects of behavior?	4.28 ± 0.14
Did this lab help me understand the link between hormones and aggression?	4.39 ± 0.18
Was this lab interesting?	4.83 ± 0.08
Did this lab make me want to do more science?	4.11 ± 0.20

Table 2. Student responses to survey.

Specific student comments included “it was cool to use real animals and watch their behavior and interaction,” “I have never really liked science, but this lab gave me a different overall experience.” “I felt like it went above and beyond a normal lab setting for non-majors. It was very interesting!” “Aggression is something people deal with everyday so being able to quantify it in these crayfish was very fun and interesting.” The students liked doing surgery, and they liked working with live animals, and they liked being able to relate what they saw to real life.

Despite the overall favorable experience, the lab could be improved technically and organizationally. Students

sometimes had a difficult time finding the vas deferens and the androgenic gland. This process could potentially be facilitated by ablating the eyestalks a week or two before the initial fights and surgery (Khalaila et al., 2001). This procedure causes the androgenic gland to hypertrophy, making it easier to spot during dissection. However, this technique adds a new variable that must be controlled.

Students also found it hard at first to record behavior every 15 seconds. If possible, videotaping behavior and scoring behavior afterwards would lead to more consistent results and make possible the use of blind scoring. Additional factors that would have helped would have been to have the same teams score the same animals before and after the surgeries.

On their own, students made the leap to hormones and aggression in humans. The students were fully engaged, asking about putative hormone levels in aggressive world leaders and about emotional effects of steroids used as performance enhancers. While the lab did not produce statistically significant results this time around, I believe that it is largely a matter of small sample size and inconsistency of scoring. In terms of student motivation, interest, and learning, I feel that the lab was a success.

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