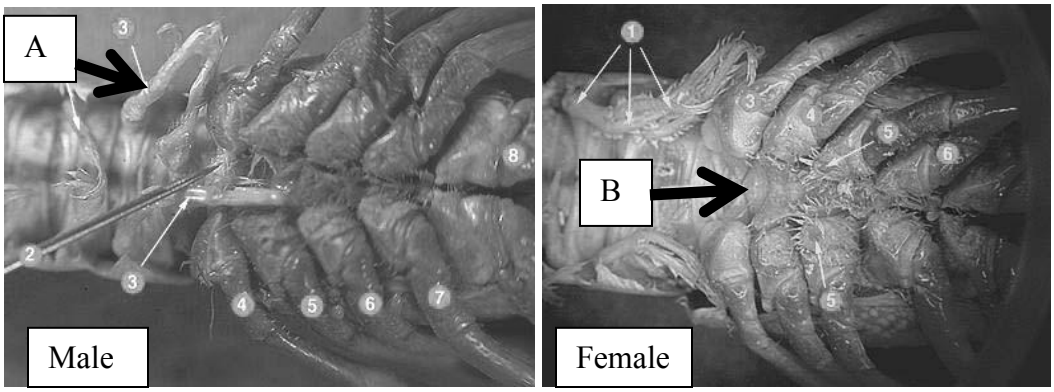
Biol 106 – Animal Behavior Lab **Spring 2008**

Lab: Behavioral Interactions of Crayfish

In this lab we will observe interactive and aggressive behaviors of crayfish (*Procambarus clarkii*). Understanding complex interactions between individuals within a species can be investigated through behavior analyses. We will observe both male and female crayfish as they interact with each other and describe the types of behavior involved in those interactions.

Background:
Males and females are sexually dimorphic in this species. In the male, the first two pairs of swimmerets are longer, and prong-shaped (Labeled A in the image below). They serve as a sperm transfer organ during mating. Females have a seminal receptacle. The sperm received from the male is stored here until the female releases her eggs (Labeled B in the image below).



Most crayfish are nocturnal, coming out at night to search for food. During the day they remain either in burrows excavated in soft muddy bottoms, or hidden beneath rocks, logs, or other debris. Crayfish defend their burrows or hiding places vigorously against all intruders, especially other crayfish. Territorial behavior occurs in many species of animals.

Why do organisms exhibit territorial behavior? List several possible reasons.

Agonistic (fighting) behavior of many decapod crustaceans features a variety of distinct, behavior patterns. Interactions start when animals advance to within one body length of another individual and overtly react to its presence. The approaching animal is termed the initiator. As the interaction progresses, animals may use a variety of behaviors in an encounter. Crayfish exhibit the following behaviors when fighting:

1. Meral spread – when individuals exhibit a posture, characterized by raising the anterior portion of the body

2. Wrestling

3. Clawing – an individual actively uses claws in an interaction (pinching or jabbing with claw)

Another behavior you are likely to observe is called retreating, where one individual attempts to move away from the other. This will not be considered an aggressive fight behavior; however you should make note when this behavior occurs.

Observation:

Before biologists begin to analyze behaviors by using experimentation, they must first observe the behaviors and become familiar with those behaviors. Above, we have listed focal behaviors for fight behavior in crayfish. We will do an observation trial prior to conducting our experiments, to familiarize ourselves with the behavior of our study organism.

*****Precautions for handling crayfish*****

Wash your hands before and after handling the crayfish. Please wear gloves provided in lab.

To pick up a crayfish, approach from behind and gently grasp the animal by the carapice (never by the extremities). The crayfish will flail its legs when you pick it up, so be prepared!! Do not drop the animal!! It cannot pinch you if you are holding it properly.

Procedure: Observation of fight behaviors in crayfish
(NOTE: The observation trial will be conducted together with another lab group)

1. Prepare a test arena: Add water **(Note: Treated tap water, TAs will show you how to prepare water for the experiments)** to the **fill line** indicated on your test aquarium.

2. When ready, add both individuals simultaneously to the test arena (one on each end of the aquarium). Allow the individuals to acclimate to the tank for 5 minutes. Observe behavioral interactions between the two individuals for approximately **10 minutes**. Discuss the different types of behaviors you observe. Identify the specific aggressive behaviors 1-3 and retreating behavior for individuals. You will likely need to discuss the different behaviors you observe with your group members to make sure everyone identifies specific behaviors the same.

Observation is an important step for animal behavior studies and will provide the basis for further investigation. Once everyone is the group can identify each behavior, then we are ready to conduct the experiments. This procedure allowed us to remove a possible source of error (repeatability between observers).

Why is repeatability important?

Experimentation:

In this lab we will conduct two different experiments investigating behavioral interactions between individuals.

Experiment 1: Same Sex Interaction (e.g. Male-Male or Female-Female)

In this experiment we will observe the interactions between two crayfish of the same sex. Whenever two individuals compete for a territory, there is likely to be a winner and a loser.

Your null hypothesis for this experiment is that there will be no differences in the number of fighting behaviors observed between the winning individual and the losing individual.

Based on the behaviors listed above, write a testable hypothesis for what you expect to see with this interaction.

Do you expect to see some behaviors more often than others? Will winners and losers differ in the behaviors they use? Why or why not?

Procedure for experiment 1: Same sex interaction

You will use two crayfish of the same sex, acclimated to individual territories within an aquarium.

1. Prepare a test arena: Add water **(Note: Treated tap water, TAs will show you how to prepare water for the experiments)** to the **fill line** indicated on your test aquarium.

2. When ready, add both individuals simultaneously to the test arena (one on each end of the aquarium). Allow the individuals to acclimate to the tank for 5 minutes. Observe behavioral interactions between the two individuals for **10 minutes**. Record the sex of the individuals in your experiment. Record the number of times you observe aggressive behaviors 1-3 for each individual in Table 1 below. Also record the number of times each individual retreats in the margin of the page. (You may want to use the number of retreats to help decide which individuals are winners and losers.) Two members of the group will record behaviors for individual 1 and the other group members will record behaviors for individual 2. **(Note: When recording behaviors, one person should watch an individual crayfish continuously and verbally indicate when behaviors occur. The other person should record the number of times the behavior occurs)**

Table 1: Number of fight behaviors observed in same sex interactions

Aggressive Behavior	Individual 1	Individual 2
Meral Spread		
Wrestling		
Clawing		

3. Determine whether there was a winner or a loser. Which individual seems more dominate? Assign a plus score to the winner and a minus score to the loser.

4. What sex where the individuals in your experiment? Do you think there could be differences in behavior between male-male vs. female-female interactions? Explain.

Experiment 2: Male-Female Interaction

In this experiment we will observe the interactions between a male and female crayfish.

Your null hypothesis for this experiment is that there will be no differences in the number of fighting behaviors observed between individuals.

Based on your previous experiment, write a testable hypothesis for what you expect to see with this interaction.

Do you expect to see some behaviors more often than others? Why or why not?

Do you expect to see different behavioral interactions between opposite sex individuals than those you observed for same sex individuals? Why or why not?

Procedure for experiment 2: Male-Female interaction

You will be given one male crayfish and one female crayfish, acclimated to individual territories within an aquarium.

1. Prepare a test arena: **Remove the water** from the first experiment, then add fresh water **(Note: Treated tap water, TAs will show you how to prepare water for the experiments)** to the **fill line** indicated on your test aquarium.

2. When ready, add both individuals simultaneously to the test arena (one on each end of the aquarium). Allow the individuals to acclimate to the tank for 5 minutes. Observe behavioral interactions between the two individuals for **10 minutes**. Record the number of times you observe aggressive behaviors 1-3 for each individual in Table 2 below. Also record the number of times each individual retreats in the margin of the page. (You may want to use the number of retreats to help decide which individuals are winners and losers.) Two members of the group will record behaviors for the male and the other group members will record behaviors for the female. **(Note: When recording behaviors, one person should watch an individual crayfish continuously and verbally indicate when behaviors occur. The other person should record the number of times the behavior occurs)**

Table 2: Number of fight behaviors observed in Male-Female interactions

Aggressive Behavior	Male	Female
Meral Spread		
Wrestling		
Clawing		

3. Determine whether there was a winner or a loser. Which individual seems more dominate? Assign a plus score to the winner and a minus score to the loser.

4. Did you observe any different behaviors (not listed) for the male-female interaction that you did not observe during the male-male interaction?

Data Analysis:

Your TA has collected the data for your entire class, and you will analyze this combined data set. Fill in the tables below using data from the entire class.

Table 3: Same sex interaction (Total number fight behaviors)

Category	Observed (total # of fighting behaviors)
Winners	
Losers	
Total	

Table 4: Male-Female interaction (Total number fight behaviors)

Category	Observed (total # of fighting behaviors)
Winners	
Losers	
Total	

We want to use these data to test our hypotheses that crayfish use fighting behavior to obtain a territory. This hypothesis makes the following prediction, which we can test with these data. The hypothesis predicts that winning crayfish will use more fight behaviors than losing crayfish.

This prediction can be statistically tested using a chi-squared-goodness-of-fit test. For this test, we first need a "null hypothesis", which in this case is that there is no difference in the amount of fighting behaviors used between winning and losing crayfish. Under this null hypothesis, we should see winners and losers use the same amount of fighting behaviors. First, fill in the table below with the observed values from the same sex experiment: the "observed" column is the class data from Table 3 above, and the "expected" column is found by splitting your total sample size in half for each category (e.g., if you had a total sample size of 20 crayfish behaviors, you would expect 10 behaviors for winners and 10 behaviors for losers). The totals of the two columns should be the same. Leave the last column blank for now.

Table 5: Chi-Square of same sex interactions (Winners vs. Losers)

Individual Type	Observed (total # of fighting behaviors)	Expected (total # of fighting behaviors)	Statistic
Winner			
Loser			
Total			

We can now calculate our chi-squared test statistic: the chi-squared value for each category (winner or loser) is $(O - E)^2/E$, where O is "observed" and E is "expected. Calculate these values and fill in the table. The total chi-squared value is the sum of these values. Calculate this value and put it in the box marked with heavy lines.

Now we need to evaluate the test statistic. If the chi-squared value is low (below a critical value), then observed values and expected values are pretty similar to each other and we conclude that there is no reason to reject the null hypothesis. If the chi-squared value is very high (above a critical value), then the observed and expected values are very different from each other, and we reject the null hypothesis. In this latter situation we conclude that winners and losers do not behave the same, and we need to look back at the data to see which group has more fight behaviors.

For reasons we don't need to go into here, the critical value for this particular test is 3.84. **Is your calculated chi-squared value above or below this value? Is the prediction of our hypothesis supported or not?**

Now we will do the same calculation for Male-Female interactions. Fill in Table 6 with the observed values from the male-female experiment: the "observed" column is the class data from Table 4 above.

Table 6: Chi-Square of Male-Female interactions (Winners vs. Losers)

Male Type	Observed (total # of fighting behaviors)	Expected (total # of fighting behaviors)	Statistic
Winner			
Loser			
Total			

Is your calculated chi-squared value above or below this value? Is the prediction of our hypothesis supported or not?

Summarize your results of the two experiments using a few sentences. Make some conclusions about fight behavior in crayfish.

Further Investigation:
We have investigated whether winning and losing crayfish exhibit differences in the amount of fight behavior used, but are there differences in the types of behaviors used by male and female crayfish?

Do males and females differ in the types of fight behaviors used?
Use the class data to fill in Table 7.

Table 7: Total number of times behaviors occurred for each sex in experiment 2.

Behavior	Male	Female
Meral Spread		
Wrestling		
Clawing		

Based on the observed numbers of each behavior for males and females, do you see any differences in the amount of behaviors for each sex? Why or why not?

What problems are associated with this data? Why is it not appropriate to test for differences among male and female behavior?

Can you think of a better way to test for differences in fight behavior of male and female crayfish?

Note: This lab write-up was written by Letitia Reichart and adapted from other laboratory exercises from the following sources:
www.bio.davidson.edu/courses/invertzool/lab/ex8**crayfish**.pdf
http://caspar.bgsu.edu/~courses/Ethology/Labs/CrayfishAgon/
Escape behavior in cockroaches – Mike Webster, Washington State University