You and your teammates should now be familiar with the natural history and basic behaviors of *Betta splendens*. It is now time to consider a relevant, interesting animal behavior question to address, using this species as your model organism.

I. Research Tool: The Ethogram

To begin your ethological studies, you will first create an ethogram for *Betta splendens*. An ethogram is a comprehensive list or catalog of all the behaviors that an organism exhibits. Before you begin to quantify behaviors in an experiment to test a behavioral hypothesis, you must first gain an understanding of what is typical behavior for the species. What behaviors should you seek to observe? What behaviors occur in the environmental context that interests you? What sorts of behaviors are specific to your species? An ethogram represents the first step of the scientific process, making observations. You will follow in the footsteps of many natural historians before you in making observations, taking careful notes, and defining and categorizing the behaviors that you observe.

Spend time watching the *Betta splendens* individual in the bowl at your lab station. Observe the fish’s behavior and document what you see. For each behavior, name the behavior and give a clear definition that describes how that behavior can be differentiated from other behaviors. Feel free to observe multiple fish, but watch individuals long enough to get an idea of all of their behaviors. Begin by watching the fish undisturbed. When you have watched the individual undisturbed for at least ten minutes, and feel confident that you have documented many behaviors, you can investigate agonistic behaviors with a little manipulation of your fish. The sight of its own reflection in a mirror is enough to stimulate agonistic activity in a sufficiently aggressive male. (They do not recognize themselves, and think they see another male.) To observe the behaviors associated with agonistic display, slowly and carefully place a small mirror against the flat side of the bowl.

As you observe and attempt to define each behavior, consider exactly how the fish accomplished each movement. What anatomical parts of the animal were involved in the behavior? What position does the fish assume with respect to its reflection (head-on? sidelong?)? What was the context of the behavior? What other movements or behaviors does the fish employ in his display? Try to fully describe the action that the animal carries out, but avoid attempting to assign a function to that behavior.

As an example, consider observing a wild songbird. You might document several different behaviors. Perhaps you see the bird capture and eat an insect. This could be classified as two different behaviors (capturing; eating). Consider how the bird moves around. Does it fly from branch to branch? Does it hop? These might be 2 different locomotion behaviors. Does it make any sounds? Give displays? Some example behaviors for a songbird are listed in Table 1. Note that the definitions of each behavior include descriptions of what anatomical parts may be involved, and clearly differentiate between similar behaviors.

Observe the *Betta splendens* in the bowl at your station, and use the space below to list and define the behaviors you see. You should spend at least 10-15 carefully observing the fish, and document at least a dozen discrete behaviors. When you have finished cataloging the behaviors of the *Betta splendens*, you can begin to organize...
your list of behaviors. Think about the different types of behaviors you learned about in the previous lab. You may attempt to classify behaviors into broad categories (agonistic behaviors, foraging behaviors, social behaviors, reproductive behaviors, etc.)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description of behavior</th>
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<tbody>
<tr>
<td>Prey Capture</td>
<td>The bird pursues and catches an insect in its bill. May or may not include flight.</td>
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<tr>
<td>Feed</td>
<td>Feed – The bird is consuming food. This includes chewing the food.</td>
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<tr>
<td>Call</td>
<td>The bird produces simple, short vocalizations</td>
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<tr>
<td>Sing</td>
<td>The bird produces complex vocalizations of longer duration than calls</td>
</tr>
<tr>
<td>Wing Wave</td>
<td>The bird raises one or both wings, vibrating them away from the body. Occurs in agonistic situations</td>
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</table>

When you have completed documenting all of the behaviors of *Betta splendens*, focus on one specific individual for five minutes. **List the behaviors you will watch for in the first cell of each column of the ethogram (Table 2) at the end of this chapter.** Document which behaviors occur. You can use tally marks for behaviors that occur within the first 15 seconds of observation, the second 15 seconds, etc.

**II. Experimental Design: The Effect of Different Visual Stimuli on *Betta splendens* Behavior.**

Each team will be supplied with male and female *Bettas* in individual bowls. **NEVER PLACE TWO FISH IN THE SAME BOWL. SUCH IRRESPONSIBLE BEHAVIOR WILL RESULT IN YOUR BEING DISMISSED FROM THE LAB AND RECEIVING A ZERO FOR THIS LAB PROJECT. FURTHER DISCIPLINARY ACTION IS ALSO POSSIBLE.**

Also available are materials such as paper cutout models ("puppets") of male and female Bettas of different colors, different sizes, and different positions, mirrors of various sizes (and convex/concave). You might also consider using videos you find online, and display these to your fish on the screen of your pad or laptop computer. (This may require some engineering of a small "stage": a set-up that will allow you to (1) display the stimuli to your fish in a repeatable, consistent manner and (2) prevent damage to your computer.)

Consider what you have observed about Betta behavior, and ask a question related to the fish's behavior. It might be about aggression, agonistic display, mate choice/sexual selection, the “trade off” between conflicting evolutionary needs (e.g., the need to attract a mate vs. the need to avoid attracting predators). This will be for you and your team to decide. You might wish to spend five minutes with each team member writing down ideas based on your literature search and lab observations, and then share the ideas afterwards. You can then decide which ideas to use, and modify as appropriate. Devise an overall hypothesis based on your observation and question. Finally, devise mutually exclusive experimental hypotheses that can be tested with a rigorous experiment of your own design.
A. General Instructions for Observing Betta splendens behavior

1. Some individual fish are particularly sensitive or aggressive, and these may be stimulated even by the sight of brightly colored or patterned clothing. Avoid wearing such clothing to lab. Pale-colored clothing is the least likely to interfere with fish behavior.

2. Position your fish where it cannot see neighboring animals until you are ready to begin your experiment. Keep the visual barriers in place unless you are actually making observations of fish behavior.

3. Avoid abrupt movements when near the fish, and speak quietly. **Do not tap on the side of the fish bowl, as this creates a very loud, stressful noise for the fish.**

4. When recording the positions and movements of your Betta's display, note that the male will employ most of its fins, its gill opercula, and the associated branchiostegal membrane. A particularly energetic male may bend his body in tight angles. Note the orientation of the fish to its stimulus and record any changes in the coloration of your fish (for example, watch for color to fade or become brighter, or for color streaks to appear on various areas of the body.

5. Record the length of time of each behavior you observe, as well as its subjective aspects (for example, you might rate the strength of the display ("-" for weak, "+" for medium, "++" for strong—or variations on that theme, perhaps using numbers to rank degree of energy shown by the fish.) Do not stimulate the fish for longer than one minute for each trial, as longer trials may result in habituation to the stimulus.

6. Wait least 5 minutes between trials, allowing the fish to calm down completely. Between trials, be sure to block your subject's view of other fish, and avoid fast movements or loud noises. (Sound travels much more easily through water than through air. So don't say anything rude about the fish. They can hear you.)

7. It may be helpful to record the sequence of movements the fish uses in a full display.

8. Replicate each trial at least 3 times. Note any differences between replicates. What might cause such differences as time goes on?

9. Paper fish models will also elicit a response from males, but because they are stationery, they might not elicit as strong a response. However, the subject will also not habituate as quickly to a static model as to a mirror (why might this be the case?)

10. When using a paper model, move it slowly up to the subject and then wave it slightly to attract the subject's attention. Try to use similar technique and movement in each trial, to avoid introducing human error into your experiment.

11. Male Bettas respond strongly to the sight of another Betta, whether male or female. Consider the responses of your fish to same sex or different sex stimulus.

12. Refer to the fish anatomy diagram (Lab Chapter 2) when designing your ethogram.

B. Ethical Treatment of Experimental Subjects

Be kind to your fish, and we will be kind to you. Any student witnessed abusing animals in the laboratory will be immediately dismissed from the lab, receive a ZERO on the lab project and presentation, and be subject to possible additional disciplinary action.

Animals of any species used in experimental studies should always be treated with respect, and given the proper care and maintenance at all times.
C. Behavioral Research: An Example
Consider this imaginary example (you won’t be tempted to do anything similar):

Observation: All unicorns have horns. Male unicorns have either curly or straight horns, while females have small, straight horns.

Question: Does the shape of a male unicorn’s horn affect his reproductive success?

Overall Hypothesis: The shape of a male unicorn’s horn affects his attractiveness to female unicorns.

Experimental Hypotheses:
1. The shape of a male unicorn’s horn will not affect the number of females who are interested in mating with him.

2a. The shape of a male unicorn’s horn will affect the number of females who are willing to mate with him. (two-tailed)

2b. A male unicorn with a curly horn will attract more mates than a male unicorn with a straight horn. (one-tailed)

The next step would be to design an experiment to allow you to choose between your null and alternative hypotheses via statistical data analysis. If your data indicate rejection of the null hypothesis, you must then consider possible explanations for your results. (e.g., What is the shape of the male’s horn “telling” the female about his suitability as a mate?) This should lead you to additional questions and new hypotheses about this system. A lifetime of research awaits you!

D. Betta splendens Behavior: Developing Your Project
In designing your experiment, consider some of the following questions, and also try to conjure original questions of your own.

• What are the possible functions of agonistic display in male Betta splendens?

• Does agonistic display between two males change if there are females observing the contest?

• How might certain aspects of the display behavior been adaptive in the wild ancestors of these fish?

• Why do males bother with display? Why not just launch into battle?

• Wild Betta splendens are not as brightly colored, and do not have fins as long and showy as the domestic variety you are observing. How might artificial selection have affected the behavior of these fish? Do you think some or all of your results can be applied to wild populations? Which ones, if any, and why?

• You may or may not be aware that even species that can distinguish different wavelengths of light as “color” may not see colors the same way we do. An animal’s spectral sensitivity is the range of wavelengths of light that elicit a response in the visual system. Hue discrimination is the ability to distinguish different wavelengths of light as different stimuli (“colors”). If you plan on doing anything with color, then you must familiarize yourself with the literature on Betta splendens spectral sensitivity, hue discrimination, and other aspects of vision.

• What other environmental factors might affect the agonistic display of Betta splendens? Of what evolutionary and/or ecological significance are these factors?
Once your team has decided on its course of action, complete the items below.

**Observation:**

**Question:**

**Overall Hypothesis:**

**Null Hypothesis:**

**Alternative Hypothesis:**

Use the space below to describe your experimental methods. Include the type of statistical test you will use to analyze your data.

What is your prediction?

What parameter(s) will you record/measure?

How many replicates will you perform?

Describe your experimental methods:

What statistical test will you employ to analyze your results, and why is it appropriate?

When all teams have decided on their experiments and finished the outline of the design, each team should take five minutes to briefly explain to their colleagues/classmates what they intend to do, and why. Critique from the TAs and from classmates should be used to fine tune the experiment and make final changes before the experiment is actually performed.
Table 2. Ethogram for *Betta splendens*

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<th>Behavior</th>
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Table 3. Behavioral observations of *Betta splendens* during a five-minute period

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<th>Behavior:</th>
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