Agonistic Behavior in *Betta splendens*
Creating an Ethogram for *Betta splendens*

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You are now ready to develop an **ethogram** for your model organism, *Betta splendens*. Before you begin, please note some important ground rules.

I. Ethical Treatment of Experimental Subjects

Be kind to your fish. Not only is this the right thing to do, it will foster reliable experimental results. A stressed fish is not likely to exhibit normal behaviors, and your job as a researcher is to maintain conditions as stress-free and conducive to normal behavior as possible in a laboratory setting.

DO NOT

- handle the fish in any way
- move fish containers without your instructor's assistance
- introduce anything into the fish bowls, including food
- tap on the fish containers
- move heavy objects on the lab benches (avoid transmitted vibrations!)

Animals of any species used in experimental studies should always be treated with respect, and given proper care and maintenance at all times. We go to great lengths to ensure that the fish you will study have excellent food, water quality, and stress-free conditions. Please be sure to do your part. **Be familiar with the IACUC Guidelines in the PowerPoint presentation you have seen, and follow all rules your instructor gives you with respect to interaction with the fish.**

Any student found guilty of intentionally mishandling or abusing animals in the laboratory will (1) be **dismissed** from the lab immediately, (2) receive a **ZERO** on the lab project and presentation, and (3) be subject to possible additional **disciplinary action**.

II. Observing *Betta* behavior: General Instructions

A. For Best Results…

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

2. **Keep the visual barriers in place unless you are actively observing fish behavior**.

Position your fish where it cannot see neighboring animals until you are ready to begin your experiment.

3. **Gauge your behavior to minimize stress to the fish.**
   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.***

B. Observing Behavior of Fish at Rest

Before you begin, be sure you are familiar with *Betta* external anatomy. Refer to the fish anatomy diagram (last week's lab manual chapter) and describe behaviors precisely when designing your ethogram.

Spend some time watching the *Betta splendens* individual in the bowl at your lab station. Observe the fish's behavior and document what you see. **Spend at least 10-15 minutes carefully observing the fish, and document at least a dozen discrete behaviors.**

When you have finished cataloging the behaviors of the *Betta splendens*, organize your list of behaviors. Use the **Ethogram Template** (.docx) linked to the online syllabus for this week's lab to record this information. (The template also appears at the end of this chapter, but as a .pdf it is only for your information.)

For each behavior,

- **name the behavior**
- **give a clear definition** describing how that behavior can be differentiated from other behaviors.
- **attempt to classify a specific behavior into a general behavioral category** (agonistic, foraging, social, reproductive, etc.).

Feel free to observe multiple fish, but watch individuals long enough to get an idea of all of their behaviors.

When you have finished your observation, slowly and carefully replace the cloth cover over the bowl, blocking the fish's view of the outside world.

C. Observing Behavior of Fish in Response to Stimulus

After you have observed fish at rest for 10-15 minutes, you are ready to subject the fish to a stimulus to elicit agonistic behaviors. Obtain a mirror from the front desk and bring it to your station.

When all team members are ready, gently remove the cloth cover from the fish bowl and carefully press the mirror against the flat side of the bowl so that the fish can see the reflection and you can see its reactions.

Observe and record the fish's behavior for one minute.

1. A male *Betta* will employ most of its fins, its gill opercula, and the associated branchiostegal membrane in his displays. A particularly energetic male may bend his body in tight angles. Record
   - which body parts are involved in the display, and how
   - the fish's orientation to the stimulus (head on? sideways?)
   - the body's position and shape
   - the sequence of movements the fish uses in a full display

2. **Record any changes in the coloration of the fish. Watch for**
   - color to fade or become brighter
   - color streaks to appear on various areas of the body.

3. **Record the duration of each behavior you observe.**

4. **Record all subjective aspects of the behavior.**
   For example, you might rate the strength of the display
   - "-" for weak
   - "+" for medium
   - "++" for strong, etc.
   - or variations on that theme (e.g., ranking with numbers)

5. **Do not stimulate the fish for longer than one minute.**
   A longer trial may result in **habituation** to the stimulus.

At the end of one minute, remove the mirror and gently replace the cloth cover over the bowl. Allow the fish at least five minutes of recovery before making any other observations. Use this time to **enter the fish's observed agonistic behaviors in your ethogram with complete descriptions**.

When your ethogram is complete, *each team member should keep an electronic copy of it*. You will be referring to your ethogram to select particular behaviors to quantify when your team designs its research project.

Be thorough, and your ethogram will serve you well.

D. Timed Observations of Fish Behavior

Now that you have a complete ethogram, you can use it to perform a few practice experimental trials. You will perform **four practice trials**. This time, each team member should assume a specific role:

a. **Timer** (calls out 15 second intervals)
b. **Stimulus Presenter** (elicits behavior by showing fish a stimulus)
c. **Behavior Reporter** (watches the fish and calls out behaviors)
d. **Behavior Recorder** (records behaviors in table)

1. Select two or three **specific behaviors** (from your ethogram) to quantify.

2. Enter each of these in the far left (blue) column of the **Timed Observation Template** (linked to the syllabus for this week's lab).
   (***You may modify this template to suit your needs for your research project.***)

3. When all team members are in position and ready, the **Stimulus Presenter** should gently remove the cloth cover from the bowl and carefully press the mirror against the flat side of the bowl, as before.

4. Once the mirror is in position, **Timer** should start the timer.

5. **Behavior Reporter** is responsible for observing the fish and calling out behaviors as they occur.

6. Practice including duration. For example,
   At the onset of a branchiostegal membrane flare
   - **Reporter** calls "membrane flare start"
   - **Timer** calls flare onset time (in seconds)
   - **Recorder** writes flare onset time

   At the end of a branchiostegal membrane flare
   - **Reporter** calls "membrane flare stop"
   - **Timer** calls flare end time (in seconds)
   - **Recorder** writes flare end time

7. **At the end of one minute, end the trial**.
   - Remove the mirror
   - Gently cover the bowl
   - block your subject's view of other fish
   - avoid fast movements or loud noises.
   - Sound travels much more easily through water than through air.
   - Don't say anything rude about the fish. They can hear you.

8. **Wait least 5 minutes between trials.**

9. **Perform three more timed trials.**
   Observe a different fish (and use a new template) for each trial.

10. **Team members should switch roles for each trial** so that everyone gets a chance to practice each role at least once

IMPORTANT: Before you record observations for your research project, assign each team member the role s/he was best at performing in these practice runs.
Once your project is begun, team members should no longer switch roles. (Why?)

Submitting Your Results

Tally the total number of times each behavior occurred during your trial.

Before you leave lab today, your team must electronically submit a completed copy of each of the following to your lab instructor.

- **Ethogram**
- **Timed Observations (four trials)**

Do not leave the lab before your instructor confirms receipt of your two team assignments, or your team will be docked five points per member.

Ethogram Template (Sample – Usable template is linked to the syllabus)

Behavior	Description of behavior	General Category