

# Agonistic Behavior in *Betta splendens*

## Data Collection

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For the next two weeks, you will be performing experimental trials on *Betta splendens*. Your team should have its protocol ready, its spreadsheets prepared for data recording, and all team members aware of their roles and duties.

Before you begin, please recall some important ground rules.

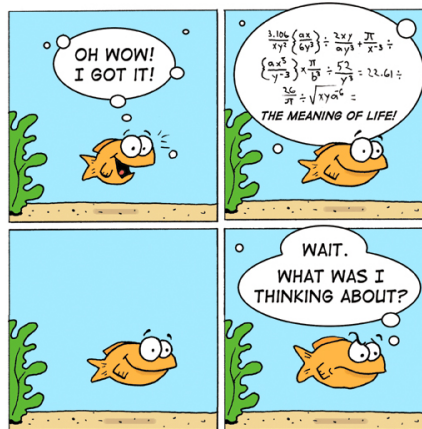
### I. Ethical Treatment of Experimental Subjects

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!)

**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.** Fish have excellent visual acuity. They could identify you in a lineup. If only they could remember things for longer than three seconds.\*



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THE TRAGEDY OF A THREE SECOND MEMORY

\* One of those cultural genes (memes) that goes around is the myth that fish have a memory lasting only three seconds. (Remember Dory from "Finding Nemo"? That was the joke.) In reality, some species have been shown capable of remembering certain things for five months. So don't think the Statute of Limitations will protect you.

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.

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 research days.**

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. General Instructions for Observations**

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.

#### **1. 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.
- all subjective aspects of the behavior

- the sequence of behaviors

**4. Note any changes in behavior as the trial progresses.**

What might cause such behavioral differences as time goes on?

**5. Do not stimulate the fish for longer than one minute for each trial.**

Longer trials may result in **habituation** to the stimulus.

**6. A one-minute behavior trial will count as one replicate.**

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

Allow the fish to calm down completely. When finished with a trial,

- block your subject's view of other fish
- avoid fast movements or loud noises.
- Sound travels much more easily through water than through air.

### **III. Trials and Replications**

There are **six numbered stations**. Each has

- One **male fish** in a bowl on the **left** (the **left fish**)
- One **male fish** in a bowl on the **right** (the **right fish**)
- One **female fish** in a rectangular container
- All fish have **ID codes** written on their containers in wax pencil.

**You will NOT be moving fish from station to station.**

Instead, each team will perform its experiments at one station for a set amount of time, and then the teams will rotate to change stations and use different fish for the first 12 trials.

In the second round, you will present the **alternative stimulus** to each of the fish you have already used in the first round, performing 12 more trials.

**Do not slide bowls along the lab bench surface, as this creates vibrations that can stress the fish.**

**If you need to move a fish container for a particular trial, ask your instructor for assistance.**

**All teams will perform a *paired* test on each subject.**

**Each fish will be the subject of two separate trials, each with one of two stimuli.**

**The order of the two stimuli must be varied from subject to subject to ensure random exposure to stimuli (and the confounding effect of habituation or other effect of two sequential trials). This is why you will flip a coin to determine the order of stimulus presentation at every other station.**

Your paired test will employ two different stimuli. As before, we will refer to them here as **stimulus A** and **stimulus B**. Your team may have chosen different labels, but the idea is the same.

One **station session** will consist of your team running a single one-minute trial on each of two fish at the station. You will flip a coin to determine the order of the stimuli.

Let's say the coin toss said use **stimulus A** on **left fish**, and **stimulus B** on **right fish**.

### **First Station:**

**Trial 1: left fish** receives **stimulus A**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 2: right fish** receives **stimulus B**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

At the end of two trials, your instructor will tell teams when to move to the next station. Each **station session** should last about 10-15 minutes.

### **Second Station:**

**Trial 3: left fish** receives **stimulus B**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 4: right fish** receives **stimulus A**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

**Notice that you have changed the order of stimuli at the second station.**

**This controls for the effects of a fish behaving differently simply because a stimulus is the *second one* to which it is exposed, and not because the stimulus was *different*.**

### **Third Station:**

Flip a coin again to determine the stimulus order for Trials 5 – 8.

Let's say this time the coin said to use **stimulus B** on **left fish**, and **stimulus A** on **right fish**.

**Trial 5: left fish** receives **stimulus B**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 6: right fish** receives **stimulus A**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

### **Fourth Station:**

**Trial 7:** left fish receives stimulus A

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 8:** right fish receives stimulus B

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

### **Fifth Station:**

Flip a coin to determine the stimulus order for Trials 9 – 12.

Let's say this time the coin once again said to use stimulus B on left fish, and stimulus A on right fish.

**Trial 9:** left fish receives stimulus B

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 10:** right fish receives stimulus A

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

### **Sixth Station:**

**Trial 11:** left fish receives stimulus A

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 12:** right fish receives stimulus B

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

## **When your team has completed 12 trials, you're halfway done.**

At the next change, you should be back at the station where you started.

Review the stimuli you presented to each of these fish in the first round.

You will now present each fish with the *alternative* stimulus, the one it did not receive in the first round. This will comprise your paired sample.

For example:

### **If you did this in the first round (first station):**

**Trial 1:** left fish receives stimulus A

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 2:** right fish receives stimulus B

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

**Then you will do this in the second round (first station):**

**Trial 13: left fish** receives **stimulus B**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and begin preparing for Trial 2.

**Trial 14: right fish** receives **stimulus A**

Five minute rest period begins at end of timed stimulus.

Cover the fish bowl and wait for instructor signal to change stations.

Do this for another 12 trials. At the end, you will have 2 trials for each fish, each with a different stimulus.

You will perform this same protocol again next week.

Before you start your second week of data collection, the fish in each laboratory room will be swapped. By the end of your two weeks of data collection, you will have paired trial samples from 24 different, individual fish.

**And then the fun begins! Data analysis!**