Designing an Authentic Behavioral Research Experience in the Classroom Using Siamese Fighting Fish, *Betta splendens*


Ms. Souza

Gianne Souza and Dr. Ronald M. Coleman

California State University, Sacramento Department of Biological Sciences


Dr. Coleman

MOTIVATION

Recently there have been major shifts in biology education towards emphasizing the scientific process to more closely reflect actual scientific practices. **Creating an authentic research experience that appropriately scaffolds the development of these complex skills remains challenging** in classroom settings. Common challenges include limited time available for working with large numbers of students, cramped working areas, and limited funding and materials resources. Using the Siamese Fighting Fish, or *Betta splendens*, **we have created an economically viable authentic animal behavioral research experience that is emotionally and intellectually engaging while emphasizing the development of skills critical to the scientific process**. Students will work collaboratively in pairs and as a class to perform novel research **investigating the relationship between temperature and surface air breathing behavior of *Betta splendens***. This basic animal model can be easily adapted to incorporate student creation of novel questions and student-driven design of experiments to address those questions.

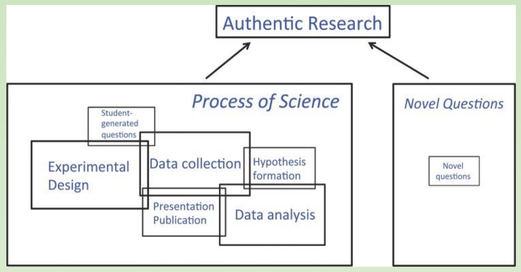

Figure 1. ***Two conceptions of authentic basic research.*** © 2014 R. M. Spell *et al. CBE-Life Science Education* © 2014 The American Society for Cell Biology. A national survey asked 279 Biology faculty to define an authentic research experience. Two overall themes emerged – the more common theme emphasizes the Process of Science and its constituent skill sets, while the less common theme emphasized creation of novel research questions.

SETTING UP BETTAS IN THE CLASSROOM

Housing for Bettas must be safe, space-saving, cost-effective, support maintenance of animal well-being, be easy to manipulate for different testing conditions, and facilitate clear observation of animals during experimentation and data collection.

Figure 2. ***Setting up Bettas in the Classroom.*** *A*: 1 gallon (3.8L) containers housing individual Bettas are submerged in a larger heated water bath to maintain constant temperature. *B*: Plastic water bath is protected from 100W submersible aquarium heater by shard of ceramic pot. Air stone circulates water to evenly heat containers. *C*: Bettas are visually blocked from one another to prevent exhaustion. *D*: Male bettas build bubblenests in ideal conditions.

Estimated Start Up Costs for a Classroom of 30 Students	Estimated Cost ($)
Bettas (1 per pair of students + 1 extra = 16 total) (est. $3/each)	$50
Containers (2 boxes of 12 CCW Plastic Square Jars, size SG)	$94
Tubs for Water Baths (3 Sterlite 28 qt/27L Tubs at $14 each)	$42
Aquarium Heaters (1/Water Bath = 3 total) (EHEIM Jager Submersible Aquarium Heater 100 Watt with Thermostat at $30 each)	$90
Air Pump (Tetra Whisper 10g Air Pump)	$5-10
Air Control (Aquarium Airline Tubing $3, AccuAir 4-Way Gang Valve $3, and Penn Plax 4-pack Air Stones $4)	$10
Cards (use scrap cardboard or paper) and laminate	FREE
Food (1 jar betta pellets)	$5
Small fish net	$3
Bucket (Home Depot 5-gallon Painter's bucket)	$3
Water Conditioner (Small bottle of Prime) (optional – dependent on tap water)	$5
Microfiber Cloths (Package from Dollar Store)	$2
Salt (Small box non-iodized Kosher salt from grocery store)	$2
Tank Decorations (optional)	VARIABLE
Total Estimated Costs	**$300 - 350**

OBSERVING AGGRESSIVE DISPLAYS IN BETTAS AT LOW (27°C) AND HIGH (30°C) TEMPERATURES

When two male bettas see one another, they typically begin an aggressive territorial display characterized by flaring (opening gill plates and spreading fins). We were interested in behavioral differences at different environmental temperatures. Twelve blue male veil-tail bettas were housed individually in the housing system shown in Figure 2. Animals were visually isolated from one another outside of test sessions.

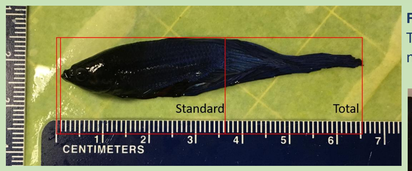

Figure 3 (left). ***Measuring Bettas.*** Standard and Total length (cm) of each betta was used to size-match sparring partners.

Figure 4 (right). ***Experimental Set-up.*** Containers of combatants were removed from the water baths and placed next to each other on the table for observation. Temperature of each container was recorded prior to removal of visual barrier. Every encounter was tallied in real time and video recorded. Containers were placed back into the water bath after the contest was observed.

Bettas were subjected to two different temperature treatments (Low 27°C; High 30°C) for at least 3 days prior to testing. Each male was observed in 10 minute long paired contests with 6 other males of comparable size, during which we quantified air breathing and observed flaring and tail beating. Bettas that consistently displayed frightened or disinterested behavior were excluded.

Figure 5 (left). ***Surface Air Breathing.*** Bettas can obtain oxygen from the air via surface breathing. Here the betta in the front has just taken a breath at the surface while displaying to an opponent. The leftover air bubble clinging to the surface of the water is circled in red.

EXPERIMENTAL RESULTS

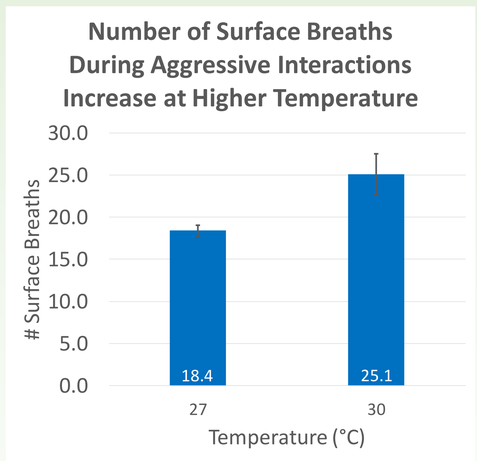

Figure 6. ***Number of Surface Breaths During Aggressive Interactions Increase at Higher Temperatures.*** 12 male bettas were maintained at target temperatures for at least 3 days prior to testing. Contests between size-matched pairs of males were observed for 10 minutes and number of surface breaths recorded. Each male was tested against 3 novel opponents 3 at each temperature.

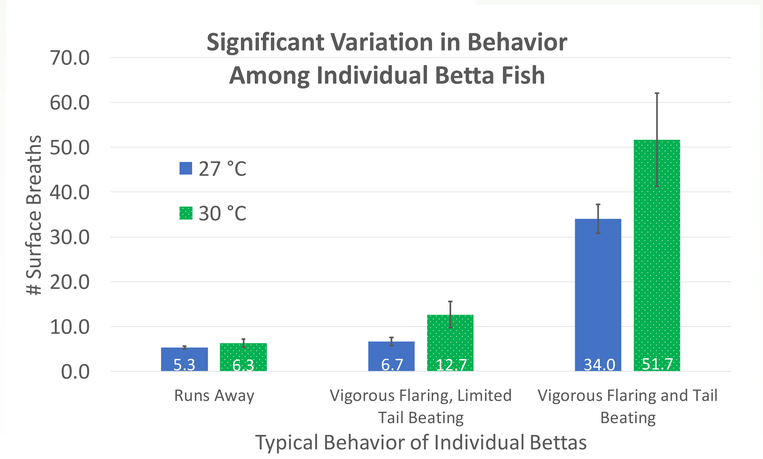

Figure 7. ***Significant Variation in Behavior Among Individual Betta Fish.*** Individuals exhibited consistent behavioral patterns during encounters, regardless of opponent. Bettas that consistently "run away" avoid all visual contact with opponent and swim away from the central barrier. Bettas that exhibited "vigorous flaring, limited tail beating" were consistently engaged with their opponent but did not tail beat often. Bettas with "vigorous flaring and tail beating" often beat their tails against the visual barrier. Three individual bettas of each category were chosen as representations above. Results are an average of 3 trials against different opponents at each temperature.

CONCLUSIONS

- Bettas at 30°C took on average 6.7 more surface breaths than bettas at 27°C during a 10 minute aggressive interaction.
- Individuals displayed unexpected variation in behavior patterns compared to other bettas ("running away" vs "vigorous flaring, limited tail beating" vs "vigorous flaring and tail beating") and were consistent across trials, opponents, and temperature.
- Bettas that consistently ran away took few surface breaths at both low and high temperatures.
- Among bettas that vigorously flared during encounters, those that were heavy tail beaters were also more likely to take more surface breaths, while those that did not tail beat often were less likely to breathe at the surface. Both types increased frequency of surface breathing at higher temperatures.

DISCUSSION

Experimental Results

Like most fishes, bettas are ectothermic and are entirely dependent on the surrounding temperature. As the temperature of the water increased, bettas that were actively engaged in aggressive displays also increased in the number of surface breaths. Bettas are facultative air breathers and can utilize gaseous oxygen. This increase in surface breathing at higher temperatures may be linked to higher metabolic demands during aggressive displays in warmer water.

Interestingly, individual bettas showed surprising variation in behavior during aggressive contests compared to their peers. Out of the 12 bettas tested, 2 showed consistent avoidant behavior and ran away from their opponents. These bettas had no significant difference in the number of surface breaths at low or high water temperatures. There were two different types of aggressive bettas: limited tail beaters and tail beaters. Bettas that tail beat often during contests also took more surface breaths, perhaps because tail beating is energetically taxing.

Classroom Considerations

The built-in thermostats on the aquarium heaters allow for easy manipulation of the environmental temperatures of the experimental bettas. Additionally, discussions on the effects of climate change on animal behavior and ecology can be easily tied in.

Counting the number of surface breaths during an aggressive interaction is relatively easy, but requires intense observation and attention to detail. The first 5 minutes of an encounter yield a usable number of breaths for student observations.

Additional classroom considerations include: unexpected diseases that may infect the bettas; potential impact of ambient room temperature; and statistics may be required for analyzing results. These considerations highlight the authenticity of this behavioral research experience and can be discussed as inherent considerations in science with the students.

REFERENCES

Spell RM, Guinan JA, Miller KR. Redefining Authentic Research Experiences in Introductory Biology Laboratories and Barriers to Their Implementation. CBE Life Sci Educ. 2014 Spring; 13(1):102-10.

ACKNOWLEDGEMENTS

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