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3D-Printed Fish Models for Testing Guppy Mate Choice

John F. Stewart[1], Michael E. Moore[1], J. Lance Forshee[1], Kathryn D. Boyles[2], Moria G. Harmon[1], Jason N. Bruck[3], and Donald P. French[1]

[1]Oklahoma State University, Life Sciences, West 501, Stillwater OK 74078 USA
[2]Stillwater OK 74078 USA
[3]Scottish Oceans Institute, East Sands, St. Andrews Fife, UK
(**john.stewart@okstate.edu; moomi@okstate.edu; jaylf@ostatemail.okstate.edu; kdboyle@ostatemail.okstate.edu; moria.harmon@okstate.edu; jb296@st-andrews.ac.uk; dfrench@okstate.edu**)

A study of proximate causation of mate choice can help to engage students in the topic of sexual selection. We developed a new and flexible method for observing mate choice behavior in guppies (*Poecilia reticulata*.) Students observe male preference for 3D-printed fish models of female fish of different sizes, colors, and shapes to determine what traits are important in sexual selection. In their first week, the students expose a male guppy to a standard suite of different 3D-printed models. They then request modifications for the next week and the week afterward. The investigation engages students' abilities to carefully observe animals, quantify those observations, and use evolutionary reasoning to communicate their findings in a scientific manuscript peer-reviewed by anonymous new scientists. The use of 3D printers overcomes a hurdle (crafting accurate models that can be manipulated) making this type of authentic research accessible to a large and broader audience.

Keywords: Guppies, Ethology, Sexual Selection, 3D-Printing

Introduction

Objectives:

In this laboratory series, students will:

1. Learn about guppy mate choice behavior.
2. Conduct an experiment testing guppy mate choice using 3D-printed female guppy models.
3. Generate a hypothesis about what kinds of features attract male guppies to mates.
4. Request a modified guppy model to test their hypothesis and then use that model.
5. Write about their results and discuss how they fit into our understanding of guppy mate choice.

In this investigation, groups of students spend 3 class periods collecting data and writing about male guppy mate choice. We designed the investigation to incorporate 5 groups of 2 to 4 students in a mixed-major introductory biology laboratory course at a large land grant university. In the associated lecture, students concurrently learn about evolution and sexual selection.

In our course, students write three drafts of manuscript-style laboratory reports over the course of 3 weeks. They give their first week draft to their laboratory instructor (typically a graduate student) for review. In the second and third weeks, they improve their work and submit their manuscript to a modified journal website for double-blind review. Their reviewers (generally instructors for other laboratory sections) request revision in the second week and grade the paper with a standard rubric in the third week.

Instructors will need to include institution-appropriate approval for including live vertebrates for teaching. Additionally, for long-term use of the investigation, the instructors will need to arrange for animal husbandry of the animals. Prior to conducting the laboratory, instructors will need to furnish presentation apparatuses for displaying the fish models to the subject fish. The instructors will need to print—or arrange to 3D-print—the models.

Student Outline*

Background

The Center for the Study of Sexual Selection in Fishes (CS^3F) is dedicated to understanding the ecological, evolutionary, physiological, and behavioral factors that shape the mating systems, mating strategies, and mate choices associated with fishes. CS^3F is currently focused on proximate causation for mate selection in guppies, *Poecilia reticulata,* especially the role of visual cues in mate choice. Proximate causation refers to the mechanisms involved (what stimuli, neurons, hormones, sensors play a role and how do they cause the behavior). The alternative, ultimate causation, refers to the factors involved in the evolution of the behavior. An excellent example of work on ultimate causation area is that of Endler (1980), who examined the effect of the presence of predators on guppy behavior and color patterns.

The general questions currently under study are those related to "Why are some guppies chosen as mates more frequently than others?" including:

- What visual characteristics of female guppies do males use to select mates?
- What visual characteristics of male guppies do females use to select mates?
- What behaviors influence mate choice?
- What influence do social factors have on mate choice?

Guppies, *Poecilia reticulata*, have been a research animal of interest to neuroscientists, evolutionary biologists and ethologists (animal behavior researchers) for several decades because of their small size, easy care, well-identified behaviors (Baerends *et al*. 1955), variable morphology (color, size, shape), and the excellent early work describing behavior and ecology. Guppies are sexually dimorphic (i.e., males and females look different) with males having patches of color that vary in number, size, position, hue, reflectivity, and intensity (Endler 1991, Houde 1997). Variations in color are found within and between populations in Trinidad, and these variations appear to be influenced by the presence of predators (Godin and Briggs 1996, Gong and Gibson 1996, Gong 1997, Houde 1997, Millar et al. 2006). Research on guppy behavior includes work on social interactions (Archard and Braithwaite 2011, Farr and Herrnkind 1974, Farr 1980), the effect of light on mating behavior (Endler 1987, Archard et al. 2009), the effect of size (Herdman et al. 2004, Dosen and Montgomerie 2004, Magellan et al. 2005), coloration (Houde and Torio 1992, Kodric-Brown 1993, Sathyan and Couldridge 2013), and immunity (López 1998) on mate choice.

Scientists in the CS^3F pursue research that fills gaps in or extends the understanding of mate selection and courtship behavior in *Poecilia reticulata* and more broadly to fishes in general. Similar species include other fish in the family Poecillidae, such as mollies (MacLaren et al. 2004, MacLaren 2006, MacLaren and Rowland 2006a, MacLaren and Rowland 2006b, Kozak et al. *2008*) and swordtails (MacLaren and Daniska 2008, Maclaren et al.2011, MacLaren and Fontaine 2012, MacLaren and Fontaine 2013). Work on unrelated fishes has also been informative. Among the most extensively studied are sticklebacks (*Gasterosteus aculeatus*) (e.g. Rowland 1982, Rowland 1989, Rowland 1995, Rowland and Sevenster 1985, Bakker and Rowland 1995, Rowland et al. 1995, Dzieweczynski and Rowland 2004), Siamese fighting fish (*Betta splendens*) (Dzieweczynski et al. 2006, Dzieweczynski and Leopard 2010) and many species of Cichlids (family Cichlidae) (e.g. Rowland 1975, Werner and Lotem 2006).

Recently, student researchers working with Dr. Jason Bruck in ZOOL 4413 Fish Biology at Oklahoma State University developed new research techniques to allow scientists to examine the cues that fish use to select mates. One technique involves using a 3D printer to create models to present to live fish and identifying their choices by recording their behavior toward each model. Using models to study fish behavior is a well-established technique (Rowland 1979, Rowland 1999). However, using a 3D printer is a new approach that appears to work and offers greater flexibility as your guest mentors have shown (Boyles et al. 2015). Using software, CS^3F researchers are able to expand on this work by creating models of different colors, sizes, dimensions, and shapes. Research at CS^3F has suggested male guppies are more attracted to larger females reflecting longer wavelengths (Francis et al. 2015, Majors et al. 2015).

Bruck's students also tested showing videos to the fish and observing their responses (Gregg et al. 2015, Newsom et al. 2015). These techniques have the potential to be combined in interesting ways. Many of the studies cited above used similar techniques, i.e. models (sometimes called "models") or video. In addition to their publication in *JIBI*, current and future CS^3F researchers follow a specific protocol so that all the data collected is uniform. This has allowed Dr. Bruck and his students to analyze data from previous CS^3F studies and prepare them for publication in national scientific journals. Building a database

of current and future data will allow for similar scholarly products and for you to share data for publication in JIBI. Depending on the needs of current researchers, you may be asked to make specific comparisons in your first week of data collection, then be free to test your own hypotheses in weeks two and three.

A. Recommended Skills & Knowledge

Before attending lab, new members of the CS^{3}F should be able to:

- explain the standard procedures for data collection used in the lab
- identify the anatomical features of male and female *Poecilia reticulata*
- identify all relevant courtship behaviors of male and female *Poecilia reticulata*
- record frequencies and durations of behaviors using the CS^{3}F standard protocols
- select and use Excel (or web-based applications) to perform appropriate statistical tests
- measure objects using calipers

It is important that you do the Pre-lab activities http://biol1114.okstate.edu/pre-labs/indexnew.cfm – especially those in which you watch the behavior of the fish. There are additional movies of the guppies' behavior for you to watch. You should also consider spending additional time in the LRC watching guppies. It is very important that you can recognize the different behaviors. Recording behaviors incorrectly (i.e. not in the same way other researchers record them) will affect your study and could ruin the chances that your guest mentors will be able to gather the data they need to publish their work.

It is VERY important that you can identify specific behaviors so you can record when each occurs. Please review the following short videos on the CS^{3}F web page very carefully before you try to record any behavior (or do the pre-labs).

1. Guppy Mating Overview (1 min 15 seconds)
2. Sigmoid behavior (7 seconds)
3. Sigmoid behavior with fish in outline (8 seconds)

In addition, it is recommended that you become familiar with the following terms and concepts to facilitate your searches for and understanding of research literature:

Courtship Display
Mating Strategy
Fitness
Intrasexual Selection (Competition)
Intersexual Selection (Mate Choice)
Polygyny
Good Genes Hypothesis
Handicap Hypothesis

The following journals are recommended as reference sources:

- *Animal Behaviour*
- *Behavioral Ecology*
- *Behavioral Ecology and Sociobiology*
- *Behaviour*
- *Environmental Biology of Fishes*
- *Ethology*
- *Evolution*
- *Proceedings of the Royal Society of London. Series B: Biological Sciences*
- *Journal of Introductory Biology Investigations*

B. Special Resources and Instructions

1. An important note about **lab attire** for these labs:

The presence of other objects that are brightly colored outside the tank may attract (or repel) the fish and interfere

with the experiments on the guppies' responses to different color models. Therefore, all researchers should wear only white, gray, or black clothing during these labs so they do not confound the results. The lab is equipped with some unfashionable trash bags with holes in them to cover up any brightly-hued attire. The CS^3F is not responsible for any shame that may accompany their use.

2. **Required Certification:** The CS^3F complies with government and university regulations regarding the care and use of animals. The CS^3F is monitored by the Institutional Animal Care and Use Committee (IACUC) for its adherence to the procedures identified in Animal Care and Use Protocols (ACUP) AS-10-12 and AS-14-15. All participants in the laboratory must comply with the approved procedures.

All researchers should adhere to the following guidelines for animal handling to ensure the safety of the researchers and the animals.

- Fish are not to be removed from or transferred between aquaria except by authorized personnel.
- Only models made or treated with approved substances may be placed in aquaria.
- All experimental procedures must reflect procedures in the approved ACUP.

Figure 1. Tank

3. **Experimental Tank Setup**

The CS^3F provides each research team with a 38 L aquarium equipped with a power filter and heater located at the center rear of the tank. You should carefully observe, record, and report the dimensions of, locations of objects in, and contents of the aquarium. Each tank is marked with 2 vertical lines down the front of the tank ¼ of the length from each end (Fig. 1). These designate the two "response zones" at the end and the "neutral zone" in the center. Each tank has all but one side blocked off with paper (to prevent the fish viewing other stimuli.)

4. Presentation Apparatus

The presentation device (Fig. 2) is a wooden meter stick cut to fit across the tank through. Two holes were drilled through the meter stick to hold the models in the tank at a precise distance from the side of the tank and each other. Notches cut into the ruler will help you position it correctly over the tank. Two binder clips secure the meter stick, holding the models, to the tank. These should already rest on the side lips of your tank. Tell your lab mentor if they are not.

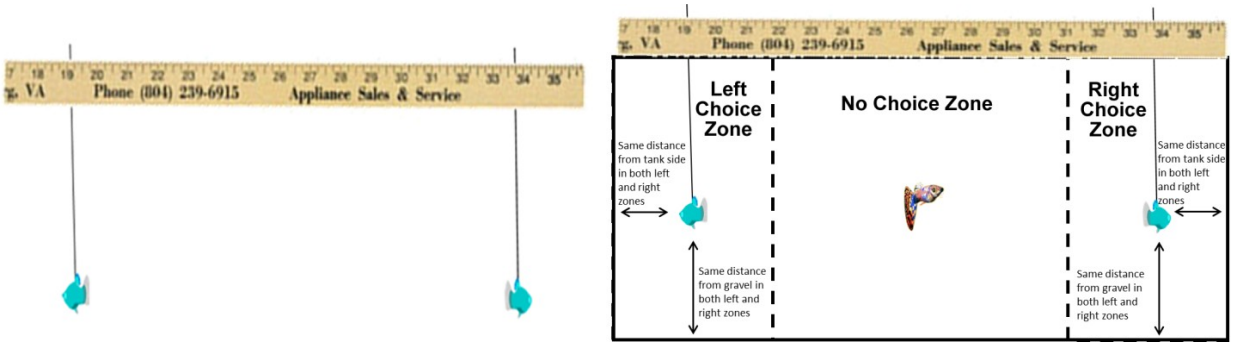

Figure 2. Presentation apparatus Left image is apparatus consisting of a meter stick cut and notched to fit across lip of tank. Right image is apparatus set into tank. Models should each rest at the same distance from the corresponding end of the tank, at the same distance from the gravel at the bottom of the tank and at the same distance from the rear of the tank. In this diagram, the heater and filter are not represented; they should be at the rear center of the tank.

5. Test Stimuli – Each lab should have a set of model fish. These "standard" models may vary depending on the

particular large scale investigation ongoing at the CS^{3}F. The Boyles-2014 (Boyles et al. 2015) set is

a. Control (2) - Intermediate size, Gray
b. Large Gray
c. Small Gray
d. Orange
e. Purple

You should carefully measure (Fig. 3) and record (Table 1) the dimensions and color characteristics of each model if you don't have them.

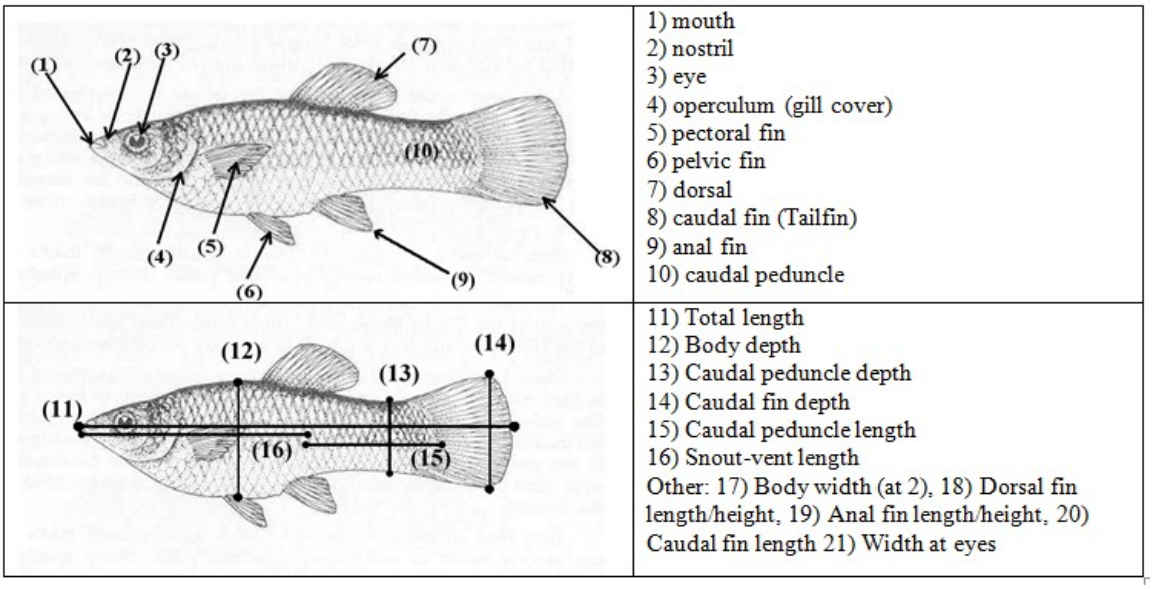

Figure 3. Guppy Model Measurement Guide.

6. Subjects

Each tank should have one male fish in it, which was placed in the tank just prior to the beginning of your lab period. You should wait at least 5 minutes for a fish to acclimate to its surroundings before you begin a trial. Additional subjects (male guppies) available for use will be in the tank labeled "Naïve Fish". Fish that have been exposed to all 5 models will be transferred into the "Used Fish" tank.

Table 1. Guppy Model Measurement Data.

Parameter	Dimensions for each fish model
Model Name →	
Total length (mm)	
Body depth (mm)	
Caudal peduncle depth (mm)	
Caudal fin depth (mm)	
Caudal peduncle length (mm)	
Snout-vent length (mm)	
Body width (mm)	
Dorsal fin length/height (mm)	
Caudal fin length (mm)	
Width at eyes (mm)	

7. Protocol – Be sure you **read and understand this whole section** before you begin anything.

a. **Preparation**

i. Do NOT begin a trial until instructed to do so.

ii. During each trial, you should present one Control model and one other model.

iii. Each fish should have all 5 pairs presented to it in the order indicated in Table 2. (Can you explain why?) One presentation is of 2 Controls. (Can you explain why?)

Table 2. Order of presentations of models by each lab group (lab table). Each lab group will present five (5) pairs of models to their subject fish.

Table	Presentation Pair 1	Presentation Pair 2	Presentation Pair 3	Presentation Pair 4	Presentation Pair 5
1	Control vs. Control	Control vs. large	Control vs. small	Control vs. orange	Control vs. purple
2	Control vs. large	Control vs. small	Control vs. orange	Control vs. purple	Control vs. Control
3	Control vs. small	Control vs. orange	Control vs. purple	Control vs. Control	Control vs. large
4	Control vs. orange	Control vs. purple	Control vs. Control	Control vs. large	Control vs. small
5	Control vs. purple	Control vs. Control	Control vs. large	Control vs. small	Control vs. orange

iv. For each trial, attach a control model at one end of your ruler. Decide which end of the tank that represents with the toss of a coin to randomize your data collection (i.e., remove observer selection bias). Attach another model to the other end of the ruler so that it will hang to the same tank depth by adjusting it such that the marks on the wire are visible against the ruler. Turn the models so the right side of each model faces the center of the tank.

v. Assign roles to each group member

1. One or two timers – one at each end zone; these team-members should record the total amount of time the fish spends in the zone the timer is watching by starting the stopwatch when the fish enters the zone and pausing it when the fish leaves.

2. One or two behavior recorders; These team-members record how many times the guppy performs one of the courtship behaviors see below) in the zone being observed and which zone it performs them in if there is only one recorder. If you have a group of two, make arrangements with your TA and guest mentor.

b. **Presentation** – each 5-minute presentation is for one pair of models

i. Wait till the fish is as close to the center of the tank as possible.

ii. Quickly lower the models into the tank without splashing and start a 5 minute timer.

iii. Each observer should begin measuring the fish behavior, immediately

1. "Timers" – should start and stop their stop watch as the male's head enters/leaves the corresponding "end zone"

2. Observers – Should record each occurrence of the following behaviors in their zone.

a. Gonopodal swings - Male guppies' sexual organs are called gonopodia. They are fins modified to deliver sperm. During courtship, a guppy may swing his

gonopodium to alert the female of his intentions.

b. Sigmoid curves (review videos of guppy behavior if you are unsure of what these look like) - guppies often curve his body into a C or S shape. Sometimes, they vibrate themselves.

c. Fin fanning - Male guppies may fan with their fins to attract female attention.

d. Biting - Male guppies often bite females in courtship. Frequently, they bite at the females' genital opening that is located posterior to the large bulge of the body on the ventral side of the fish.

iv. When the timer rings, the trial is over, withdraw the model and wait for the guppy to return to the center zone of the tank.

v. Repeat the presentation protocol with a different pair of models.

8. Post-Trial: After you have tested all of the pairs, tell your lab mentor so the guppy will be placed into the "Used Fish" tank so that it can recount the wonder and horror of his experience with his compatriots.

9. Manuscripts

Depending on the results of these first experiments, you may need to conduct more trials with more subjects or you may be ready to design more experiments. Because all CS^{3}F researchers will be sharing the results from ALL groups for this first experiment, it is unlikely that more trials will be necessary even to reach the standards for publication in professional scientific journals. For your first drafts of your manuscripts for JIBI, the shared data from all the fish in your lab will suffice.

While protocols are standard, readers of articles stemming from this research may not be familiar with the rationale behind many of the chosen procedures. Authors should include justifications for the choices of procedures in their manuscripts.

An important component for your first report will be the **future directions** in your discussion. In it, you should include the model(s) you would like to test in the future. You will follow the same protocol for displaying fish models. Therefore, each lab section can create up to 5 models; each group creates only one new model.

10. **Designing your next experiment**

You can see the information on the models CS^{3}F webpage. You can also "order" your model using the same link. You are allowed to change the color and/or one physical parameter of any of the models that have been created.

Your model will become part of a database of models to be used by others in the future. Future CS^{3}F scientists are expected to cite you, just as current CS^{3}F should cite those whose models they used to give them credit for the model design. You will also want to contact whoever created or used the model to let them know or to share data (with credit).

When designing your future experiments, remember: Each fish can only be exposed to a model once, each fish occupies a tank for the duration of the lab period, the total stock of available fish is limited, and experiments need to test multiple subjects (fish) to determine how they respond to a particular model. Therefore, CS^{3}F researchers must coordinate with the other groups in their labs so that all available fish are exposed expose to all models. A suitable plan should be included in all reports. Researchers may have to collect data over the next two lab sessions to reach a sufficient subject pool for statistical analysis.

Models are being built in 213 LSW. When the door is open, you are welcome to see models being made. If you want to learn more about the software and how to make models, your mentor can provide you with the contact information for those constructing the models.

An alternative for your future directions: It turns out that fish like YouTube, too.

Other students in Dr. Bruck's class tested the feasibility of using tablets to show videos to guppies. Newsom et al. (2015) tested mate choice in female guppies using two tablets looping video of different males. They did not find much preference, but their sample size was not high, and there was a slight but intriguing trend. Videos have been used successfully in other investigations (Trainer and Basolo 2000). Several questions emerged from the work:

- Would different guppy videos work better?
- Would testing mate choice among males using the same method give better results?

In scientific literature, papers that describe new methodologies are immensely useful (and highly cited) because they

provide standard, well tested, techniques that save other researchers time and allow them compare their work with others. The CS³F encourages researchers to consider developing and publishing work describing the best approach(es) to using videos to test guppy mate choice behavior. The CS³F has 15" tablets that can be used for presentations, however videos used by Newsom et al. (2015) were scaled to appear normal size on 10" tablets.

Surface Tablets can be checked out of the OSU library and used for this purpose.

Pursuing a different line of research, Gregg et al. (2015) used their tablets to show videos of predators to guppies. The guppies fled videos of predators and responded differently to different predators. They appeared less afraid of slow predators, even though they had been raised in captivity and never seen a real predator. Just search for videos online and find potential that guppies could reasonably fear.

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Materials

Assuming that your classroom has 5 groups conducting the experiment, for each class, you will need:

- 5 male guppies (though you should keep spare guppies, since they are not long-lived species).
- 5 10-gallon aquaria marked to indicate choice zones, plus 2 other tanks for non-experimental storage.
- Nylon small fish net.
- 5 wooden yardsticks (modified).
- 20 binder clips.
- 10 clothespins.
- Masking tape.
- 10+ 0.039 inch (0.99 mm) music wire lengths.
- Heat source, such as a Bunsen burner or a butane lighter.
- 3D-printed female guppy models.
- Means to time trials and collect data.

Notes for the Instructor

Setup Instructions

Animal Husbandry

Running these laboratories necessitates that the instructors fulfill the requirements of their institution's Institutional Animal Care and Use Committee (IACUC), because federal law mandates that educational use of vertebrates is certified as humane. The procedures in this investigation are not especially risky for guppies, but maintaining guppies for your laboratory can be difficult. You will need to decide whether you want to maintain a constant breeding stock of guppies or only use the guppies for the experiment. Both approaches include advantages and disadvantages.

To maintain a stock of fish, you will need larger (20 or more gallon) breeding tanks with both male and female fish. You will need to keep aquarium gravel at the bottom of the tank to give fry (juvenile) guppies a place to avoid the cannibalistic adults. Enrichment items in the breeding tank such as plants and ceramic models can help maintain the population over the long term. Keep a proper feeding and aquarium maintenance schedule. You will need pumps to circulate and aerate the water, heaters for constant temperature, and day-night cycled lighting to aid the guppies' circadian rhythm. (As they are native to tropical shallow water, they expect a constant day-night cycle.) You will need to include a means of filtering the water to avoid the build-up of fish waste. We have found that biofilters that use a culture of bacteria do the best job of keeping the tank fresh and healthy. Even if you maintain a breeding stock of fish, you should occasionally add newly purchased individuals to prevent inbreeding.

After purchasing guppies for laboratory use, gently acclimate them to their new environment. Carefully adjust the conditions of your aquaria to a consistent temperature, salinity, and pH (Hieronimus and Vriends 1993). Small variations can kill guppies. When introducing newly purchased fish, first lower the plastic bag in which they came into a prepared tank (aquarium lights should be turned off) to allow temperature and pH to equilibrate. Next, transfer your fish into a small fish transfer container with a 50%-50% mixture of water from the bag the fish came in and your aquarium water. Lower the transfer container into the aquarium (hook to side) to allow the fish to acclimate to this mixture before placing fish into the aquarium. Do not pour the water from the store bag or the 50%-50% mixture into the aquarium as they will be too highly concentrated in waste and can disrupt the aquarium balance.

For the best results, prior to running the experiment, separate the males for the experiment from females for at least two weeks. After this sex segregation period, they will respond better to the models. If you maintain a breeding stock, a separate tank will aid you in this endeavor. You will have to temporarily maintain a fully outfitted tank if you want to only use the guppies for one investigation, since those guppies will need to survive more than a month (2 weeks in sex segregation, plus 3 weeks for the investigation.) After the investigation, you will need to find a way to humanely dispose of the fish or make them available for adoption, unless you maintain a breeding stock. This issue must be addressed in any IACUC proposal.

Presentation Apparatus and Display Tanks

Each presentation apparatus is a simple wooden yardstick adjusted to rest easily in position during the experiment. First, you must drill holes for the 0.039" music wire into the yardstick through the top and bottom face of the ruler at the 11-inch and 25-inch marks using a 1/32" drill bit. (Generally, florists use music wire more than musicians. It was originally for piano strings, but hobbyists and others found it quite useful for all manner of activities, so it is produced for hobby shops and the like.) Because most 1/32" drill bits are not long enough to penetrate the entire height of a common yardstick, it is imperative to carefully line the holes on the top and the bottom. We have found that a drill press and clamps are very helpful in this endeavor. Tape clothespins to the tops of the yardsticks to secure the piano wire after insertion into the yardsticks. Next, cut notches at the 8-inch and 28-inch marks into the yardsticks to allow them to rest securely on the lips of the display tanks.

To attach 3D-printed models to the wire, heat the end of the wire with an open flame and press it into the

model. The wire should melt its way in. Hold the wire and the model still for a moment so that the plastic around the wire cools enough to hold the two items together. Next, use electrical tape to create a stop for the wire after insertion into the yardstick. The stop will ensure that the model is about halfway between the tank bed and water surface. (For us, that is about 17.5 cm from the point at which the wire inserts into the model.)

To prepare your display tanks, mark choice zones on the viewing side of the tank. Use a permanent marker to make vertical markings 12.5 cm from each side of the tank. During the experiment, make sure that the lids of the tanks do not cover or impede the placement of the presentation apparatus. Also, put interior features such as filters and pumps into the center of the tanks to prevent intrinsic side biases. We have had trouble with reflective glass distracting subjects. To guard against that, try using a product like SeaView Background Mounting and Illumination Solution to secure aquarium backgrounds to your tanks (See http://www.seaview info.com/ for more information). We have found that this product nearly eliminates reflection and glare in the tanks. Add two binder clips to each side of the tank, leaving a tight space in between for the presentation apparatus to rest. That space should be in the center of each side.

3D-Printing Models

You will need a 3D-printer that uses polylactic acid (PLA) plastic, which is a thermoplastic derivative of corn. Acrylonitrile butadiene styrene (ABS) is another commonly used 3D-printing plastic, but it is not as safe to use with live animals, so it will not be as easy to clear with an IACUC. You will need to become familiar with your printer's interface to provide it with printing instructions. We use three base sizes (40 mm control, 36 mm small, and 44 mm large) of models as well as 3 different colors of plastic (Makerbot Warm Gray for control and True Orange and True Purple for treatments) in the basic setup of the experiment. Print the basic 3D models (Available at http://osu-hhmi.okstate.edu/2-uncategorised/87-able-2016-supp-files) for the first trials, keeping in mind that on most printers, you can print multiple models of the same color in the same print run so long as they all fit on the print bed.

We use AutoDesk MeshMixer (See http://mesh mixer.com/) to modify models. The software is easy to use, but you will need to learn how to do so. There are a number of videos that will show you how to operate MeshMixer, and that is probably the best way to learn on your own. You can export those files into 3D model files that match a format that your 3D printer software can read.

Running the Trials

Trial tanks must be the same temperature and pH as the holding tank. Prior to a set of trials, make sure that there is a lone and healthy male guppy in each of the trial tanks. Allow them at least 10 minutes to acclimate to their new tanks. In the meantime, students should measure their models' dimensions for their records. When the students are ready, you may begin the trials. We have traditionally used one master classroom countdown timer to avoid staggered and confusing alarms, but it is likely best when the students start the trials with their guppies in the middle non-choice zone of the tanks. Recently, we developed Software (Available at http://osu-hhmi.okstate.edu/2-uncategorised/87-able-2016-supp-files) for scoring these particular trial runs, and with it, students can start their own countdown timers without interfering with other students' trials.

Before each trial, students should prepare their presentation apparatuses. Students will need to randomize the sides for each model, except in the case of the control-control pairing. For the colored models, each group should ensure that the colors appear on opposite sides from each other. Before each trial, students should prepare their presentation apparatuses. Students will need to randomize the sides for each model, except in the case of the control-control pairing. For the colored models, each group should ensure that the colors appear on opposite sides from each other in subsequent runs—i.e., if they ran purple on the left, then they should run orange on the right. Likewise, for the differently sized models, the small and the large models should be on the same sides in subsequent runs so that neither side of the tank tended to have a larger guppy for the pairings. When a trial begins, the students should lower their presentation apparatuses into the tank quickly but without splashing. They should make sure to rest the notches on the tank walls in between the preset binder clips.

During the trial, students will need to track cumulative time spent in each choice zone and count the numbers of gonopodial swings, bites, sigmoidal curves, and fin fanning events that they observe for each choice zone. It is important that the students can recognize those behaviors. Students can usually detect bites, but the other three behaviors are not as obvious (though as mating behaviors, they are usually quite clear.) When the five-minute trial is over, students should remove their presentation appparatuses and pass them to the next groups. Allow each fish at least two minutes to rest between trials. When all trials are over, transfer the guppies to a common tank. If you have more laboratory groups coming in, then make sure that the fish are in a separate storage tank to avoid reusing them.

Subsequent Trials and Data Analysis

In our laboratory class, each investigation takes three weeks, and in weeks two and three of the guppy mate choice experiment, students use models with their own modification requests. The teaching

assistants ask each group of students to provide modification requests to a postdoctoral fellow who uses MeshMixer and a 3D-printer to fulfill the requests. Printed models are available for students by the next week. The rapid turnaround for 3D- printing is what allows students to have their own personalized models in time for the second and third weeks. Each classroom can then select which models to use as controls (i.e., an orange 40 mm fish as control and an orange 44 mm fish as the treatment.) Over the next two weeks, students can collect enough data for 10 data points, assuming that there are 5 guppies that produce data over 5 trials each per week for 5 groups.

We have used Wilcoxon sign-rank tests for analysis, though we may switch to a more appropriate Friedman test. The students do not necessarily need to understand the mathematics behind the statistical tests, but they do need to understand how to interpret their outputs. Given small sample sizes, many groups of students may see nearly significant *p*-values, and they will need help interpreting them. From week to week, you will likely need to reuse guppies, something that technically results in pseudoreplication, so you will need to either ignore it or mention to the students that it would be a potential problem for a real research study.

In our investigations, students write one paper over the course of the three weeks. At the end of the first session, they turn in a draft to their TA who provides feedback. In week two, they submit their manuscript to a modified journal webpage, and the editor distributes the manuscripts to sets of three anonymous reviewers (who TA other sections) for comments. At the end of the last session, the students resubmit their work to the journal website, and the reviewers grade those papers according to a common rubric. For the guppy mate choice investigation, students write about the background of mate choice, how they conducted their experiment, their results, and a discussion with interpretation of their results. For guppy mate choice, they need to connect what they observed to sexual selection and evolution.

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About the Authors

John Stewart is a postdoctoral fellow at Oklahoma State University and works to create authentic research experiences for undergraduates in the classroom and through helping students get involved in research with faculty.

Michael Moore is a Ph.D. candidate at Oklahoma State University studying how students respond to flipped classrooms.

Lance Forshee is a Ph.D. candidate at Oklahoma State University who studies the interrelation of students learning chemistry concepts and introductory biology concepts such as the citric acid cycle.

Kathryn Boyles is a recent Oklahoma State University graduate in Fisheries who helped to originally conceive the experiment and design the base 3D models for the models.

Moria Harmon is a lecturer at Oklahoma State University and lab coordinator for BIOL 1114 Introductory Biology who helped with the related animal husbandry requirements in the experiments designed for ZOOL 4413 Fish Biology and with developing the CS3F investigation in BIOL 1114 Introductory Biology.

Jason Bruck was a visiting professor at Oklahoma State University researching cetacean ethology who developed the investigation in ZOOL 4413 Fish Biology and helped design the original experiment.

Donald French is a professor at Oklahoma State University who serves as the coordinator for Biol 1114 Introductory Biology and conducts research focused on active learning and authentic research as mechanisms for increasing persistence and performance in introductory biology courses.

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