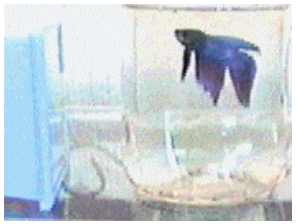
Effect of Estrogen on Male Fighting Fish Behavior



INTRODUCTION

Ethology is the zoological study of animal behavior. Ethologists are generally interested in innate or instinctive responses (genetically-programmed behaviors with little or no learned component). There are several advantages of instincts over learning. The animal does not require experience to perform the behavior; this is especially important if the initial response of the animal is vital to survival. Secondly, instincts do not require extensive neural coding for information storage and modification (and is therefore appropriate for animals with simple nervous systems). As an example, orb-weaving spiders build a perfect web on their first attempt (despite having no prior experience with webs for most species). Not to build a web, or to take the time learning to modify an imperfect web would result in starvation since spiders require webs to capture prey. A major cost of instincts over learning is their inflexibility in the face of environmental change. Innate responses control to one degree or another the feeding, sexual, and social behaviors of all animals (including man). For many animals these mechanisms drive them to choose a particular habitat or food. The study of communication is one of the more interesting aspects of animal behavior. Although much of our own language depends on learned responses, most organisms rely instead on genetically-coded rules of grammar and vocabulary. Three major forms of communication have been described among animals: chemical, visual, and mechanical (including touch and communication by sounds or vibrations). Of these three methods, transmitting messages by chemicals is probably the most primitive.

During the past decade or so, it has been clear that pollution in the environment affects the behavior of a variety of animals. Light pollution from cities disrupts migration in birds and insects, while thermal pollution from power stations changes the mating season for animals as diverse as fish, frogs, and manatees. Pesticides, industrial waste, and seemingly innocuous products (such as many plastics) leach into the environment and affect behavior directly (hormone-disrupters, neurotoxins), or indirectly (illness, interfering with development, etc.). Over this time researchers have identified a number of "environmental hormones" (also known as "hormone mimics" or "endocrine disrupters"). In most cases these compounds affect estrogen hormone systems and have led to the feminization of males; although some animals, including female polar bears, have been masculineized by exposure to hormone-disrupting chemicals. Bird, alligator, frog, and fish populations have been shown to respond to these chemicals in the environment. Among humans, research is currently underway to determine if these pollutants may be related to rises in prostate and ovarian cancers. The ever-growing list of these agents include several restricted or banned pesticides--such as DDT, kepone, heptachlor, dieldrin, mirex, and toxophene. Some polychlorinated biphenyls (PCBs) exhibit these disruptive properties, as do certain combustion pollutants, ingredients in plastics, breakdown products in detergents and a number of pesticides and herbicides. Even trace amounts of natural estrogens released in human female urine have been shown to affect fish populations.

A review of hormone disrupting chemicals can be found here. (http://www.mindfully.org/Pesticide/Hormone-Mimics-In-Food.htm). Related information on a variety of animal taxa are at these links:

- More frog troubles: Herbicides may Emasculate Wild Males (http://www.anapsid.org/frogdecline2.html)
- Source of Chemicals that Feminize Lake Mead Fish Discovered (http://www2.nature.nps.gov/YearinReview/yir98/chapter04/chapter04pg5.html)
- The Gender Benders (Alligators and birds; http://www.sciencenews.org/pages/sn_edpik/ls_7.htm)
- Mandelson's Birds Face Hormone Disruption Threat (http://www.foe.co.uk/resource/press_releases/19980121094053.html)
- Polar bears (http://news.bbc.co.uk/1/hi/sci/tech/906832.stm)
- The Demise of Human Sperm (http://www.alkalizeforhealth.net/Lspermdamage2.htm)

In today's laboratory we will explore the effects of estradiol on the behavior of male Siamese fighting fish (*Betta splendens*). In preparation for the lab half of the males have been exposed to a 0.10 mg solution of estradiol for one week (this is equivalent to the dose of two birth-control pills). Estradiol enters the fish (most likely through the gills) and is expected to decrease the normally aggressive behavior shown by these males.

MATERIALS NEEDED:

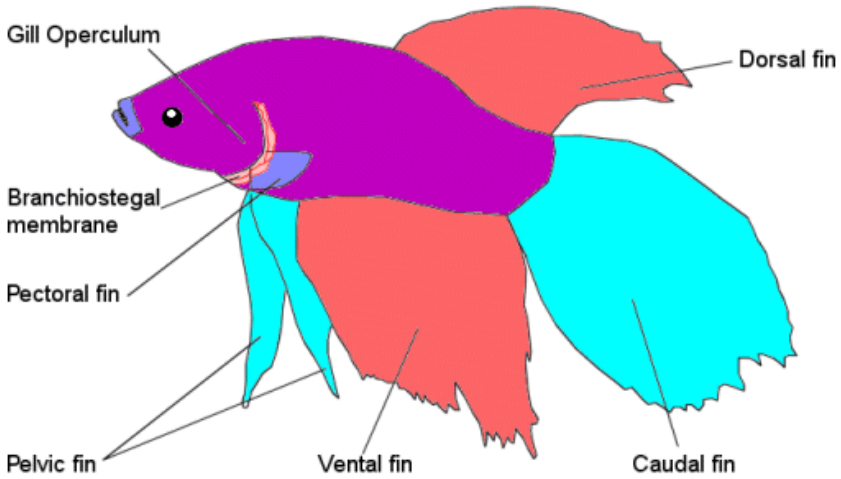
- Male Siamese fighting fish (*Betta splendens*); one dosed, one unexposed to estradiol per group of two or three students (Take an even and an odd-numbered fish; you will find out which have been dosed at the completion of the laboratory).
- Mirrors, and one- two liter flat-sided containers with water.
- Depending on the availability of time, a few female *Betta splendens* to serve as stimuli to males.

Several terms are commonly used in the ethological literature to describe the behavior patterns of animals. Fixed action patterns are genetically-determined sequences of movement. These stereotyped behaviors vary little from one individual to another and are often species-specific. Releasers are found in the environment external and send out stimuli that release fixed action patterns. In the previous experiment, the courtship behavior displayed by your male roaches varied little from one male to another and represents a fixed action pattern. The pheromone deposited

by the female roaches was the releaser for the males' courtship display. Releasers can be more complex than the simple presence or absence of a chemical as in the above example. In this exercise, we will explore the combination of complex visual stimuli that serve as releasers to trigger the fixed action pattern of aggressive (agonistic) display by male Siamese fighting fish (*Betta splendens*). The exchange of aggressive signals allows the fish to set up dominance-subordinate relationships. If the two combatants are not confined to a single bowl, the subordinate animal can flee and the conflict is resolved without the danger of bloodshed. As you shall see, visual communication is an effective way of showing varying levels of motivation and is an example of a graded response.

Low-level Aggression by Male *Betta splendens* A movie is here

High-level aggression by male *Betta splendens*
Movie of transition from low-level aggression (alert) to high-level aggression (here)



Appendages used during an aggressive display by male *Betta splendens*.

PROCEDURE:

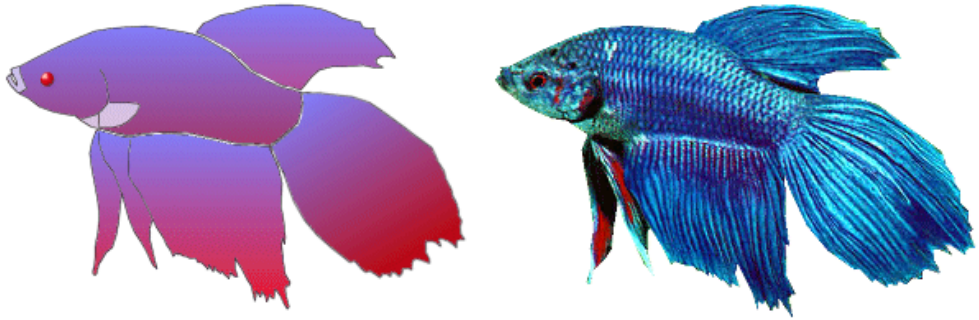
CAUTION: Students with brightly-patterned clothing may excite some male fighting fish. Put on a lab coat or smock to hide these colors from the fish during the experiment.

1. Work in groups of two or three. Collect the following materials: two male fighting fish (even- and odd-numbered), a mirror, and stopwatch. Do not position your fish where it can see neighboring animals. Avoid abrupt movements when near the fish and speak quietly. Do not tap on the side of the fish bowl.
2. Response of Male Siamese Fighting Fish to Mirror Stimulation**.** Male fighting fish vary in their aggressive response and the best results will be from the more aggressive males. For aggressive animals, mirror stimulation is enough to elicit an agonistic display. To assay the relative aggressiveness of your animal and to demonstrate the behaviors associated with agonistic display, slowly move a mirror to the flat side of the bowl and observe the response of the fish to its own reflection. Do not hold the mirror near the bowl for more than a minute or so. If the fish is over-stimulated, habituation to the stimulus will occur and the animal may not respond during the later parts of the experiment. Note: One of the fish may not respond fully to mirror stimulation. This is expected, since one animal has been exposed to estrogen.
3. Record the movements associated with the agonistic display. An aggressive animal will use most of their fins and gill structures in the display. The opercula will open and the branchiostegal membrane will swell. Highly aggressive males will often bend their bodies at an agile close to 90Ú (to display both the open opercula and the flared fins). Pay attention to the orientation of the animal to its mirror image and record any changes in the coloration of your fish (look for either "bleaching" or brightening in color). Remove the mirror and record the time it was removed. This fish will be used again and should be allowed at least 5 minutes to calm down completely. To the best of your ability, record the sequence of movements associated with full display. Do the gill opercula open first; or are these structures one of the last to be displayed during an agonistic encounter?
4. Next, repeat procedure 3 with your second fish and note any differences in the aggressive behavior between the two.
5. Return to the first fish and redo the procedure outlined in step three. This time, record the time it takes for the fish to notice, then approach the mirror, followed by the other components of the display. Also record the strength of the display ("-" for weak, "+" for medium, "++" for strong). Record the time for the strongest response your animal shows for each of the appendages. Stop after five minutes, even if the fish doesn't fully respond. If your fish shows a full response within the five minutes, you can stop at that time (don't continue to harass him). A data sheet is located at the end of this page or use the Excel-format sheet found here. Record the end-time so that this fish can rest at least five minutes before being used again.
6. Now run procedure five with the other fish. Record the time and strength of the display as before.
7. Response of Male Fighting Fish to Models. For the remainder of the experiment, paper fish models are used to stimulate a response from males. Models are preferable for long-term testing since the males will not respond as strongly, will not continuously escalate their response, and will not habituate as quickly. Cut out the fish model shown below, and then tape an applicator stick to the model. Move the models slowly up to the fish and then wave them slightly to attract the male's attention. Try to present the models in the same way (same speed of presentation, and similar movements). Record the response of the male to the models as before. You may record up to five minutes if the male does not exhibit a full display. If the male shows a full display sooner, you should stop the presentation of the model and then go to the nest step.
8. Run step number 7 for the other fish. Record the time and strength of the displays as before.
9. Re-run step 7 for the first fish.
10. Re-run step 8 for the second fish.
11. Encounters Between Your Male and Another Fish. When you are finished with the models, move your fish near another group's male and observe the response to a living animal. Pair an

even-numbered fish with an odd-numbered fish. Do not transfer the fish to the same bowl! Determine which animal is the most aggressive. Compare the data your neighbors collected for the models with your own data and determine if the winner in the encounter was generally more responsive. If available and time permits, a female (or another species of fish) can be used as a stimulus for one of the more aggressive males. Briefly describe the behavior of the male to the female. Record the "winner" (the animal with the more-aggressive display).

12. Run procedure # 11 pairing the other two fish with one another.

More on the behavior, ecology, and feeding of fighting fish is here. *Betta* breeding is here and here.



Model *Betta splendens* to be used for puppets. Cut me out! Try the model first. If your fish doesn't respond, use the realistic model instead and indicate in your data that you are using the realistic fish instead of the model. If you have to use the realistic fish, that means that your fish has lower aggression than usual.

Fish Number	Odd or even?	Stimulus	Time until Notice	Time until Approach	Time of Pelvic Fin Flare	Pelvic Fin Intensity (-,+,++)	Time of Ventral Fin Flare	Ventral Fin Intensity (-,+,++)	Time of Caudal Fin Flare	Caudal Fin Intensity (-,+,++)	Time of Dorsal Fin Flare	Dorsal Fin Intensity (-,+,++)	Time of Operc. Flare	Operc. Flare Intensity (-,+,++)	Time of Branch. Membrane flare	Branch. Mem. Intensity (-,+,++)	Time until 90° body response	Intensity of 90° body response (-,+,++)
_____	Odd	Mirror	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	Odd	Puppet	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	Odd	Puppet	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	Even	Mirror	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	Even	Puppet	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____
_____	Even	Puppet	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____	_____