Science Unit: ***Urban Biology***

Lesson 2: ***Can Fish Detect Odours?***

School year: 2007/2008

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Grade level: Presented to grades 6-7; suitable for 4-7 with age-appropriate modifications.

Duration of lesson: 1 session, lasting 1.5 hrs

Notes: **This lesson has been written as if the class is finishing "Stream to Sea" (formerly "Salmonids in the Classroom"), but it is applicable to other species as well.** The differences are mainly in how the home tank and test tanks are set up, as different species have different requirements for temperature, oxygenation, and pH. If not using salmon, the easiest subject species would be goldfish. Set up the home tank as directed by the pet store.

If using salmon fry, test them at least a week after the yolk sac has been absorbed. Goldfish purchased from pet stores are usually adults and can be tested when they appear comfortable in their home tank.

As soon as appropriate after the experiment, the fish should be released (the Stream to Sea website listed in the Reference section gives deadlines for release of coho or chum fry) or donated (aquariums, schools, families with fish tanks, even some pet stores), unless the school using the lesson has a permanently maintained aquarium of its own.

Objectives

Learn some facts about fish anatomy, especially olfactory organs (organs of smell).

Understand the importance of olfaction to fishes, and how fishes are affected by human pollution of waterways.

Set up and run an experiment in animal behavior, using the scientific method.

Learn how to care for living animals before, during and after an experiment.

Background Information

Most fishes use smell (olfaction) more than vision to find food. Olfaction is an essential sense for fishes for other reasons as well:

a) Catfish may use smell to detect dominant individuals.

b) Goldfish, minnows and guppies (among others) use smell to detect an injured comrade and the presence of a predator.

c) Salmonids use smell to help find their way back to their natal streams for spawning.

d) Males of many species also use smell to detect ripe females (when the females ovulate they release a fluid from their ovaries that leaks into the water). Female goldfish release another substance as well that causes an increase in milt (sperm) production by males.

e) Many fishes exhibit parental care. At night, they maintain knowledge of their brood's location through smell.

f) Many fishes rely on olfaction for migration at night, when vision isn't useful.

Fishes can detect chemical substances by smell or taste. In the case of olfaction, the sensory cells are situated at the bottom of two pits (one L and one R) in front of the eyes. Each pit is connected to the outside by a single opening or a pair of openings (the nares). Water enters the pits through the nares by natural currents, by swimming movements, or by rhythmic changes in pit shape caused by the respiratory motions of the nearby mouth. The olfactory nerve relays stimuli to the brain.

The position of the nose (inside rather than outside, like our own) contributes to streamlining and efficiency during swimming. (For the same reason, fish lack external ears!)

Pollutants can hide smells, making food-finding, predator avoidance, mate recognition and night migration more difficult. As well, pollution can mask odours that salmonids need to find their home streams at spawning time. Everything soluble that we dump into water can be perceived by fishes and can affect them, often unfavourably. Students should make the connection between clean streams, healthy fish, and human behavior, and should understand that we all affect ecosystem health in the world beyond the classroom.

Vocabulary

Olfaction	Sense of smell
Chemoreception	Senses of smell and taste; the two are closely connected (consider how things taste when you have a cold and your sense of smell is blocked)
Nares	The openings into the olfactory pits.

Materials

- Several 10-gal (40L) test tanks (1 tank per group of students)
- One 20-gal (80L) home tank with lid and appropriate lighting, temperature, oxygen and pH; see Notes
- Styrofoam for bottom and 3 sides of each test tank (leave one long side open) if using salmon fry
- Dry or frozen fish food
- Stopwatches (2 per group)
- Thermometers (1 per group)
- Bendable straws (4 per group)
- Duct tape
- Disposable plastic pipettes (2-3 per group)
- 2-3 reservoir buckets with lids, each 25-30 gal, (100L) for conditioned water for the test tanks
- Grease pencil or heavy magic marker
- For each reservoir bucket: air stone, tubing, and pump for oxygenation
- Small dip nets
- Ammonia and pH test kits
- Dechlorinator solution (e.g. Aqua-Safe)
- 100-ml glass beakers (2 per group) for plain and food water
- Two 500-ml glass beakers (one for plain water and one for "food water"); covers all groups
- Coffee filters and funnel to strain fish food from water to make "food water"
- Data sheets and pencils
- Heaters for home tank and reservoir buckets, if needed by the species
- Frozen gel packs and frozen pop bottles (containing water) to cool

	being used (but see Notes)	the water in the test tank if using salmon fry
• White vinegar for rinsing test tanks	Cup (1 per group) to hold fish during transport from and to the home tank	Pails with handles to bring tap water to reservoir buckets.
• Clean plastic cups (2/group) to hold food-scented and plain water for pipetting		

In the Classroom

1. Introduction (to be done during the week before the experiment)

- Hook the students with surprising and interesting facts about how fishes use smell. Before presenting the facts, ask the students what they know about the sense of smell in fishes. (If they are doing "Stream to Sea" they will probably bring up homing behavior in salmon.) Ask the students if they know where the nose of a fish is located and show them diagrams (see the first two websites in the Reference section).

  The facts are as follows:

  - Sharks have an amazing ability to smell injured prey. In fact, for most fishes smell is essential for finding food. (We also can find our food by smell as well as vision, but vision is not as valuable in water as in air.)
  - The females of many fish species put out an odor advertising their readiness to spawn, and the males respond with courtship and milt (sperm) production.
  - Minnows and many other fishes can detect when a group member has been harmed (the damaged skin secretes an "alarm substance"), and they take evasive action.
  - In some species of catfish, members of a group can determine the dominant fish in their group by the smell of that individual.
  - Adult salmonids can use smell to find their way up to their home stream to spawn, once they arrive at the mouths of their home rivers. **We will be testing salmon fry to see if they have developed a sense of smell early in life.**
  - Anemone fish learn the odour of their home anemone and use their sense of smell to find it when they return from foraging.

- Point out that pollution can mask smells and also damage olfactory organs. Ask the students for examples of how they could pollute a waterbody.

- Introduce the fish they will be using, giving a thumbnail sketch: whether its habitat is clear or murky water, what it eats, and any interesting points learned from other sources (such as when visiting a pet store). If the class is using salmon, this information will have been discussed previously.

  NOTE: It is easiest if the species being used tolerates room-temperature water, as goldfish do, but if salmon are used, putting 1-2" Styrofoam around 3 sides and the bottom of each test tank should keep the temperature relatively constant for the 10 min the fish will be in the test tank.

- Tell the students that they will be learning how to set up and run an experiment in animal behavior using the scientific method, as well as gaining some experience in animal care and handling.
- Briefly describe the experiment: Each group of students will expose one fish in a tank simultaneously to plain water and food-scented water (introduced at opposite ends of the tank), and over 5 min measure how much time the fish spends on each side of the tank. (The midpoint of the tank will be marked by a vertical dark line on the outside of the glass.) Groups will pool their results and run a statistical test on them.
- List the sequence of steps in the scientific method: observation, question, hypothesis (an educated guess about the answer to the question being asked), prediction, test, conclusions. The students should understand and be comfortable with these terms by the end of the week, and should be able to locate most of them in the paper by Metzgar (1967) (see the Reference section):

  1) **Null hypothesis (H_o):** In an experiment, the hypothesis of no difference. For example, if we were asking whether a hungry fish would spend more time on the side of the test tank containing food-scented water or the side containing plain water, the H_o would state that the fish exhibited no preference. Encourage the students to state the H_o in their own words before giving them help.

  2) **Alternative hypothesis (H_a):** A hypothesis that there is a preference for one condition over the other; in this instance, that a hungry fish prefers food-scented water to plain water. Encourage the students as for H_o.

  3) **Prediction:** An "If…then…" statement that always follows from a hypothesis. Using the example above, the prediction for H_a would be "**If** a hungry fish is given a choice between food-scented and plain water, **then** it will choose food-scented water." The corresponding prediction for H_o would be "**If** a hungry fish is given a choice between food-scented and plain water, **then** it will exhibit no preference." Again, encourage the students to formulate their own predictions for H_o and H_a.

  4) **Test:** An experiment is a test of the prediction.

  5) **Control test**: The point of an experiment is to find out whether varying one condition causes a particular outcome, while holding all other conditions constant. A control test exposes the fish to the same situation it will experience during the experiment, but without variation in the condition. In this experiment, the control is a test tank containing only plain water, in which two students (one at each end of the tank) simultaneously squirt in plain water through the straws. If the fish shows no preference for either side of the tank, assume that there are no unknown factors (differences in ambient light or in the "squirting style" of the students) that could influence the results of the experiment, and continue with the test of H_a. Students may find the concept of a control somewhat confusing so it is worth spending some time on this.

  6) **Variable:** The factor being examined. In this experiment, the variable is the smell of food (present or absent).

  7) **Replicate:** A copy. If two or more groups obtain the same response, that response can be relied upon more than if there were only one group doing the work, because the sample size is greater.

- Introduce the concept of statistics and explain the Chi-square test (X^2). This statistical test must be explained during the week before the experiment; **because it must be done on the control before the experiment can be run.** See the Appendix for an explanation of how to do this test. Make a handout of this section of the Appendix for the students to work with during the week.

- Organise the students into groups. Assign each student in a group (or have them assign themselves) the tasks they will do on the day of the experiment. Assuming 6 students per group, these tasks are:
    - Timekeepers (2 students, one per side)
    - Observers (2 students, one per side)
    - Recorder (1 student)
    - Student responsible for getting the fish from its home tank and replacing it after the experiment (1)

- In the coming week, each group should do the following:
    - Write out its version of H_o and H_a as well as the prediction for each hypothesis
    - Review the statistics handout and ask any questions that arise
    - Identify the steps of the scientific method in the paper by Metzgar
    - Help set up the reservoir buckets. These buckets will hold the water for the test tanks, and must be similar to that in the home tank (temperature, oxygenation, pH). First run tap water for 30 min, then add it to the reservoir buckets. Add dechlorinating solution (e.g. AquaSafe). Each bucket will also need an airstone which should be attached by tubing to a small pump. Put a lid loosely over each bucket.

  Note that for salmonids it will not be possible to cool the water below room temperature in the reservoir buckets; it will have to be cooled in the test tanks (see section below on Setup).

2. Safety guidelines

- Mop up spills so that no one slips on the floor
- Wash your hands with soap and rinse well before and after the experiment
- No food or drink near any of the tanks
- No banging on any of the tanks or on the table
- Do not attempt to capture fish from the home tank with your hands; use the dip nets. As well, the dip net should be moved slowly through the water so as not to frighten the fish. Return the fish to its home tank as soon as the experiment is over. The well-being of the animals should always be your first priority.

3. Science experiment (written as if for one group)

a) Setup

- Do not feed the fish on the morning of the experiment. You can feed it when it is back in its home tank.
- Clean your test tank with a splash of white vinegar and tap water. The tap water should have been running for 30 min to clear contaminants in the pipes.

- Dry the outside and draw a vertical line down the middle on one of the long sides, using a grease pencil or magic marker. This is the viewing side.
- If using salmon, put 1-2" (2-4 cm) thick Styrofoam around three sides and underneath the test tank to reduce heat loss. If using goldfish, put dark paper around three sides of the test tank. (**Note to teacher: ask the students why this is a good idea.**)
- Fill each 500-ml beaker with plain water. Add a small amount (1-2 tbsp) (~20mL) of fish food to one beaker to soak, and stir occasionally.
- At each end of the tank, do as follows: Push one straw into another and secure with duct tape. Leave the top straw straight, but bend the bottom straw so that its short arm (outflow arm) faces into the tank. Attach the joined straws to the tank above the waterline, again using duct tape. The outflow arms should be at equal depths, halfway to the bottom.
- Using a plastic pipette and water from the reservoir bucket, insert the tip of the pipette into the top end of the straw and practice squirting it through each outflow arm.
- Strain the food out of the water using coffee filters (unbleached paper filters work well). Filter 2-3 times to be sure no food particles remain. The water may be slightly colored by pigments in the food but this is unavoidable. You now have one beaker with plain water and one with food-scented water.
- Fill the test tank with water from the reservoir bucket, and add 1-2 frozen gel packs and/or 1-2 frozen 500-ml pop bottles to cool the water down. You cannot run the experiment until the temperature in the test tank is within one degree (C) of that in the home tank. (If using goldfish, room temperature water is satisfactory.)
- Nothing is to be put in the test tank except water and, if required, the gel packs or pop bottles.

b) Experiment

- Introduce the fish to the test tank and give it 5 min to become accustomed to its new situation. If it stays in one corner or on one side, be patient. Fish have different "personalities" and a timid individual may take more than 5 min to adjust.
- During this time, obtain food-scented water and plain water in separate clean plastic cups. You will pipette water from these cups into the straws.
- Control: Two students, one at each end of the tank, simultaneously introduce plain water using pipettes to squirt the water down the straws. (Five squirts are sufficient.) Over the next 5 min, record how much time the fish spends on each side of the tank. One student will be responsible for timing each side. Do the χ^2 test on the results. If your control is satisfactory, you can proceed with the experiment. If not, try to solve the problem.
- Experiment: Simultaneous introduction as before, but one student will use food-scented water and the other will use plain water. Over the next 5 min, record the time the fish spends on each side of the tank.
- Return the fish to its home tank.
- In your own group, use your χ^2 worksheet to analyse the results. The data will be pooled for this part of the work.

4. Closure Discussion

- Why couldn't the control data be pooled?
- Why is it so important to minimize the stress on the fish?
- What problems did you encounter, and how did you solve them?
- Review the importance of smell to the fish species you used.

5. References

<http://badmanstropicalfish.com/anatomy.html> Badman's Tropical Fish site. Accessed May 2008.

<http://www.starfish.govt.nz/science/images-for-download/bony-fish.gif> External features of a typical bony fish. Accessed May 2008.

<http://www.salmonidsintheclassroom.ca/> "Stream to Sea" website of Fisheries & Oceans. Comprehensive information on care of salmon from egg to fry stage. Accessed May 2008.

Metzgar, L.H., 1967. An experimental comparison of screech owl predation on resident and transient white-footed mice (*Peromyscus leucopus*). J. Mammalogy 48 (3): 387-391.

Reebs, S., 2001. Fish Behavior in the Aquarium and in the Wild. Cornell Univ. Press, Ithaca.

6. Appendix

a. Introduction to the χ^2 (chi-square) test

If every time you run the experiment (using a different fish each time) the fish goes toward the food-scented water, you could conclude that these fishes have a preference directly from your observations. If exactly half of the fish go to each environment, the absence of a preference is also easy to see. However, during an experiment there is usually an unequal number of fish found in the two environments (for example, in 5 cases a fish goes to one side, in 11 cases a fish goes to the other). This makes it harder to determine if this species of fish has a preference.

Note that we are assuming here that males and females behave similarly, and we are ignoring possible age-related differences!

Statistics is the science of collecting, organizing and interpreting numerical data. Statistics are used to calculate the probability of an event occurring randomly and the purpose of statistics is to form unbiased conclusions. Statistical analysis has many diverse applications including sports predictions, the polling of election outcome(s) and analysis of experimental data.

Did your fish really have a preference for one of the environments you offered it or was the variation you saw simply due to chance? To answer this question, we will use the **Chi-square (χ^2)** test.

The χ^2 test tells us whether or not we can reject the null hypothesis (**H_o**). Remember: the null hypothesis states that a hungry fish has no preference for one environment over another. If, as a result of the χ^2 test, you **can** reject the null hypothesis, the **alternate hypothesis (H_a:** there **is** a preference) may offer a plausible explanation for the results. Alternate hypotheses are never truly accepted, but do indicate a direction for further experimentation. This is one of the central maxims of science.

If as a result of your calculated χ^2 value, you **cannot** reject the null hypothesis, the alternate hypothesis is rejected. **This DOES NOT mean that your experiment was a failure. Why?**

If you find yourself in this situation, what would be your next step?

Realize also that accepting a hypothesis does not necessarily mean the hypothesis is true. Although most of your evidence may support this hypothesis, conflicting evidence may invalidate your hypothesis in time. Remember that years ago the world was believed to be flat. The Earth was also believed to be the centre of the universe!

b. How to do the χ^2 test

Suppose you want to know whether there are different numbers of cars and bicycles passing by the window of your classroom between noon and 1 pm. The variable is the type of transportation being used.

Hypotheses for this experiment: H_a: Commuters have a preference for one type of transportation. H_O: Commuters have *no preference* for either type of transportation.

You count the cars and bicycles during this time on two different days and gather the data as in the example below:

	Cars	Bicycles
Day 1 (Replicate 1)	21	47
Day 2 (Replicate 2)	35	37
Observed Totals	56	84

Observed ratio: 56 cars: 84 bicycles

Note: You must ADD the data from replicate 1 and replicate 2 together (you do NOT average it) to get the **observed totals** and the **observed ratio.**

For the χ^2 analysis, you also need an **expected ratio.** This will be based on the null hypothesis. The null hypothesis predicts that there will be no difference in mode of transportation, and it therefore predicts an expected ratio of 1 car: 1 bicycle. To get this ratio from your data, add together the observed ratio (56 cars + 84 bicycles = 140 vehicles) and divide by 2 = 70. Since your null hypothesis states that there is no preference for one mode of transportation, there will be 70 cars and 70 bicycles (a 1:1 ratio).

The χ^2 **formula** is: $\chi^2 = \Sigma \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$

In order to facilitate the use of this formula, we can arrange our data in table form.

	Cars	Bicycles
Observed	56	84
Expected	70	70
Observed – Expected	-14	14
(Observed – Expected)^2	196	196
$\Sigma \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$	2.8 + 2.8 = 5.6 = χ^2	

The **Σ** in the χ^2 formula means that we **add** the results from the two columns in the table, 2.8 + 2.8, to yield our final χ^2 value of **5.6.**

Now that you have calculated a χ^2 value for your control experiment, compare it to a predetermined **critical value** to determine whether you can reject the null hypothesis or not. The critical values are found in the attached table. The selection of your critical value depends on two factors: **degrees of freedom** and **probability.**

Degrees of freedom: The degree of freedom (df) is calculated as one less than the number of conditions (in this case, transportation) that you are testing. The formula is written: df = n – 1. As there were two types of transportation, df = 1. Degrees of freedom are listed in the left-hand column of the table.

Probability: In any experiment, some of the results will be due to chance. Think of tossing a coin 100 times. You should get heads exactly half of the time, but you probably won't due to chance. The probability or **p-value** makes allowances for this. Scientists around the world have agreed to accept a 5% probability (p = 0.05) that their results are due to chance. Probability or p-values are tabulated in the accompanying chart. Look for the $P_{.05}$ column.

Align the $P_{.05}$ column with the df = 1 line on the table. You should arrive at **3.84**, which is your **critical value.** Since we arrive at our critical value by aligning $P_{.05}$ with the degrees of freedom, you can see that it is important that you know the correct degree of freedom in your experiment.

If your calculated χ^2 value is *greater than 3.84***,** you can *reject the null hypothesis.* The difference between your data and the null hypothesis (no preference) was too large to be explained by chance. The commuters *appear* to be demonstrating a preference for one type of transportation. The alternate hypothesis may offer a plausible explanation of your results. Remember that this does not make the alternate hypothesis true; new data may change the picture in time.

If your calculated value is *less than or equal to 3.84, you cannot reject the null hypothesis.* You have insufficient evidence against the null hypothesis to reject it. This means that any apparent preference you observed was *due to random variation or chance.*

In our car/bicycle example, our calculated χ^2 value of 5.6 is greater than 3.84. We reject the null hypothesis; the alternate hypothesis *may* be true. There was a statistically greater number of bicycles than cars passing our window during that hour. If χ^2 had been less than or equal to 3.84, we would not have been able to reject the null hypothesis. We would have to refine our experiment or test a new alternate hypothesis.

c. Analysis of your results

1) Control test

As you now know, this is set up exactly the same way as your experimental test, but without the variable being tested (in this case, the food-scented water).

Set up your data in this table to calculate the χ^2 value of the control experiment:

Control Test	Right side of test tank	Left side of test tank
Observed		
Expected		
Observed – Expected		
(Observed – Expected)^2		
Σ $\frac{(\text{Observed – Expected})^2}{\text{Expected}}$	+	=

What is the calculated χ^2 value for your control experiment? ______________________

Is your calculated χ^2 value higher or lower than the critical value? __________________

What can you conclude from these results? ___________________________________

If the calculated χ^2 for your control experiment is <u>lower</u> than your critical value, you can be confident that your control is valid and no other variables are influencing the behavior of the fish. You can now complete the experiment.

If your calculated χ^2 for the control experiment is <u>higher</u> than the critical value, you should adjust your setup (e.g. ambient light, student pipetting technique) and try again.

2) Experiment

It is important for the groups to pool their data in this section. Therefore, although each group will do the χ^2 independently, it will be on the same data set. Compare your results in a class discussion.

Variable: _________________________________

Arrange the <u>pooled</u> data from your experiment in table form to calculate the χ^2:

Experiment 1	Form of variable (food-scented water)	Form of variable (plain water)
Observed		
Expected		
Observed – Expected		
$(\text{Observed} - \text{Expected})^2$		
$\Sigma \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$	+	=

The χ^2 value for your pooled data is______

What is your df for this experiment? ____

Is your calculated χ^2 greater, less than or equal to the critical value? ______

Can you reject your null hypothesis? ______

What conclusion can you draw from these results? ____________________________________

NAME______________________ DATE______________________

MY EXPERIMENT ON FISH

What I did:

What I discovered: