Chapter 3

Size-Selective Feeding of Zooplankton by Fish

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Introduction

In this laboratory, students test the size-selective predation hypothesis of feeding by vertebrates, first proposed by Brooks and Dodson in 1965. We offered this exercise mainly to third- or fourth-year limnology students, but it would be equally appropriate for students of ecology or first-year biology students being introduced to community ecology. Laboratory sections of 2.5 hours duration are more than adequate for this exercise.

In our experience, results from this experiment have been unambiguous. These results are notable because of the myriad of variables that are often difficult to control in community ecology experiments. Employment of the electivity coefficient will help introduce students to quantitative ecology. Dissection of the fish stomach leads many students to explore the whole animal.

Materials

1. Five gallon aquaria, at least one for every three to four students.

2. Bluegill sunfish, one per student.

3. *Daphnia magna* or other *Daphnia* species of mixed sizes, at least 500 per tank.

4. Dissecting microscopes.

5. Petri dishes, each containing a circle of 1-mm graph paper.

6. Sugar-ethanol solution: a mixture of 4 g dextrose and 100 ml (70%) ethanol.

7. Aquarium nets, one per tank and one for each jar of anesthetic.

8. A net for filtering the zooplankton. (A 250-ml wide-mouth polyethylene bottle may be used. Saw off the bottom and saw out the top of the cap leaving only the ring with the threads for screwing it on. Place a piece of netting [mesh size, 75 μm] over the top and replace the cap ring. Invert the bottle and you have a fine plankton filter.)

9. Probes for manipulating the plankton, made from insect pins with the heads removed stuck into dowels the size of pencils.

10. Small dissecting scissors.

11. Anesthetic for euthanizing the fish, MS 222 made up according to manufacturer's instructions, 1 liter in wide-mouth jars is plenty. Four fish may be placed in each jar simultaneously.

Notes for Instructors

This laboratory requires the use of the bluegill sunfish, a vertebrate, and consequently you will undoubtedly need to file a protocol with an Animal Resources Officer (or equivalent) at your institution. The anesthesia MS 222 that we have suggested, was the anesthesia required by the Animal Resources Office at Cornell, and the requirements may be different elsewhere.

Bluegills of 4–5 cm may be ordered from biological supply houses such as Carolina Biological Supply Co. Other small sunfish, such as the pumpkinseed, would probably work as well, but we have never used them for the study. During warm weather, we have had several lots arrive at least partially dead, so be sure to order them in advance to be sure you have enough. It is also easy to seine the fish from a pond. Check with your state fish and game agency to see what kind of permit you would need to collect them yourself. Do not throw the fish into any pond if you have excess fish after the exercise is completed, unless you have collected them from that pond. If you want to return excess fish to a pond that they did not come from originally, check with your state game warden to make sure that it is legal and make sure that the pond already contains the species.

If you collect the fish yourself, collect enough pond water to fill their aquaria. This water should be filtered through a plankton net to remove extraneous organisms. If you have ordered fish, use spring water, filtered pond water, or aged tap water in the aquaria. Take care that your water source does not contain any toxic materials that may be harmful to either fish or *Daphnia* sp. Bluegills can be trained to eat commercial flake fish food, but make sure that they are hungry the day of the experiment.

Acquiring *Daphnia* sp. or other large Cladocera can easily be done by collecting them with a plankton net during warm weather. Select a pond that contains few small fish. It may take a few tries to find the right pond. You will need at least 500 *Daphnia* per aquarium for each four students. They can also be ordered from one of the biological supply houses. If you are buying them, order *Daphnia magna*. They are large enough to be seen with the naked eye.

Daphnia may be propagated in several gallon jars filled two-thirds with suitable spring or aged tap water. For food, *Daphnia* do well on unicultures of green algae such as *Chlorella* sp. or *Scenedesmus* sp. However, it is easy to maintain an algal culture for *Daphnia* by scraping algae from an overgrown aquarium and adding this to a gallon jar containing nitrogen-rich water taken from a fish tank. Install a bubbler and keep the culture well lit. Periodically add more fish-tank water. When the culture appears green, add about 10 ml to the *Daphnia* jars every few days. Do not place a bubbler in the *Daphnia* jars, but illuminate them so that the algae keep the water oxygenated. Bubbles under their carapaces cause *Daphnia* to float to the surface and die. For the same reason, take care when transferring *Daphnia* to aquaria. If the aquarium contains 1 gallon less than its capacity, the *Daphnia* can be gently transferred into the aquarium by lowering the jar below the aquarium water surface.

Student Outline

Introduction

There are a number of factors which influence which species are found in any particular ecosystem; for example, habitat suitability, nutrient resources, and biological interactions, among others. Among the biological interactions, predator-prey interactions may determine which species

are present and also what the size distribution of these species may be. The early observations of freshwater ecologists suggested that planktivorous fish may selectively consume zooplankton in the size ranges that they can most easily observe, capture, and handle. Brooks and Dodson (1965) hypothesized that fish can observe and therefore capture large plankton more easily than small. Therefore, in lakes containing planktivorous fish, large zooplankton would be excluded and only small zooplankton would be found. During this laboratory, this hypothesis will be tested. The bluegill sunfish, *Lepomis macrochirus*, will be placed in a tank with a population of *Daphnia* that range from small (newly hatched) to large (mature). After being allowed to feed for a while, the fish will be sacrificed and the size range of the *Daphnia* in the stomachs will be determined. This size range will be compared with the mean of the size range present in the tank before and after feeding. This mean will represent the average size available to the fish during the experiment. The ability of the fish to capture prey selectively will be determined using an index of electivity.

Procedures

1. Remove a subsample from the aquarium before the fish are added. *Daphnia* may be sampled by simply dipping a 1-liter beaker into the tank after gently swirling to assure uniformity of distribution. Pour it gently through the zooplankton filter. At least 50 *Daphnia* should be in the sample. The sample should be washed into a 100-mm petri dish with enough filtered lake water or well-aged tap water to suspend the organisms. Next, add about 5 ml of soda water to anesthetize them. After they have ceased moving quickly, add 1–2 drops of sugar-ethanol solution. Placing a circle of graph paper in the bottom of the petri dish, measure each *Daphnia* to the nearest 0.1 mm. The 50 *Daphnia* may be divided between members of the class so that any individual would measure relatively few *Daphnia*. The actual counting and measuring may be done after the experiment has been completed.

2. Each person will add one fish to a tank so that each tank will contain a maximum of four fish. Each student will carefully watch his/her fish. When the mean number of *Daphnia* consumed per fish is about 30, remove all of the fish using the nets.

3. Place the fish in a solution of the anesthetic MS 222. Allow the fish to remain quiet until all movement has ceased, about 5–10 minutes.

4. Using an aquarium net reserved only for the anesthetic solution, remove the fish from the anesthetic, rinse in tap water, and place in a petri dish to open the stomach and examine the contents. To remove the stomach, cut through the gut cavity along the mid-ventral line from the anus (anterior of the anal fin) to the gill arches (see Figure 3.1) using dissecting scissors. Take care not to cut into the gut. Now, cut from the anus dorsally until the musculature of the back is encountered. From the gill arches cut dorsally through the pectoral girdle so that you create a flap (see Figure 3.1). Snip the gut close to the buccal cavity and at the other end, near the anus. Place the gut in a petri dish containing a small amount water. Cut the gut open and swishing some water through it, remove all zooplankton with the help of a probe. Count and measure each *Daphnia* in the fish gut.

5. Pour the anesthetic solution through a zooplankton filter and examine for regurgitated *Daphnia*. Count and measure the *Daphnia* regurgitated and add to numbers found in the gut contents.

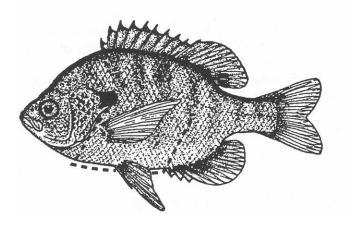6. Remove another subsample from the aquarium as in step 1, and count and measure the *Daphnia*.

Figure 3.1. The bluegill sunfish, showing the incision line (modified from Eddy, 1957).

6. As the counts are being made, draw a large table on the blackboard. Along the left margin, list sizes of *Daphnia* from 0.1, 0.2, 0.3,... to 2.0 mm. Along the top margin, list categories of the number of *Daphnia* in each size category before feeding, the number of *Daphnia* after feeding, and the number in the fish guts.

Calculations

1. Make a table of the size frequency distribution of the *Daphnia* in the aquarium. For each size category, calculate the mean number of that size at the beginning and at the end of the experiment. The average of the beginning and ending frequencies represents what the fish "saw" during the experiment, rather than just the number at the beginning or end.

2. Plot this mean size-frequency distribution of the prey in the tank.

3. Repeat steps 1 and 2 for the *Daphnia* in the fish stomachs.

4. To determine if the fish are positively selecting prey of a certain size, a measure called an electivity index has been devised by several authors. We will use Ivlev's:

$$E_i = (r_i - p_i) \div (r_i + p_i)$$

where r_i = the percent that prey size i forms in the fish diet, p_i = the percent that prey size i forms in the environment (aquarium). E_i varies between -1 and +1. If an item in the environment is always rejected by the fish, then $r_i = 0$ and $E_i = -p_i/p_i = -1$. If an item is found in the diet that is so rare in the environment that our measure does not detect it, then $p_i = 0$ and $E_i = r_i/r_i = +1$. If an item is exactly as abundant in the diet as it is in the environment, then $r_i = p_i$ and $E_i = 0$. Thus, avoided items have negative electivities, preferred items have positive electivities, and indifferent (not particularly selected) items have electivity of zero.

5. Calculate the E_i for each size-class of prey. If a size-class is found in neither the diet nor the environment, do not include it in your calculations. Plot electivity versus prey size for your experiment.

6. Dodson (1974) found that the larval salamanders (*Ambystoma tigrinum*) in the Colorado Rocky Mountains shifted from negative to positive electivity for prey of about 1 mm. Galbraith (1967) found that for the fish, *Perca flavescens* (yellow perch) and *Oncorhynchys mykiss* (rainbow trout) in Michigan lakes, the switch was for prey of 1.34 mm. He used *Daphnia* larger than this as a measure of food quality for the fish in the lakes. How do your data compare with these researchers' results?

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