

Chapter 10

Culturing Experimental Organisms for Use in Teaching Biology

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Introduction

Many organisms can be collected in the wild and cultured in the laboratory. Collecting has its advantages: it is inexpensive, and requires a prior knowledge of the organism's natural habitat and behavior—information that can be of value when culturing these organisms. There are difficulties in collecting wild specimens, most notably in obtaining a pure culture and the risk of introducing disease into your facility. In addition to collecting organisms in the wild they may also be purchased as pure cultures from biological supply companies (see Appendix A for a list of suppliers). The information to follow will enable you to set up an efficient and economical culture facility for a variety of aquatic and terrestrial organisms. The methods described in this paper were accumulated over several years. For more detailed information see Carolina Biological Supply Company (1975), Needham et al. (1937), Orlans (1977), and Perkins et al. (1981).

Culturing Parameters

Water quality is the most important factor for producing a healthy aquatic culture. Use only fresh clean well water or dechlorinated water. If this is not available use boiled water from a spring, pond, lake, or river. A neutral pH of approximately 7 is desirable. The water must be free of metals such as copper and zinc. If possible, an analysis of the water should be performed; this service can often be provided by government laboratories specializing in water quality. Chlorinated municipal water can be used for culturing with limited success. This water must be collected and aerated for at least 1 week prior to use.

Water quality is also important for terrestrial organisms. Various chemical parameters can influence metabolism or growth. For example, a culture of flesh flies is improved when dechlorinated water is made available. Animal care personnel have reported the improved culture of mice and gerbils when these organisms have been provided with dechlorinated drinking water (S. Craft, pers. comm.).

The temperature regime for aquatic organisms should be constantly maintained near the organisms natural temperature range. Temperature should not fluctuate more than 1-2°C. Environmental temperature changes of aquatic organisms should be effected over several hours or days to avoid temperature shock.

Natural photoperiod is recommended for optimal growth of most organisms; for the Northern Hemisphere, a maximum 16 hour photoperiod is acceptable. Continuous light is not beneficial, while indirect, natural day light or artificial light benefits most organisms.

A variety of containers have been found suitable for culturing organisms. These include pickle jars, mason jars, plastic pails, covered culture jars, and aquaria. Containers must be clean and free of chemicals. Before introducing organisms to a new culture vessel the container should be rinsed under flowing tap water for several hours. Soap must not be used for cleaning. Baking soda is an effective cleaning agent.

Cultures must be labelled with the identity of the organisms and the initial culture date. If possible, duplicate cultures should be maintained providing extra material in the event of failure. Cultures should be maintained in an area remote from fumes, for example away from fume hoods and chemical storage. Freshwater organisms should be cultured in an area separate from marine organisms. In small enclosed areas, excess salt in the air appears to adversely effect some of the more delicate freshwater organisms.

Each culture should be provided with its own set of maintenance equipment (for example, net, droppers, etc.). This insures decreased incidence of cross-contamination and transmittal of disease from culture to culture.

Maintain simple culture procedures avoiding unwarranted attention. Prior to handling organisms avoid using hand creams or washing hands with soap. Many invertebrates are very sensitive to the chemicals contained in these products. When required, slow gentle movements are the most effective in handling and the least stressful to the organism. In any stage of handling aquatic organisms maintain the maximum amount of moisture possible when removing the organism from its culture environment.

Terrestrial invertebrates require similar maintenance requirements as aquatic organisms: provision of clean dechlorinated water where appropriate, constant temperatures, and containers which are clean and free of detergents and pesticides.

After receiving shipments of organisms from commercial suppliers, allow the organisms time to acclimate to the culture container by allowing a gradual transfer. Inspect the health of the organism and read the culture information that is provided with the shipment.

Collection and Culturing Procedures

Protozoa

Protozoans are widely distributed in aquatic environments, especially in areas abundant with organic material and aquatic plants. *Euglena* is easily collected in the late summer from green stagnant ponds. *Amoeba* may be found on the leaves of aquatic plants, such as *Elodea*. Once specimens have been obtained it is important to establish a pure culture of the experimental organism. The steps involved in separating protozoans for a pure culture are as follows:

1. Place the sample under a dissecting microscope.
2. Using a 1-cc syringe, gently remove a few drops containing the desired organism. Release into a well-slide.
3. Clean the syringe.
4. Observe the well-slide under the dissecting microscope to confirm the presence of the desired organism.
5. Check the sample under a compound microscope for contamination.
6. If contaminated, put the well-slide under the dissecting microscope and carefully remove the desired organism and put the new sample on a clean well-slide.
7. Clean the syringe.
8. Add culture water to the well-slide.
9. Check the well-slide under the compound microscope. If it is contaminated, repeat steps 6 to 9.
10. When a pure sample has been obtained rinse the sample into the culture jar.

Paramecia (and other ciliates) and *Amoeba* have the same culturing requirements, except that *Amoeba* should be maintained in the dark. To prepare five cultures: (1) boil 1000 ml of water, (2) in a separate container of water boil 40 grains of wheat for 5 minutes. Transfer 200 ml of water to five culture jars and add eight grains of wheat to each. Cool. Add several droplets containing protozoa to each culture jar. Subculture every 3–4 weeks.

To culture *Euglena* add 5 ml of dry milk powder, 35 rice grains, and 40 wheat grains to 1000 ml of water. Boil for 5 minutes. Cool and add *Euglena*. (Note: milk powder depresses the boiling point.) Subculture every 3–4 weeks.

Hydra

Hydra may be found attached to vegetation and rocks in slow moving streams or ponds and lakes that receive clean oxygenated water. They may be extracted directly from vegetation or rocks on examination under a microscope or by placing the substrate sample in a culture jar and later removing the specimens that have migrated to the sides of the jar.

Hydra are readily maintained in an established aquarium or large culture jar. Water conditions are more stable in a large vessel, making it easier to establish a colony of hydra. Hydra may be cultured with snails and planaria, but are not compatible with fish. The culture should contain some plant material, such as *Elodea* or an aquatic moss. Mechanical aeration is not necessary in a hydra colony that has well-established plant growth. If it is necessary to maintain hydra for several weeks without feeding, they can be transferred to jars with loose fitting lids and stored in the refrigerator.

Hydra should be fed daily with either *Daphnia* or rinsed brine shrimp, in quantities that can be consumed in 1–3 hours. Snails maintained in the same culture as hydra can be advantageous in that they will consume the dead brine shrimp not consumed by the hydra. Surface water contamination and unconsumed food must be removed daily.

Planaria

Planarians may be found and collected in the same manner as required for hydra. "Baiting" is also a successful technique for collecting planaria. Strips of fresh liver are tied to a string and left in a stream for 15–20 minutes. Planarians are attracted to the liver, where they can be picked off and placed in the culture jar.

There are several methods for culturing planaria. One method utilizes a white enamel dish containing a rock for the planarians to escape beneath, as they are photo negative. Planarians are daily fed small pieces of liver. Water is changed 1–2 hours after feeding. Once a week the planarians are removed and the dish is wiped clean. This method, although widely used, requires that the animals be handled a great deal, and may not be as successful as the following: Culture the planarians in an aquarium with a gravel bed, corner filter, and very gentle aeration. Include aquatic plants and a few snails which help to provide a stable environment. Feed daily with rinsed brine shrimp, an earthworm, or a piece of liver. Always remove uneaten earthworms and liver.

Vinegar Eels

Vinegar eels (*Turbatrix* sp.) take very little time and effort to culture. Place one quarter of an apple in a jar with 200 ml of apple cider vinegar and one dropper of vinegar eels. Maintain away from direct sunlight at room temperature. Subculture every 4–6 months.

Aquatic Snails

Snails are easily collected on rocks, vegetation, and gravel beds in ponds, streams, rivers, and lakes. They are readily cultured in an aquarium or large-mouth jar but can become potential pests by over-populating the container. Snails should be fed small amounts of tropical fish food on a daily basis; they will also eat potato skins and maple leaves that have been soaked in water for several days. Chalk should be added to the culture to provide the required calcium for shell growth.

Daphnia

Daphnia may be collected using a plankton net in stagnant ponds and transferred immediately to a culture jar. They are readily cultured in large numbers as an experimental animal or a food source for other organisms. The most convenient way of culturing *Daphnia* is to set up a large-mouth jar with water, and add 1 mg of any of the following: fish food, egg yolk, or baker's yeast. In 2–3 days inoculate with *Daphnia*. Feed every second or third day. Once the culture has been established daily remove some *Daphnia* to avoid over-crowding and stress-induced population decline. Remove surface contamination and do not aerate.

Brine shrimp

Brine shrimp (*Artemia* sp.) eggs may be obtained from pet stores or biological supply companies. If kept frozen, eggs will last indefinitely. Any vessel is suitable to culture brine shrimp, but a long column with aeration at the bottom is most successful. Salt water (28–32%) is added to the column, gentle aeration is started, and brine shrimp eggs are added. Twenty-four to 36 hours are required for the eggs to hatch, depending on the temperature of the water. To separate the free-swimming larvae from the egg cases, turn off the aeration and set up a light near the top of the column. The free-swimming larvae are attracted to the light, making it possible to remove them. If they are being used as a source of food for freshwater organisms, pipet them into a funnel lined with a tissue (Kimwipes or Kleenex), then rinse them in fresh water. Invert the tissue in a beaker of freshwater and remove the brine shrimp. Brine shrimp will live for several hours in freshwater.

Brine shrimp can also be maintained as adults. Segregate a few free-swimming larvae and place in a culture jar or aquarium without aeration. Add a few grains of baking yeast every second day, this will produce a healthy culture of bacteria which the brine shrimp will consume. Brine shrimp are very sensitive, making it important that they are fed and the water level is maintained and left undisturbed.

Frogs

Frogs are easily collected from ponds, marshes and lakes using a long-handled dipnet. The best time to collect frogs is in the early spring. Environmental regulations prohibit the collection of amphibian species in some areas.

Frogs require live food, including flesh flies, *Tenebrio*, and earthworms. Construction of a culture facility is required (Figure 10.1). Frogs are housed in stainless steel tanks with sloping floors, providing both a dry and a moist environment. Heat lamps are provided to maintain a temperature of 18–20°C. Water is changed daily. A screen frame is constructed with 1/2" PVC plastic pipe fitted with 1/2" plastic tees and elbows; the frame is covered with fiberglass screening which is cut to fit the frame and stitched together. The frame sits on the stainless steel tank and is sealed with silicone. The door to the facility is sealed with velcro. Frogs are daily fed *Tenebrio* larvae or adults, or adult flesh flies. It is important to note that frogs cannot digest flesh fly larvae.

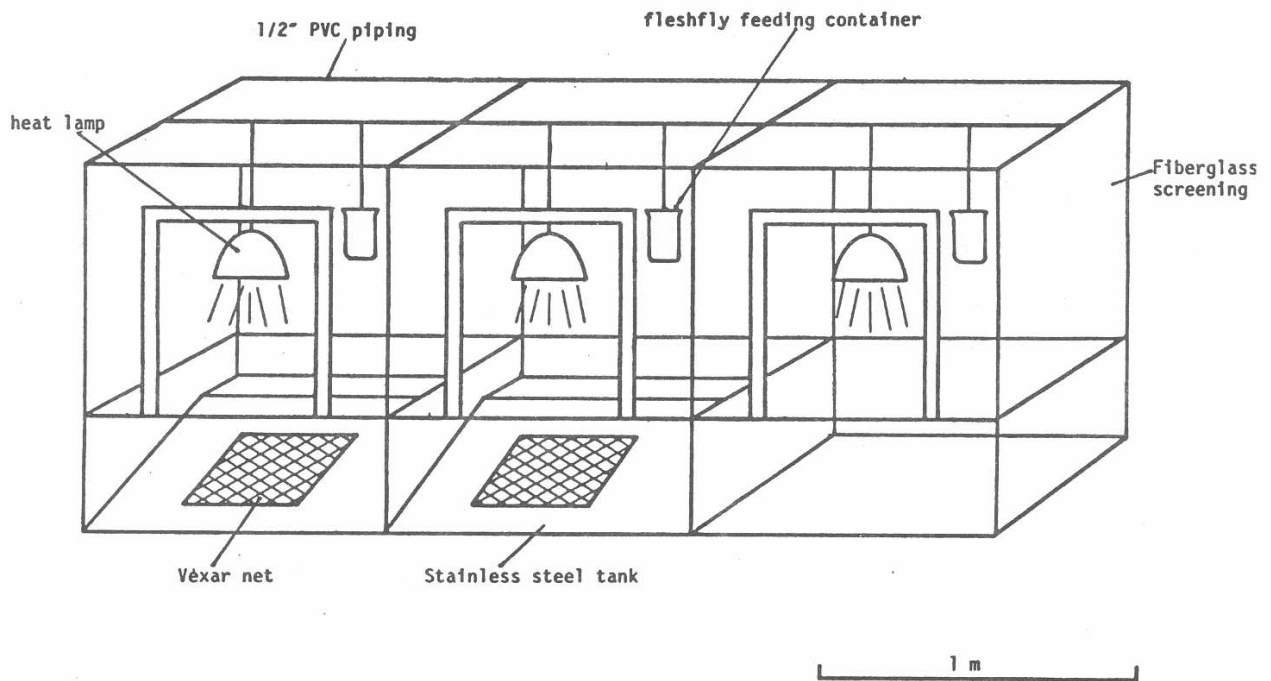


Figure 10.1 Rearing facility for frogs.

Turtles

Since some turtle species require relatively warm water (18–20°C) and others require saltwater, a recirculation system is required (Figure 10.2). Two fiber glass tanks are connected with a platform of 3/4" plywood. A frame is constructed out of 1" × 3" plywood and covered in Vexar netting. A ramp descending into each tank is made from plastic, fluorescence light covering, called egg carton. All wood surfaces are painted with epoxy paint. The drains are 2" ABS piping. The collection bucket is a 50 liter plastic garbage pail with a small filter bucket containing polyester fiberfill; filter wool is changed daily. A 4MD "Little Grant" pump is used. The head tanks are 50 liter garbage pails filled with biorings (coke rings). The hose from the pump to the head tanks is garden hose, the hose from the head tank to the turtle tank is Tygon tubing. To prevent possible over-heating due to reduced H₂O flow, the pump is fitted with a float valve. There is a heat lamp over the drying platform to provide surrounding air temperatures of 18–20°C. The facility in Figure 10.2 will maintain 20 to 30 turtles measuring 15 cm in diameter. Weekly there should be a 10% water renewal to maintain clean water. Turtles are fed canned cat food daily.

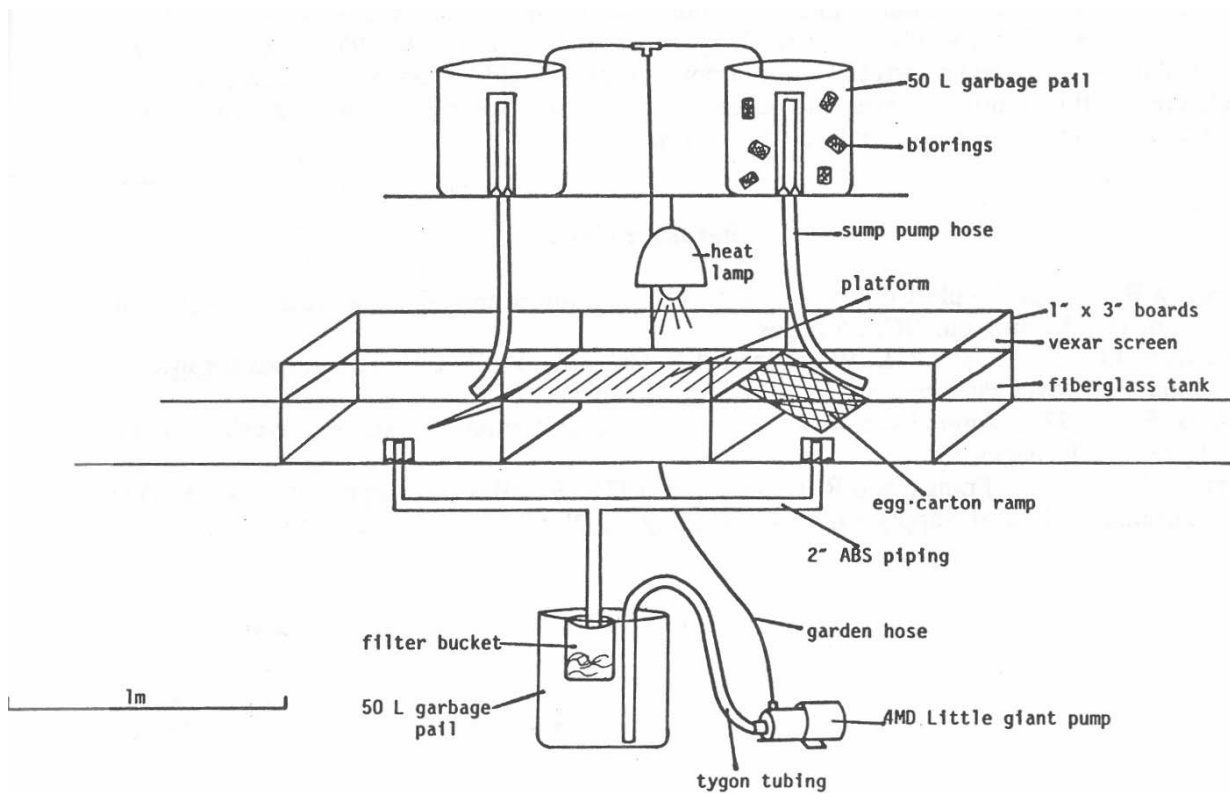


Figure 10.2 Recirculation system for rearing turtles.

Flesh Flies

Flesh flies (*Sarcophaga bullata*) are readily maintained with few supplies (P. Sivasubramanian, pers. comm.). Adults may be housed in commercial fly cages or in inexpensively constructed containers made of dowelling and cheese cloth. Adult flies are fed sugar cubes and have water present at all times. Three to 6 days after the flies have emerged provide a small piece of liver, remove the liver the following day. Approximately 10 days later, place another small piece of liver in the cage, and remove it 5–6 hours later. This piece of liver will contain the first instar larvae; place liver in a tinfoil cup which contains more liver. Place the tinfoil cup in a large container of sawdust. Within approximately 10 days the larvae will emerge from the liver and into the sawdust where they will pupate. The pupae may be sieved from the sawdust and placed in a cage where the flies will emerge in approximately 10 days. When a culture is no longer required remove the source of water and within a few days the flies will expire.

Tenebrio

Tenebrio are readily cultured in any smooth-walled container, such as a pail or aquarium. Mix equal amounts of bran, whole wheat flour, rye flour, and corn meal to a depth of 15 cm. Add 25 to 50 adult *Tenebrio* and a carrot. Carrot or potato slices should be added every few days to provide moisture. Within 3 months larvae and pupae should appear. Subcultures are made when new adults appear, insuring a continual supply of organisms.

Literature Cited

- Carolina Biological Supply Company. 1975. Carolina lower animals. Carolina Biological Supply Company, Burlington, NC, 25 pages.
- Needham, J.G., F. E. Lutz, P. L. Welch, and P. S. Galtsoff. 1937. Culture methods for invertebrate animals. Dover Publications, New York, 590 pages.
- Orlans, F.B. 1977. Animal care from protozoa to small mammals. Addison-Wesley, Don Mills, Ontario, 374 pages.
- Perkins, K. W., R. L. Franks, and R. H. Whitten. 1981. Reptiles and amphibians: care and culture. Carolina Biological Supply Company, Burlington, North Carolina, 32 pages.

APPENDIX A
Addresses of Suppliers

Boreal Laboratories
1820 Mattawa Ave.
Mississauga, Ont. L4X 1K6
(800) 387-9393

Carolina Biological Supply Co.
Main Office and Laboratories
Burlington, NC 27215
(800) 334-5551 or (919) 584-0381

Carolina Biological Supply Co.
Powell Laboratories Division
Gladstone, OR 970297
(800) 547-1738 or (506) 656-1641

Northwest Laboratories
Western Division
3581 Shelbourne St.
P.O. Box 6100, Station C
Victoria, B.C. V8P 5L4
(604) 592-1341

Northwest Laboratories
Eastern Division
61 Malcolm Rd.
P.O. Box 1356
Guelph, Ont. N1H 6N8
(510) 836-7720
FAX: (519) 836-4105

Ward's Natural Science Ltd.
1840 Mattawa Ave.
Mississauga, Ont. L4X 1K1
(800) 387-7822