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HYDRA AND PLANARIA CULTURE: PRACTICAL APPLICATIONS FOR TEACHERS

Dorothy A. Jones
Department of Biology
Saint Joseph's College
Rensselaer, Indiana 47978

ABSTRACT: Increased public pressure for biology lab procedures that avoid unnecessary use of vertebrate material provides a stimulus for expanding student and teacher awareness of invertebrates as systems for the study of life processes. Hydra and planaria, long familiar to biologists for both research and teaching purposes, can easily be maintained live in a classroom situation over a period of several weeks to months and used in a variety of ways to encourage middle school and high school student research projects. Lab culture of both these organisms is cheap and requires only a few minutes a day.

INTRODUCTION

The Office of Technology Assessment of the Congress of the United States (OTA) has recommended that alternatives to the use of animals be sought in the areas of biomedical and behavioral research, toxicity testing, and education in the life sciences. Furthermore, the OTA has defined animals as "nonhuman vertebrates: mammals, birds, reptiles, amphibians, and fish," (Gibbons, 1986). Increased study of life processes in invertebrates seems to be indicated. This article summarizes techniques developed for inexpensive and relatively foolproof longterm culture of two aquatic invertebrates, hydra and planaria. Both animals are widely used in research and teaching. Both are widely known for their regenerative powers. Both are easily obtained through biological supply houses or by field collecting. It is assumed that the reader is at least casually acquainted with these organisms. If that is not the case, please refer to any invertebrate zoology text (eg., Pearse, Buchsbaum *et al.*, 1987).

HYDRA CULTURE

A basic requirement in culturing hydra is that the containers be totally free of detergent. To ensure that this is the case, use disposable plastic Petri dishes for working with very small numbers of hydra. Newly purchased transparent Pyrex cake dishes are ideal for larger cultures. I have used 8″ x 8″ x 2″ size. To keep dust off, heavy duty aluminum foil may be folded into a cover that is easily labeled with a permanent marker. This is what I recommend for cultures kept by elementary school and middle school students. Hydra do well in water 1.5 to 2 centimeters deep. (With this amount of water there is little loss from spilling when the dishes are moved around.) The foil cover should be folded in such a way as to stay up out of the water. Hydra are sensitive to metal ions. For high school and college student mass cultures, glass plate covers are more satisfactory. 9″ x 9″ x 9″ plates with ground edges are available through Carolina Biological Supply, or can be cut to order by a glazier.

Hydra tolerate a broad temperature range. They do well at temperatures between 10 and 25 degrees Centigrade (about 50-80 degrees Fahrenheit), but like most organisms, are more sensitive to sudden temperature changes than to gradual ones.

Hydra are aquatic, and need good culture water. They do well in freshwater aquaria, sometimes well enough to achieve pest status. I have collected local *Hydra littoralis* from the Iroquois River that runs through Rensselaer and have grown other hydra in river water, with success.

Recycled plastic gallon milk bottles, rinsed out a few times with tap water, make cheap detergent-free water collecting and storage bottles. River water can be filtered by passing it through a detergent-free (i.e., purchased for this use) plastic kitchen funnel containing a wad of polyester aquarium filter fuzz. I set the funnel in the neck of a recycled 2 liter plastic pop bottle. When the filtrate fills the bottle, an aquarium pump and plastic air line tube are used to bubble air through it for a few hours. Discard the wad of filter fuzz after it has been used and rinse the funnel with tap water. After thoroughly aerating a bottle of filtered river water, screw on its own cap, label it with laboratory marker, and store for use when needed. As the bottles begin to accumulate scale that precipitates out of the river water, I simply discard them and use new ones. The scale isn't harmful to the hydra, but it's messy. It chips off the inside of the bottle and forms flakes in the culture dishes, making it hard to see the animals.

River water is usually satisfactory for growing hydra, but it may show wide pH swings. My readings on aerated Iroquois River water ranged from pH 6.7 to 8.5 over a period of several months. Howard Lenhoff's book, *Hydra: Research Methods,* (1983) recommends several artificially prepared solutions for growing hydra. I chose "M" solution, in which sodium and calcium ions are at a final concentration of 10^{3} M, magnesium and potassium ions are at a final concentration of 10^{4} M, and the whole solution is buffered to pH 7.6.

Modified "M" solution may be prepared according to the following procedure, shared with me in 1989 by Dr. Joann Otto of Purdue University Department of Biological Sciences.

For 2 Liters of 4X Stock "M":

4.0 mL	2M NaCl
0.4 mL	2M KCl
0.8 mL	1M $MgCl_2$
8.0 mL	1M $CaCl_2$
16.0 mL	0.5M Tris (Tris-HCl buffer, pH 8)

Bring volume to 2L with distilled water and adjust pH to 7.6 with HCl. Keep 4X Stock refrigerated, as Tris buffer is not stable for long periods at room temperatures.

For 1 L of "M" solution, use 250 mL 4X Stock "M" and 750 mL distilled water. Deionized glass distilled water is preferred, but any glass distilled water will serve the purpose, including the bottled distilled water that is sold in pharmacies and grocery stores.

Lenhoff (1983) has written about the importance of chemically pure distilled water in hydra culture. I found that both bottled Shurfine brand distilled water and our ordinary laboratory distilled water serve well as a basis for "M" solution. The distillation should

have been done in a glass-lined system, not in a copper tubing still. I haven't used any ion exchange column treatment, although both Lenhoff (1983) and Otto (1989) recommend it. Ion exchange cartridges need constant replacement, if they are operating directly on tap water, as would be necessary with the distillation apparatus available to me.

Lenhoff reports that water prepared through a process of reverse osmosis is also satisfactory as a basis for "M" solution (Lenhoff, 1983). Culligan company purifies water that way, but then some ions are put back in, to get a good drinking water taste. I have tried to find out which ions are put in, and to what molar concentrations, but have not been able to obtain that information. Shurfine was the only brand of bottled distilled water for which I was able to get a detailed water analysis, though I sent letters of inquiry to several companies.

I routinely aerate the "M" solution. *Hydra littoralis* (North American common brown hydra) seem to do better in aerated water. This is not surprising for a species that normally inhabits streams and creek beds. Recycled seltzer bottles can be used for aerated "M" solution. One 10 ounce seltzer bottle is usually enough for an 8″ square culture dish water change. Several hundred hydra may easily grow in one 8″ x 8″ x 2″ culture dish, if the water is changed daily. I maintain fewer hydra per dish, and need fewer changes.

Libby Hyman (1939) and Whitten and Pendergrass (1980) have recommended that hydra be fed *Daphnia,* but then the *Daphnia* culture has to be fed, as well. I prefer to use *Artemia* nauplii, baby brine shrimp. *Artemia* cysts can be purchased by the can or vial from aquarium supply stores under the name "Brine Shrimp Eggs". If kept dry, the cysts are stable at room temperatures for months.

"Instant Ocean", or any other brand of artificial sea water is best for hatching *Artemia*. It can be made up with tap water by the gallon, once again in recycled milk bottles. I found artificial sea water far more reliable than the alternative rock salt/epsom salt saline recommended in the culture guide issued by the supplier (San Francisco Bay Brand, Inc., 1987).

Using artificial sea salt mix in tap water with constant aeration, I found no need for constant illumination of the hatchery box during the 48 hour hatching period, even though such illumination is recommended by the San Francisco Bay Brand, Inc. *Artemia* culture guide. Ordinary diurnally variable laboratory lighting served the purpose. I have not tried hatching brine shrimp in conditions of total darkness.

To obtain *Artemia* nauplii, fill a brine shrimp hatchery box (San Francisco Bay Brand Company's "Shrimpery Brine Shrimp Hatcher") to within an inch or so of the top with artificial sea water and tape an aquarium plastic tubing line to the inside of the box so that the solution can be continually aerated. Turn on the air pump. When air is bubbling through the water, sprinkle *Artemia* cysts on the surface. A really good hatch can be reliably obtained from a quarter teaspoon of cysts, but fewer can be used.

Forty-eight hours or so later, the hatchery box will be teeming with nauplii. Detach the aquarium tubing and move the box to a place beneath a bright light or near a sunny window. Fill the transparent hatchery cylinder vial with aerated river water or "M" solution, cap it, and invert it over the hatchery box, fitting it to the space provided. The nauplii, attracted to the light, will swim up into the cylinder, rinsing themselves free of excess salt. After 15 to 30 minutes, a crowd of nauplii will have collected in the vial. Remove the vial from the hatchery box. Take off the cover and use a Pasteur pipette to dispense the baby brine shrimp to the hydra. By means of a such a pipette,

the hydra may be fed individually and feeding behavior may be observed using a dissecting microscope, or large numbers of nauplii may transferred to the hydra culture for mass feeding. For mass feedings alone, an ordinary medicine dropper will suffice. *Artemia* nauplii will remain active in fresh water for a few hours, long enough for the hydra to find them, whether they are hand fed or not.

Four to six hours after feeding, hydra need a water change. To accomplish mass culture changes, pour off the "M" solution or river water from the hydra dish into a quart size transparent Pyrex measuring cup or bowl. Swirl the cup, spinning the hydra toward the center. As they settle, rinse the cake dish with fresh aerated "M" solution or river water, and add the rinse water to the cup. Many hydra may remain attached to the cake dish. For a simple water change, these are left undisturbed and fresh "M" solution or aerated river water is poured onto them. The hydra in the measuring cup are then pipetted up and squirted back into the dish, too.

Individual Petri dish water changes require that the hydra not be lost as the water is poured off. The animals may be saved by being temporarily pipetted into the dish lid, or the entire contents of each dish may routinely be transferred to a 6-ounce Pyrex custard cup, from which the hydra may then be returned to the Petri dish.

It's easier to see the hydra over a dark background. If the lab table where I'm changing the animals isn't black, I use a sheet of black construction paper. This method is cheap, but messy when wet, so I wipe the bottom of the cups or dishes dry before placing them on a paper background.

A hydra colony can be fed as often as daily or as seldom as weekly, depending on the rate of colony growth desired. The more often a colony is fed, the more rapid its growth rate will be. Colonies grow exponentially at room temperature with daily feeding and good care. The rate of budding reflects the rate of mitotic cell division, which depends on temperature, pH, ion concentrations, and oxygen and carbon dioxide concentrations. Mitotic rate is also sensitive to a wide variety of metabolic inhibitors (Lenhoff, 1983).

Hydra secrete mucous and leave small blobs of undigested material on the culture dish bottom, so it is necessary to change the Petri dishes or completely clean each large colony dish at least once a week. For large cultures, use your bare fingers to rub the hydra free of the dish bottom, then pour them off into the measuring cup and swirl it. While the hydra settle out of the old water, run hot tap water into the culture dish and rub a clean paper towel or your hand all around the bottom and sides of the dish until the slimy mucous is gone. Rinse with cool tap water to bring the dish temperature back to normal, add fresh "M" solution or aerated filtered river water, and replace the hydra.

The *Artemia* Shrimpery box also needs to be kept clean. As soon as the fresh water cylinder vial has been removed from it, run hot tap water into the box to thoroughly rinse it out. Use a clean paper towel to wipe any scum from the sides and floor of the Shrimpery. Rinse again and allow to air dry thoroughly. If the corners of the box accumulate scum, scrape it out carefully with a paper towel wrapped around a clean formalin-free and detergent-free skewer, dissecting needle, or forceps.

Using the methods and simple equipment described, I've maintained a colony of brown hydra (*Hydra littoralis*) in the Biology Department at Saint Joseph's College for ten months. During that time the room temperature was as low as 17 and as high as 29 degrees centigrade. Some animals were kept several months in a cool box at 11 to 15 degrees Centigrade. At this cooler temperature they survived a two-week period of starvation and a week with no water changes while I was away on vacation.

There has been no sign of sexual reproduction in any of my cultures of hydra, to date. I have made no attempt to induce it, although there are references in the scientific literature to techniques for doing so (Lenhoff, 1983).

I've successfully used hydra regeneration exercises with Saint Joseph's College Introductory Biology students and introduced both hydra and planaria to a delighted eighth grade advanced science class and also to third, fourth and fifth grade science classes at Saint Augustine Elementary School. The children in the elementary school were not only enthusiastically receptive to what they learned, but also capable of surprisingly sophisticated scientific concept formation and retention. Furthermore, they were careful in their handling of the expensive dissecting microscopes made available to them for the study of small invertebrates.

There is a wide variety of currently active research programs that center around these animals. To obtain the sample hydra bibliography which I have prepared in the course of this investigation, send me your name and address and two dollars for postage and copy costs. If you prefer, send a 5.5″ double-sided, double density floppy disk, plus two dollars for postage. I will be copying to it from MSDOS, version 3.2 system, Norton Textra Writer, version 2.0, using an IBM compatible PC.

PLANARIA CULTURE

Planaria culture is simpler than hydra culture in some respects, more difficult in others. These aquatic fresh water animals, like hydra, also seem to require detergent free dishes. I have successfully grown them in the same cake dishes that I use for the hydra, using the same covers. Planaria, unlike hydra, are negatively phototropic. That is, they prefer darkness to light. I have kept them on low shelves in dim room light with success.

Another way in which planaria differ from hydra is that they are not as able to withstand warm temperatures. They do best at temperatures below 20 degrees Centigrade, although they tolerate a few degrees higher.

Planaria, like hydra, are predaceous, and will eat one another if they get hungry, but brown planaria (*Dugesia tigrinum* and *Dugesia dorotocephala*) thrive on a diet of raw beef liver. The most practical way to maintain a steady supply of liver for a planarian colony is to buy a package of fresh-sliced beef liver and cut it into tiny cubes (approx. 0.5 cm square) with a single-edged razor blade. Depending on how many planaria are to be fed at a time, one, two, or three cubes of beef liver can be wrapped in small squares of plastic wrap, folded with other such packets into a plastic sandwich bag, and quick frozen. Several months' supply of liver is then easily stored in a condition ready for quick use.

When it is time to feed the planaria, remove a packet of liver from the freezer, warm it in the hand for a few moments, and unwrap the liver cubes. Drop one into each dish of planaria and leave it. After a few moments, the planaria will be attracted to the liver, swim over, and begin feeding. After two to four hours, remove the liver. If some planaria are still on it, gently rub them off. The liver must be removed from the dish before it fouls the water. The planaria should have a water change at this time.

To change the water, I use a technique similar to that described for the hydra. Pour the old water off into a quart measuring cup. The planaria do not need to be swirled. They will tend to settle or even to adhere to the cup. Any that are floating can be induced to settle by merely touching them. After the culture dish water has been changed,

carefully pour off most of the water from the cup and wipe, swirl, or push the planaria into the culture dish. I use my bare fingers to wipe any stragglers out of the cup.

Planaria continually secrete a mucous film on which they glide. Culture dishes must be thoroughly cleaned for planaria at least twice, maybe three times a week, depending on how crowded the dishes are, and regardless of how often the animals have been fed. To clean the dishes, use hot tap water and your bare hand or a clean paper towel for rubbing away the mucous. As described for cleaning hydra dishes, rinse with cool tap water and add fresh aerated filtered river water to a depth of 1.5 to 2 centimeters before replacing the planaria.

I have kept brown planaria successfully for ten months in aerated Iroquois River water. I collected some of these animals from the river last summer and maintained them easily, noting that they increased asexually by fission pinching off pieces of their tails after being fed long enough to achieve a certain size. These tail pieces then grow new heads, and the parent piece regenerates a tail within a matter of several days. Certain varieties of *Dugesia* have long been known to exhibit this curious behavior, whereas other planarians do not (Pennak, 1978, and Kurabachi and Kishida, 1988).

In species that also reproduce sexually, fission is less common at the time of egg production. It is best observed by school classes in the fall term. Planaria obtained February through April (in the Northern Hemisphere) are likely to lay eggs. Children in the Van Rensselaer Elementary School third grade in Rensselaer, Indiana, were thrilled in February, 1990, to observe tiny planaria hatching from some eggs laid in their classroom cultures. Young planaria are predaceous from the time of hatching. I have seen them attack and consume as many as four or five *Artemia* nauplii per feeding, when the planaria themselves were not much wider than the shrimp.

Ann Feil, a Saint Joseph's College student, tried culturing planaria in both aerated "M" solution and in aerated water from Lake Banet, a natural reservoir on the Saint Joseph's College campus that has been developed from a sand and gravel pit. It appeared from her work (1990) that planaria do better in "M" solution or in 5% Instant Ocean, when tested for their regenerative capacity, but further study is needed for definitive results.

I have maintained a small number of white planaria (*Procotyla fluviatilis*) for a ten month period in aerated Iroquois River water. These animals do not regenerate, as the brown ones freely do. They are exclusively predaceous, ignoring raw liver when it is offered to them. White planaria do well on a diet of baby brine shrimp, however, and also will eat hydra. Since they did not reproduce asexually as freely as the hydra and the brown planaria, the number of white planaria has dwindled over the months. I gave several away to some eager eighth grade science students, and was left with only two for the entire summer and fall. One lived for ten months.

I purchased several hundred brown planaria from Carolina Biological Supply Company recently, for work with local elementary school children. It has been necessary to keep about a hundred animals in each 8″ x 8″ x 2″ culture dish for a period of a few weeks. They have shown no ill effects, except that when they first arrived, a few climbed above the water level and dried out. Also, when they were not fed twice a week, they began eating each other. With regular feeding, they began reproducing by fission, as described above. Regular water changes and dish cleaning have been absolutely necessary.

Individual planaria may be moved around using widemouth medicine droppers. They adhere to glass, but may be loosened by rapidly squirting water in and out of the

dropper at them, or by directing a stream of air through an aquarium air line into the culture. It's best to work fast. The tiny regenerating tail end pieces are particularly sticky and can easily get caught inside the dropper. Another way to manipulate planaria is by picking them up on the bristles of a new watercolor paint brush. Even very cheap brushes work well.

A current bibliography for planaria can be obtained by sending me your name and address and two dollars for postage and copy costs. If you prefer to send a floppy disk, the specifications are the same as for the hydra bibliography.

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