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CULTURING FRUIT FLIES (*Drosophila*), AND OTHER LIVE FISH FOOD

Part One of Two Parts

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MOST AQUARISTS agree that live food is good for fishes. In the experimental laboratories at the University of Texas we culture *Drosophila, Daphnia magna,* microworms, infusorians, and Houston white worms. Detailed culture methods, using a banana mash medium, have been worked out for the first three forms and are presented in the following paragraphs.

Fruit Flies

Fruit flies *(Drosophila)* form one of the few live foods that can be raised in quantity by the aquarist. Their culture can be carried out in glass containers that occupy very little space. With reasonable care the cultures will maintain themselves and produce large volumes of living food.

In our original work we found that *Drosophila* served as an excellent aquarium fish food. We used excess fly larvae from the Genetics Laboratory at the University of Texas. As our investigations widened, we found that this excess supply was not sufficient to meet our needs, and experiments were initiated to determine methods for raising larger quantities. We have received much assistance from the genetics workers and others during the investigations.[1]

[1] Dr. Marshal R. Wheeler, Assistant Professor of Zoology at the University of Texas read and criticized the *Drosophila* discussion. Dr. John M. Cairns, Assistant Professor at the University of Oklahoma Medical School aided the early development of the feeding techniques.

Previous Use of Fruit Flies to Feed Fish and Other Animals

Information on feeding *Drosophila* to fish is scanty in aquarium literature. Feeding vestigial winged (flightless) adults, a mutant strain of *D. melanogaster,* has been recommended by Poppel (1951). Mutant strains usually do not culture as well as normal or wild type flies. Axelrod (1952) briefly mentions using *Drosophila* for aquarium feeding. Neither Innes (1952) nor Stoye (1935) mention *Drosophila.*

Likewise, there is little published information on feeding *Drosophila* to other animals. Burger (1937) recommends feeding adults to black widow spiders. Hoffman (1924) mentions the ease of raising adults and the difficulty of capturing them for feeding waterbugs. Breland (1949) was successful in raising carnivorous mosquito larvae on a combination of larval and adult *Drosophila.* Making use of the facilities at the Genetics Laboratory of the University of Texas, and the kindness of the geneticists, other laboratory animals have been raised on fruit flies. Dr. John M. Cairns, then on the staff of the University of Texas Department of Zoology, fed salamanders on *Drosophila* larvae. Graduate students working under Dr. W. Frank Blair have raised other amphibians on adult *Drosophila,* as follows: Dr. Wilmot A. Thornton, toads of the genus *Bufo;* Aaron 0. Wasserman, spadefoots of the genus *Scaphiopus; William* F. Pyburn, cricket frogs of the genus *Acris;*

and Hague L. Lindsay and John S. Mecham, tree frogs of the genus ***Pseudacris.***

The Culture Medium

Although there are many media used for raising and feeding *Drosophila,* we employ only the one used by the genetics laboratory at the University of Texas. As the cultured non-gas-forming yeast used in the laboratory is not available to aquarists, a simplified method of preparation is given below.

Ingredients for one liter' of medium:

1) 90 cc water
2) 20 grams agar (can be obtained from any biological or chemical supply house such as Turtox Laboratories or E. H. Sargent & Co., 4647 West Foster Avenue, Chicago 30, Illinois).
3) 25 cc malt extract (Blue Ribbon)
4) 25 cc syrup (Karo)
5) 30 grams brewer's yeast (usually found in any health food store).
6) 100 cc water
7) 2 lbs. bananas (green to overripe)
8) living yeast (Fleischman's fresh-active yeast)

To prepare the medium:

a. Heat and stir 1 and 2 to dissolve agar.
b. Heat 5 and 6 in pressure cooker for 45 minutes.
c. Mix all but 8 and boil gently for 15 minutes in covered container.
d. Place in sterile' (boiled) bottle to depth of about %" (less depth permits drying and greater depth is a waste of medium) and cover with sterile cloth.
e. After cooling (heat kills yeast) inoculate with a pinch of living yeast and plug tightly with sterile cotton. Do not remove plug except when flies or larvae are being transferred (keeps out undesirable mold, bacteria, flies, and ants. Keeps in *Drosophila.* Keeps medium moist.) Refrigerated, the medium will last in first class condition for two weeks, and can be used during the subsequent two weeks.
f. Place in refrigerator until about three days before using. It is ready for use when a heavy growth of yeast is on surface. The yeast forms a more or less pasty covering over the medium. It grows very close to the medium, seldom becoming high enough to be seen from the side. (Be careful of mold. Do not use moldy cultures. Molds form a more or less powdery covering over the medium. It often forms a fine thread-like covering over the medium that usually can be seen easily from the side. Yeast is usually white, molds often dark colored. *Experience is the best teacher. If the larvae flourish the growth is yeast, if not it may be mold.* Mold may be prevented by chemicals such as 5 cc of propionic acid added before step c.)

Other methods and media for culturing *Drosophila* may be found in Sturtevant (1937), Spencer (1950), and Fletcher (1953). We feel that the method employed at the University of Texas is the most satisfactory because it does not leave particles of the medium

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The metric system of measurements is used in this article. It is more convenient and logical than the better known English system. The following are the equivalents of the metric units used here:

454 grams = 1 pound
1057 cc = 1 quart
1000 cc = 1 liter

Many of the bottles and measuring devices for preparing baby formulas are marked in cc.

[3] A sterile item is one that has no living organism on it. Non-sterile items are sterilized when all of the living animals and plants on them are killed. Heating in a pressure cooker for 20 to 30 minutes is a simple method of sterilization. One must be careful not to contaminate the sterile items when they are removed. When removing bottles, do not touch the inside or the outside near the opening. Sterile cotton may be purchased in drug stores.

with the larvae after washing. Difco Laboratories, 920 Henry Street, Detroit 1, Michigan, is experimenting with a commercial *Drosophila* medium.

The Culture Bottles

Many kinds of bottles may be used to raise *Drosophila.* Most laboratories studying *Drosophila* use half pint milk bottles or smaller vials. The small containers are less satisfactory because too few *Drosophila* can be raised in each container. It is possible to raise fruit flies in larger containers, but efficiency is lost with the increased size of the container. As the surface area exposed is a major limiting factor, an increase of height is of no significant value. Increased diameter is a handicap when the flies are shaken from bottle to bottle, as the jelly-like medium is more likely to come loose if the bottle diameter is large. Moreover, the cotton plug must be larger (and more expensive) with the increased diameter of the mouth of the bottle. The increased amount of cotton is far greater than the increased production of *Drosophila.* Bottles with necks narrower than the base use less cotton and the medium is less likely to fall out of the bottle during fly changing. The half pint milk bottle is not only satisfactory but also inexpensive.

In the half pint milk bottle is not available almost any bottle will suffice. The culture techniques can be used for any bottle type.

Kinds and Sources of Fruit Flies

Only a few of the 613 species of *Drosophila* listed by Patterson and Stone (1952) are suitable to culture for fish food. According to W. S. Stone (personal communication, 1953), *Drosophila melanogaster* Meigen, *D. simulans* Sturtevant, *D. virilis* Sturtevant, and *D. hydei* Sturtevant are among the species most likely to survive the variable conditions found in homes. (See Fig. 1.)

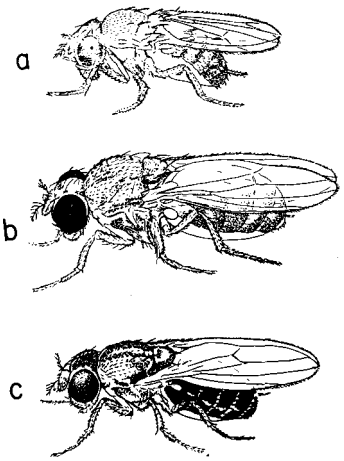

Fig. 1 — Adult males of *(a) Drosophila melanogaster,* (b) *Drosophila hydei,* and (c) *Drosophila virilis,* all ten times natural size. The small fly at the bottom is a natural size *D. melanogaster.* Redrawn from Patterson and Mainland (1944) by Grace Hewitt.

They not only will tolerate moderately high temperatures, but will also grow well on a banana mash medium. The so-called vestigial-winged fruit fly is a variant of *D. melanogaster.*

If particular, aquarists may get fruit fly "starters" from reputable biological supply houses such as Turtox Laboratories, 761-763 East 69th Place, Chicago 37, Illinois. If not particular, the aquarist can collect wild flies in a *Drosophila* culture bottle (see section on culture bottles). Many types of bait have been recommended in the literature. That proposed by Dobzhansky (1936) is relatively simple: "Fermenting banana mash is most satisfactory as bait. Ripe bananas are mashed with the aid of a spoon or a fork; some drops of a fresh yeast solution is added, and the bottle is left standing for about 24 hours before use. The bait remains good for at least four or five days after first used. The traps (culture bottles) are exposed in such a wav as to be readily accessible. and left undisturbed for a few hours; no useful purpose whatever is accom-

plished by leaving them exposed for days." We feel that the use of a bottle containing one-half inch of the culture medium described above will serve to capture wild *Drosophila.* After the flies have entered the bottle, a cotton plug can be inserted to keep them from escaping.

The wild flies can be captured in almost any outdoor locality, both in cities and in rural regions. Large numbers of flies are to be found around fruit markets. Those flies are more likely to be forms that are easy to culture. It is difficult to capture flies at temperature extremes. Temperatures between 60 and 80° Farenheit are best. When the outdoor temperatures are colder, flies might be captured during warm afternoons; and when it is warmer, dawn and dusk are the better collecting times.

Growing the Flies

The new flies are placed on fresh medium. After one or two days the adults may begin to lay eggs, either on top of the medium or on the side of the bottle immediately above the medium, depending upon the species. Of the flies used in our work only *D. virilis* lays eggs on the bottle. The elongate eggs are nearly white. If a black pepper grain were elongate it would be about the same size as a *Drosophila* egg. Frequently the eggs are concentrated in one small area, and give a whitish cast to that area. When the eggs are discovered the transferral of parents should be started.

When one or two days have passed the eggs hatch. The young *Drosophila* is a small maggot and when first seen is feeding on the surface of the medium or on the yeast growing on the bottle adjacent to the medium. Figure 3 is an enlarged drawing of a large larva. As the larvae grow they begin to "work" or soften the upper layer of the medium. The "worked" part is easily recognized by being darker than the unused part.

After a few days the larvae will have reached maximum size and will begin to form pupae. Unlike the larvae all the pupae are motionless. Pupae are darker than the larvae. Figure 2 illustrates pupae of three species.

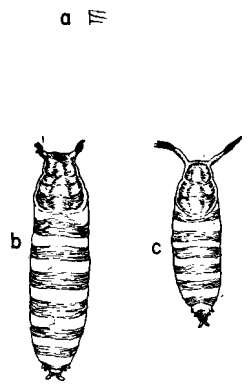

Fig. 2 — Pupae of *(a) Drosophila* ***melanogaster,*** **(b)** *Drosophila* ***hydei,*** **and (c)** *Drosophila virilis, all ten times* **natural size. Redrawn from Patterson (1943) by Grace Hewitt.**

Fig. 3 — Large larva of *Drosophila hydei, ten times* **natural size. Drawn by Grace Hewitt.**

When the adult emerges from the pupal case, the animal for the first time looks like a fly. Normally the males emerge before the females. If one looks closely the sexes can be recognized by the larger abdomen of the female. Ordinary room lighting is satisfactory for all developmental stages of *Drosophila.*

Transferring the Flies

For the adults to lay many eggs they must be fed well. To achieve best results the adults should be transferred to fresh media with a heavy growth of yeast. Daily changes are recommended for D. virilis and D. hydei and every-other-day-changes are best for D. *simulans* and D. melanogaster.

To transfer adults, invert the bottle containing the flies tightly over the new bottle and shake the flies into the new bottle. An up and down shaking motion is better than side to side. A sudden stop at the end of a downward plunge often succeeds when the flies do not move otherwise. If the medium is not firm one should not jar the bottles during shaking as the old medium will fall into the new bottle. Cold flies transfer easier than warm flies.

Larval Stage is Best to Feed to Fish

As stated above, during its life history Drosophila passes through four stages· developing egg, growing larva, metamorphosing pupa, and breeding adult.

We use fruit fly larvae (not adults) to feed native Texas darters and minnows. All of the minnows and most of the darters tested take larvae readily. Young largemouth bass grow well on them. Angel fish and bettas are fond of the larvae — in fact, bettas prefer them to daphnia. Local aquarists find that most tropicals will feed eagerly on Drosophila larvae.

Drosophila is most satisfactory as a food for fish of guppy size or larger. Depending upon the food habits of the fish they will continue growing on larvae for some time. Large angel fish flourish on Drosophila while bass in excess of three inches do not grow on such small food items, and above five inches ignore them.

Drosophila larvae can be fed to young fish. We have fed them to newly born guppies and to native Texas darters of about one-half inch in length. Drosophila is not very satisfactory for feeding to young fish that need a constant supply of live food as the larvae do not live long enough under water. The maximum yield of larvae is from the larger sizes. Thus feeding small larvae to young fish is less efficient than feeding large larvae to medium sized fishes.

The larvae from two D. virilis bottles per day (one for morning and one for night feedings) should be more than adequate for most aquarists if the raising techniques described below are followed. Four dozen half-pint milk bottles should be all that most aquarists need. Each aquarist, however, must check to see if his Drosophila cultures are adequate for his particular needs.

Harvesting Larvae

Harvesting larvae just prior to pupation gives the maximum yield. We harvest the cultures after the first pupae appear. If a culture is refrigerated, development ceases; death eventually results from prolonged refrigeration. Thus cultures may be maintained in a harvestable stage for about one week if placed in a refrigerator immediately after the first pupae appear. Care must also be taken not to let the flies get too warm. A Farenheit temperature above the high 80's results in sterilization of the flies, and a higher temperature kills them.

When the first pupae appear or the larvae have reached maximum size, the top of the mash will be semi-liquid. This semi-liquid medium with all of the larvae is freed from the firm remainder by a stream of water. This mixture is poured through a fine meshed net (nylon nets last longer) to separate the larvae from the liquid mash. The larvae should be rinsed under a faucet to remove all mash remnants. If some particles of the medium get into the net it must be

picked out or broken up by hand. These cautions are necessary to prevent fouling aquaria. If several bottles of larvae are used, many larvae will work their way through the net and fall into the washings. Recovery can be achieved by pouring the washings through a net. These larvae are usually smaller; they make better food for small fish.

The washed larvae are ready to be fed to the fish. As they do not live under water for more than an hour or so, care should be taken not to feed more larvae than the fish will eat. Scavengers should be present to eat the left over larvae. Mystery snails, *Corydoras,* and *Hoplosternum* are examples, but like all scavengers tested, they also feed on live larvae. A living supply of *Drosophila* can be made available to the fish by placing the larvae on a mat of floating vegetation such as *Riccia.* The larvae will live for several days and the fish can eat them when hungry. A "raft" made by tightly covering a feeding ring with nylon nettings serves the same purpose.

CULTURING FRUIT FLIES *(Drosophila),* AND OTHER LIVE FISH FOOD

Part 2 *(Conclusion)*

Saving Brood Stock

Larvae to produce a breeding stock should be removed from the net and placed on fresh medium (about every 10 days for D. hydei and D. virilis and weekly for the two smaller species.) It is possible to rear adults of D. simulans and D. melanogaster by setting aside the desired number of cultures containing harvestable larvae. Larval development of the other two species makes the medium so liquid that adult transfer is nearly impossible. In general it is safer to transfer breeding stock larvae to a fresh medium prior to pupation.

Between 200 and 500 flies (the larger number for smaller species) will produce sufficient eggs for a half-pint bottle. One bottle of larvae should produce enough adults to supply breeding stock for at least one and probably two bottles, following our instructions for transfering the flies. If one does not intend to regularly transfer flies to new bottles a much smaller breeding stock should be used as the crowded flies will die.

The adult flies cannot produce eggs immediately after emerging from the pupal case. While the adults are becoming ripe for egg laying, they must have an adequate supply of yeast for food. Adequate food supply may best be given by transferring the flies to fresh bottles of media every other day.

As stated above, after eggs first appear the adults should be transferred regularly. The adults survive better and lay more eggs if this is done. If the adults are left in the bottle, they eat most of the yeast. The adults need a constant food supply for high egg production. Not only is egg production lowered while the food supply is insufficient, but the adults do not lay as many eggs during the next day or two, even if given sufficient yeast. Moreover, if the number of flies recommended is in a culture bottle, they will lay more eggs than the bottle will support of larvae in one day (D. virilis and D. hydei) or two days (D. simulans and D. *melanogaster).*

The few bottles at room temperature which do not contain larvae three days after the eggs have been laid may be considered barren. Failure of the eggs to hatch may be caused from several factors: (a) it may be that the brood stock has been sterilized by too high temperatures; (b) the culture may be moldy; (c) the first eggs laid by the flies might not hatch, or (d) the culture might be all females. One must be careful when transferring the newly emerged adults as the males emerge before the females. It is possible to set up culture bottles in which most of the individuals are the same sex.

Relative Usefulness of Different Fruit Flies

Our experiments were restricted to four species: D. melanogaster, D. simulans, D. virilis, and D. hydei. Wildtype D. melanogaster and D. simulans are

similar in size, appearance, and value as fish food. About two to four cc (4.9 cc=one teaspoonful) of damp and packed larvae can be raised on banana mash in an upright half-pint milk bottle. If the bottle is laid on its side, 3 to 6 cc of larvae can be raised. The amount of surface exposed is one limiting factor. Still another is the yeast to feed the larvae. If brewer's yeast is sprinkled in the bottle daily after larval growth is noticed, "upright" production is raised from 5 to 9 cc. Six to 10 cc of larvae are raised when the bottle is on its side.

More volume can be raised using either ***D. virilis*** or ***D. hydei*** as both flies and larvae are larger. As the larvae need to breathe, only as much medium is eaten as the larvae can reach without going below the surface for extensive periods. They can "hold their breath" for short periods, but this does not appreciably increase the amount of medium used. If held under for long, they drown as in water. *D. virilis* in a half-pint milk bottle produces: (about) 5 cc upright without yeast, 6 cc on side without yeast, 8.5 cc upright with yeast, and 9.5 cc on side with yeast.

Even more volume can be produced by ***D. hydei.*** Not only is the larva large, but also the female lays more eggs per day, i.e., about 150 vs. 50 for *D. virilis.* Other factors may apply as *D. virilis* production has never exceeded the volume of larvae produced by *D. hydei* from one day's supply of eggs, even when *D. virilis* females remain in the cultures for two or three days. *D. hydei* production in a half-pint milk bottle: (approximately) 7 cc on side without yeast, 16 cc upright with yeast, and 18 cc on side with yeast. (One sample contained 24 cc.) As dried yeast has a detrimental effect on young *D. hydei* larvae, the yeast must be moistened before being placed in the culture bottle with young larvae. Occasionally *D. hydei* individuals pupate in the medium. If a culture of this species is harvested after many pupae form, it is difficult to separate the larvae from the pupae.

The duration of the life cycle also affects the relative value of the four species. At room temperature, usually between 76 and 82° F., individuals of *D. melanogaster* required a minimum of about nine days from egg to egg; ***D. simulans*** 11 days; *D.* ***virilis*** 16 days; and ***D. hydei*** 18 days. Cultures with large numbers of larvae grow more slowly than unproductive cultures. Lower temperatures will slow down growth; higher temperatures may sterilize or kill. The temperatures cited above are higher than the 70 to 75° F. recommended by the geneticists. Thus one generation of *D. virilis* and *D. hydei* takes about as long as two generations of *D. simulans* and *D. melanogaster.* One must be especially careful with ***D. melanogaster*** and *D. simulans* as the short larval stage may end before the aquarist has noticed that the culture is ready for harvesting.

If small sized larvae are more desired than volume, ***D. melanogaster*** and *D. simulans,* which never reach large size, should be selected. Of course, *D. hydei* and *D. virilis* larvae can be fed at a small size.

Many aquarists will dislike the odor produced by yeast-fed ***D. hydei*** cultures. If the yeast is added only after the culture is well advanced, the aroma is not so unpleasant. Non-yeast-fed ***D. hydei*** and the other species do not have as noticeable an odor.

Vestigial-Winged Fruit Flies'

We have also experimented with vestigial-winged ***D. melanogaster.*** First of all, the number of larvae produced is

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Le Bron's Biologicals, 600 N. Kenilworth, Oak Park, supplies vestigial winged flies to aquarists. — The Editors.

less than in wild type flies. Moreover, the flies do not transfer well with our culturing techniques. While wild type flies produce better after several transfers (until old age), vestigial-winged fly populations produce fewer larvae after each transfer. Because they cannot fly, vestigial-winged flies must spend much time on the medium. Frequently, they get stuck and die, especially if the larvae have "worked" the medium. A fold of paper towel is recommended for vestigial-winged flies to climb on, but this interferes with washing the larvae.

The only advantage of vestigial-winged flies — they cannot fly from the surface of the water — disappears *when larvae are fed.* A single culture of *Drosophila* probably has the greatest amount of food material immediately before pupation. Prior to pupation, growth increases the size of the larvae. Much food must be expended by the *Drosophila* during the metamorphosis which occurs in the pupa. Moreover, not all individuals survive pupation and most fish will not feed on pupae. Obviously, each fly is of less food value than a full grown larva. Feeding larvae also shortens the culture period. (If desired, winged adults may be heat killed and fed to the fish.)

Microworms

Care must be taken to keep microworms out of *Drosophila* cultures. They do well on banana mash and compete with the fruit flies. For those who dislike the odors which are given off by the usual microworm cultures, the little nematodes grow with little odor on a banana mash medium. When the culture medium is ready for use in raising *Drosophila,* it can also be used for microworm cultures. A series of new microworm culture bottles can be started by putting a few drops of water into a thriving culture and then pouring two or three drops (now containing many worms) in each new culture bottle. The culture bottles should be tightly stoppered with cotton except when worms are being harvested.

For harvesting, pour a few drops of water in the bottle and then pour the water into the aquarium. Put the bottle back on the shelf until a new crop of microworms develops.

We have found that the technique described below produces a larger volume of microworms for amount of time spent tending the culture. We now use 9 x 15 inch enamel pans that are two inches deep. The culture medium is placed in the bottom to a depth of about one eighth inch. Two or three smooth wooden strips 13 x 1 x 3/16 inches are placed in the pan. The small worms crawl up on the sides of the pan or onto the strips and can then be scraped off for feeding to the fish. The pans must be tightly covered to keep out fruit flies.

We have successfully used two culture media (the oatmeal and brewers' yeast described by Innes and a combination of tortilla mix' and brewers' yeast) in the enamel pans. We mix one part of brewers' yeast with about 10 parts of cooked oatmeal or tortilla mix. We have found the tortilla mix and brewers' yeast medium most satisfactory as it does not go bad so fast. It frequently lasts long enough so that if worm production drops, it pays to sprinkle on more yeast.

Daphnia

We have had success raising *Daphnia magna* indoors roughly following methods outlined by Gordon (1950). The pessimistic view of Innes (1952) has not been substantiated by our work. Strong aeration together with frequent and

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We use Tamalina, a product of B. Martinez and Sons Company, San Antonio, Texas, for the tortilla mix.

heavy feeding is necessary for high production. If sufficient food is added to enable rapid daphnia production without aeration, the water will become toxic to daphnia. As daphnia are susceptible to low oxygen and high carbon dioxide concentrations, aeration is necessary if large quantities of food are used. If no aeration is available, daphnia may be raised by careful feeding. (The airstones may become clogged. If so, soaking in Clorox will clear them.) Both the left-over solid banana mash and the washings from fruit-fly cultures are superb food for daphnia. If yeast is added to the Drosophila cultures, the wash water is likely to kill daphnia unless used very sparingly. Small daily or twice daily feedings are better than large infrequent feedings. Not only are many daphnia produced, but only waste food material from Drosophila cultures need be used.

The daphnia should be harvested with a fine meshed net. If a coarse meshed net is used only the larger ones will be harvested. Harvesting only the breeding stock lowers production. A more serious difficulty with harvesting only large individuals occurs when other animals such as copepods are in the culture. After a short time copepods become abundant and the daphnia do not prosper unless a fine meshed net is used.

Our Daphnia magna cultures, similar to those of Drosophila, have done best at temperatures near 70° F. We had poor results when the temperature exceeded 85° F.

Although we have had success with daphnia in the two-inch deep enamel pans we use to raise microworms, it is better to use deeper containers as less air is needed to stir the water. Currently we are using wooden barrels for daphnia. Sizes range from 10 gallons to 55 gallons. The volume of daphnia produced appears to be in direct proportion to the volume of water contained in the barrel. The left over banana mash and wash water from one Drosophila culture bottle will serve to feed a 55 gallon daphnia barrel twice daily. If only one feeding is made, the mash from two Drosophila culture bottles may be used. Proportionally smaller amounts should be used for smaller barrels. The individual aquarist can experiment with different feedings and find out what quantities maintain highest daphnia populations.

Containers with a greater length (not height) are superior to barrels (we use left over specimen barrels). The extra length permits a settling region at one end of the container. Aeration causes a circulation of small particles that are continually carried about in the water if there is no still area. Not only do the daphnia do better without the particles, but the particles are caught with the daphnia. When barrels are used, the aeration cannot be turned up to optimum for the daphnia as the particles will not settle out. Keeping the airstone two or three inches off the bottom of the barrel helps the settling. Strong aeration does not appear to harm daphnia if no particles are in the water.

When winter eggs (they look like black spots on the body) appear in the culture, adverse conditions are present. If this is a result of overcrowding, feed more daphnia to the fish. If not, partial drainage of bottom sediments is recommended by Gordon, 1950. (These sediments, and those from vigorous cultures, contain large concentrations of infusoria which may be used for small fish.) Refill with new water, preferably old aquarium water; however, if none is available, tap water may be used. Unless more than half of the water has been removed, Austin tap water (0.8 parts

per million of residual chlorine) is safe as the organic material in the culture renders chlorine harmless. Daphnia is very susceptible to chlorine and serves as an excellent indicator of whether tap water is dangerous for fish.

When setting up a new culture, it is best to siphon half of the contents of a vigorous culture into the new container, and fill with water. Also, the addition of a few pond snails is advantageous as they eat a slimy substance which traps and kills many daphnia. The slime often develops on the sides of the cultures. As we have found that daphnia cultures do much better exposed to light, place them in well-lighted locations. More than one culture is recommended, for despite the best care, population fluctuations occur. By the use of several cultures, a more constant supply is probable. For maximum production, a large breeding stock is essential. Once a high population density is attained, only the daily increase should be used, as harvesting breeding stock results in decreased future yield.

Caution must be observed in feeding daphnia in unaerated aquaria, as the daphnia may produce sufficient carbon dioxide to kill the fish.

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